A Study of Continuous Monocropping Obstacles of Sesame (*Sesamum indicum* L.) for Sustainable Production on Upland Field Converted Paddy

(水田転換畑におけるゴマ (*Sesamum indicum* L.)の持続的生産のための連作障害に関する研究)

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DEDICATION

In memory of;

Sister, Stella Atimango,

Mother, Margaret Angeicon

&

Father, Ayweka Felix

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CHAPTER ONE

General introduction

1. Introduction

Sesame (*Sesamum indicum* L.) belongs to the family of *Pedaliaceae*. It is an oilseed crop cultivated throughout the tropical and subtropical regions of the world for its edible oil and uses in foods (Ashri, 1989). Sesame has the same diploid chromosome number 2n = 26 with the wild relative *S. malabaricum* (Thangavelu, 1994; Hiremath and Patil, 1999; Kawase, 2000; Kumar and Hiremath, 2008). These wild relatives are widely distributed in Africa and India making these regions believed as the origin of sesame (Sani et al., 2014). For instance, the genus of sesame contains 16 genera and 60 of the species occur in Africa (Ashri, 2007).

Sesame is an annual plant that can grow between 50 cm and 200 cm in height depending on the cultivars, with opposite leaves 4-14 cm long with an entire margin (**Figure 1**). Sesame leaves are broad lanceolate whereas the flowers are bell-shaped, pink, purple or white in colors which appear in the leaf axils. The fruit is a capsule that usually dehiscences upon drying leading to shattering of seeds depending on the cultivars. Each seed capsule may contain 80 to 100 seeds depending on the cultivar. The seeds are very small measuring about 3 to 4 mm long and 2 mm wide, 1 mm thick weighing about 20 to 40 mg.

Sesame seed is very nutritious and confers health-promoting benefits when included in the diet. The seeds are rich in edible oil (46-50%) and contains 83-90% unsaturated essential fatty acids mainly linoleic acid (37-47%), oleic acid (35-43%), palmitic acid (9-11%), stearic acid (5-10%), proteins (20%), 9.6-20.51% carbohydrates, 5.11-6.94% ash content and vitamin E, minerals, lignans (sesamolin and sesamin) as well as tocopherols (Fukuda et al., 1985; Unal and Yalcin, 2008; Hassan, 2013). In sesame oil, oleic and linoleic acids are the main unsaturated fatty acids while others include palmitic and stearic acids with little content of linolenic and arachidic acids (Asghar et al., 2014). The oleic acid (43%), linoleic acid (35%), palmitic acid (11%) and stearic acid (7%) occupy 96% of total fatty acids in sesame seed (Saydut et al., 2008).

Figure 1. Sesame plants and its seeds

Furthermore, the mineral composition include K (815mg/100g), P (647 mg/100g), Mg (579 mg/100g), Ca (415 mg/100g) and Na (122.5 mg/100g) (Nzikou et al., 2009). However, the compositions of minerals as well as fatty acid contents in sesame seed varies with the cultivar type. The raw seeds contain high amount of γ-tocopherol (744-1333 mg kg^{-1}) as vitamin E (lipophilic, phenolic compounds of plant origin) (Yalcin and Unal, 2011). It has been known that sesame oil is very resistant to oxidative deterioration due to these tocopherols and also its bioavailability can be increased by sesamol which can only be found in sesame seeds making it a very special oil crop (Wu, 2007). Therefore, the high nutritional contents in sesame that makes it consumed as a health food is attributed to its antioxidant activity (Nakano et al., 2008). These anti-oxidants in sesame oil known as lignans could be as high as 11.5 mg g^{-1} and about 82% is sesamin whereas 15% are sesamolin all available in sesame seeds that promote health benefits (Wu, 2007). Usually, sesame seed may be white, grey, brown, chocolate or even black in colour. It is also known that sesame cultivars with white seed coats usually contain high sesamin and sesamolin contents than black sesame (Wang et al., 2013). Researches indicate that the major functional ingredients in sesame seeds are these lignans that contribute to the nutraceutical importance and health benefits of such as protecting against hypertension, hypercholesterolemia, dermatological diseases, carcinogenic substances, neurodegenerative diseases like Alzheimer’s disease and acting as an antioxidant in the body (Kanu et al., 2010; Prasad et al., 2012). Hence, it is important to include sesame seeds in the diet.

Due to the nutritional and health benefits of sesame seeds as mentioned above, its production is gradually increasing throughout the world. Recently, it is estimated that sesame is cultivated on an area of about 10.6 million hectares, with Sudan, India, Myanmar, United Republic of Tanzania and South Sudan contributing 20%, 18%, 14%, 9% and 6% respectively of the world’s total area where sesame is harvested (FAOSTAT, 2016). Therefore, Africa and Asia accounts for over 97% of the world’s sesame production (**Figure 2**).

Although Asia is among the top world producers of sesame, Japan sesame production is still low. According to Yasumoto and Katsuta (2006), the domestic production was negligible but now gradually increasing since sesame has been considered as a local specialty crop. Over the past two decades, sesame seed production peak was only observed in 2003 in which 65 tons of sesame seeds was produced from a total area of 125 ha after which sesame production stagnated at 11 tons of sesame seeds produced from an area of 21 hectares (FAOSTAT, 2016) (**Figure 3**).

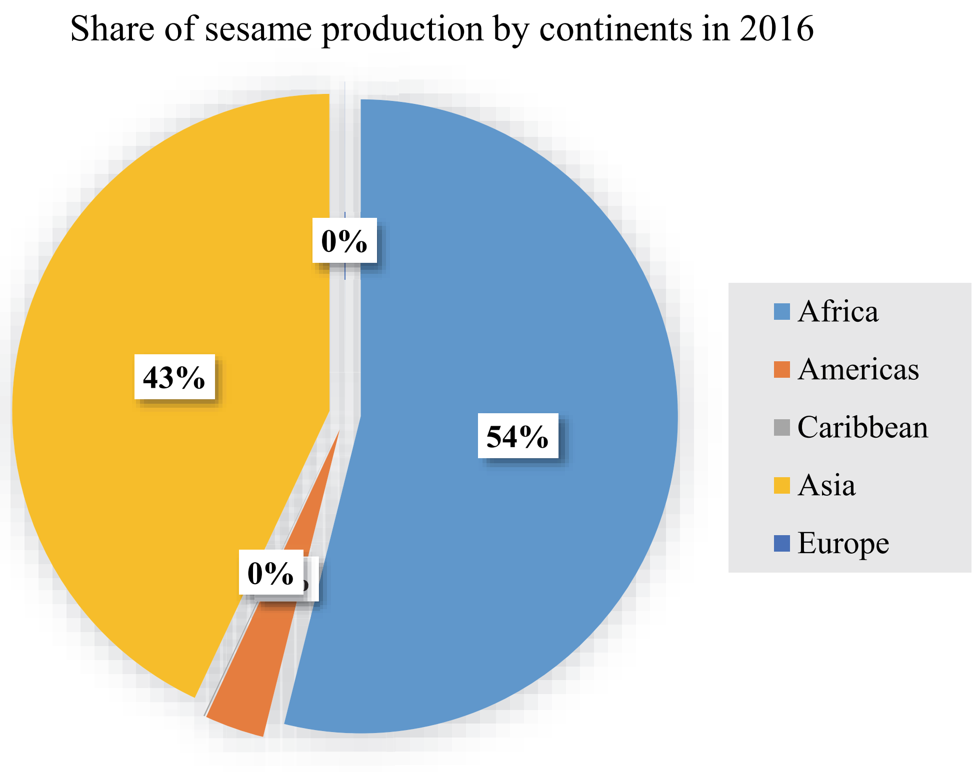


Figure 2. The sesame production share in the world.

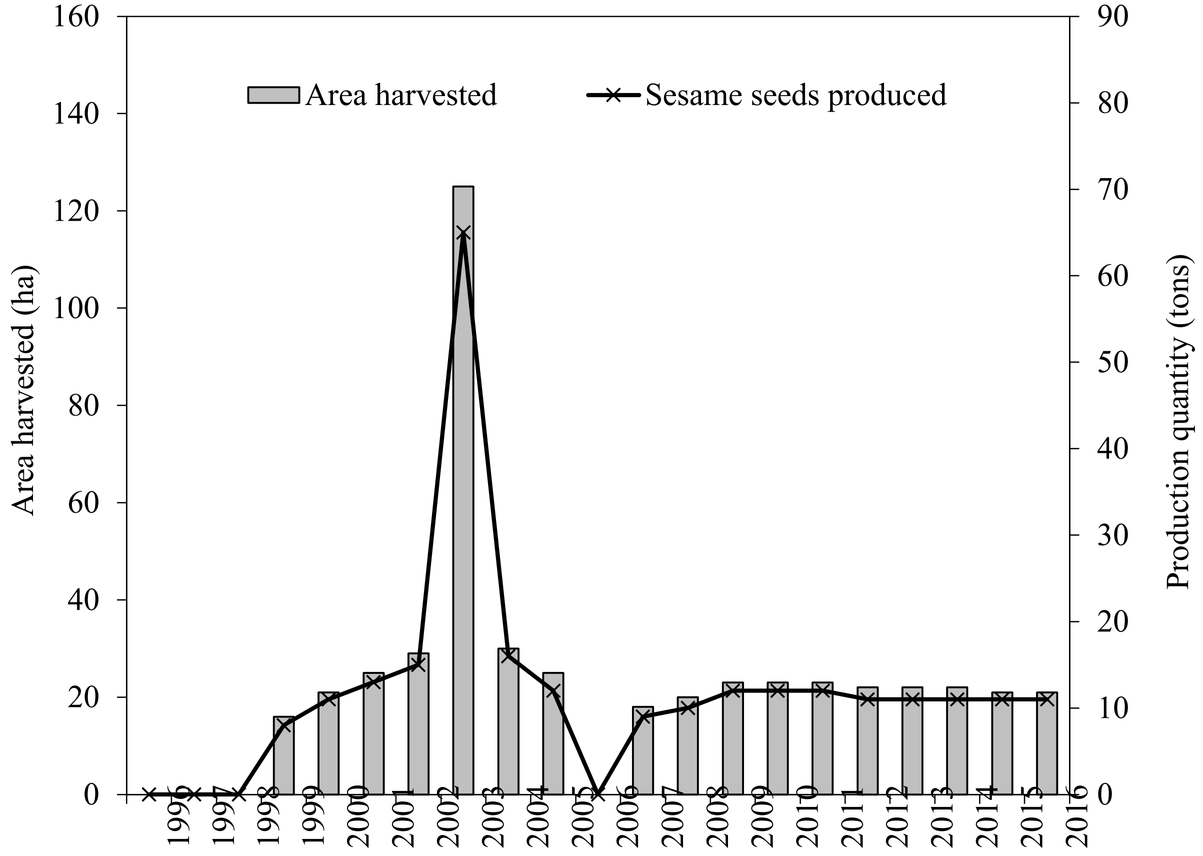


Figure 3. Japan's area under sesame cultivation (area harvested) and production of sesame seeds in the period 1996-2016.

Hence, the low production of sesame in Japan makes it among the largest sesame seed importers in the world to satisfy the rising domestic demand for sesame products. Therefore, most of the sesame seeds consumed in Japan is imported (**Figure 4**). For instance, according to FAOSTAT, Japan imported 152,101 tons of sesame, being the second largest importer after China (931,159 tons) in 2016 mainly imported from the top world exporters such as Ethiopia, India, Sudan, Nigeria, Burkina Faso etc. and spending over 200 million USD (FAOSTAT, 2016).

Although sesame seed production is low in Japan, recently its cultivation could be promoted through utilization of abandoned paddy fields. By the year 2010, an estimated 396,000 ha of agricultural land including paddy fields were abandoned nationwide (MAFF, 2014). This could be attributed to the shifting economics and declining rural population of Japan since the 1970s resulting into increased number of abandoned paddy fields (MAFF, 2001). Therefore, the government encouraged farmers to convert these abandoned paddies to the production of upland crops such as soybean, barley, wheat and sesame among others under a fallow system (MAFF, 2001; Chono et al., 2012). Given the profitability of sesame production, it is likely to increase from the utilization of these abandoned paddy fields. However, with intensification of production coupled with limited land area, farmers will have to increase yield of crops including sesame on the same piece of land, so continuous monocropping is expected to become more popular or even inevitable (Tilman et al., 2011; Alexandratos and Bruinsma, 2012). Hence, a better understanding of the influence of continuous monocropping on sesame productivity will be necessary to learn the most appropriate number of consecutive crops.

Continuous monocropping is the practice of cultivating the same crop in consecutive years on the same piece of land (Nafziger, 2009). This practice is not usually recommended due to decreased growth, yield and quality termed as ‘continuous monocropping obstacle’, ‘replant problem’, or ‘soil sickness’ (Rice, 1984; Bennett et al., 2012; Huang et al., 2013). There are reports that continuous monocropping obstacles occur in several crops such as aerobic rice (Dobermann et al., 2000; George et al., 2002; Peng et al., 2006), cowpeas and mung beans (Ventura and Watanabe, 1978), maize (Stanger and Lauer, 2008), and soybean (Johnson et al., 1992). For instance, low yield of soybean has been reported in continuous monocropping, ranging from 9.9 to 35.4% decline in 2-4 years cropping in Northeast China (Liu and Herbert, 2002). In addition, it was reported that soybean seed yield decreased by 9-14% yield in continuous monocropping (Johnson et al., 1992).

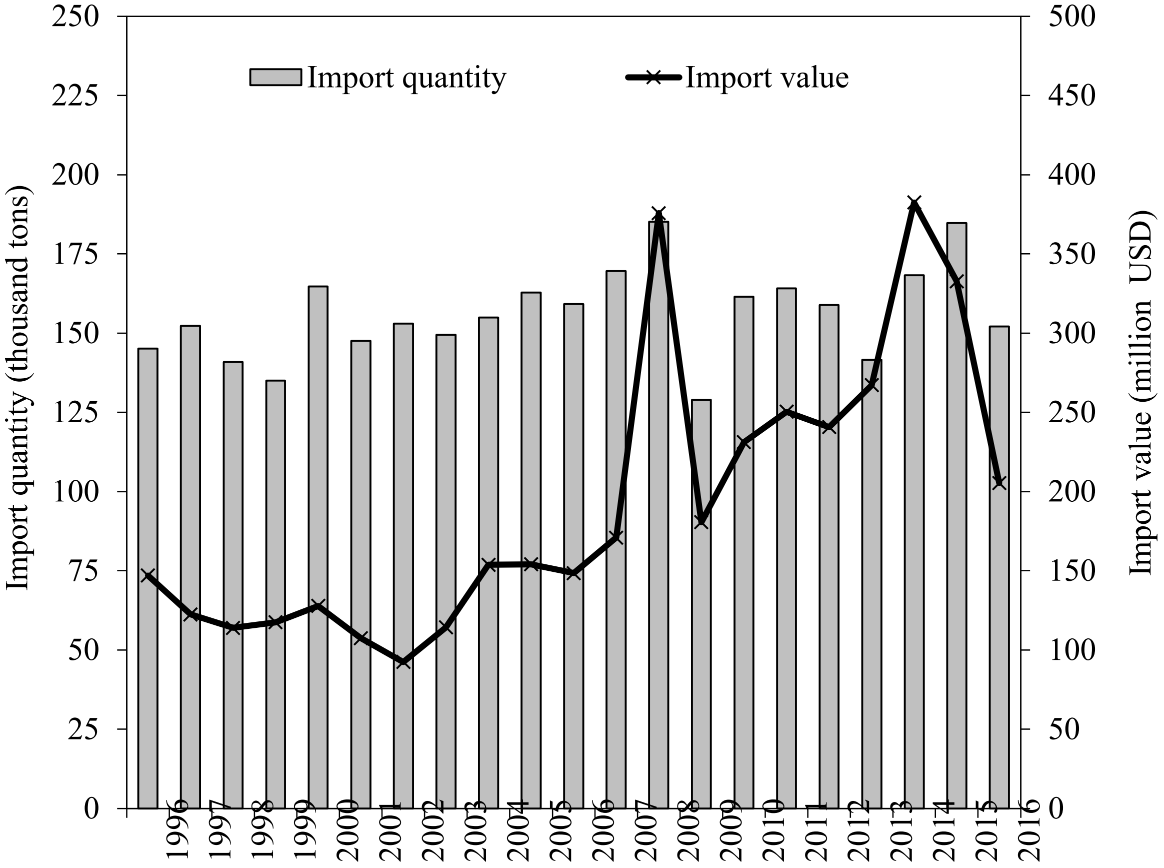


Figure 4. Japan's sesame import quantity and import value over the past two decades (1996-2016).

Furthermore, Dobermann et al. (2000) reported decline in aerobic (upland) rice yield in a long term continuous monocropping from 1968 to 1991 in Philippines. It was also reported that the overall grain yield of upland rice decreased by 73% in the second to third season compared to the first seasons in a three year experiment involving two seasons per year at different locations in Phillipines (George et al., 2002). Later, Peng et al. (2006) also reported a significant decline in aerobic rice yield by 40% after seven seasons of continuous monocropping compared to the first season and observed yield variation caused by the number of seasons, weather and variety type. Therefore, continuous monocropping is detrimental to crop yield and growth (Ventura and Watanabe, 1978; Zhou and Wu et al., 2015). Although sesame is an important oilseed cropping, it is reported to suffer yield declines in continuous monocropping (Ashri, 1989). Chung et al. (1989) reported that in continuous monocropping of sesame for 4 years, seed yield decreased by 33% compared to that after 1 year of cultivation in Korea. Furthermore, Alebiosu (2011) also reported lower seed yield in continuous monocropping (246.03 kg ha^{-1}) when compared to rotational (283.3 kg ha^{-1}) in Nigeria suggesting sesame productivity is limited by continuous monocropping obstacles. However, there is lack of information on continuous monocropping obstacles of sesame on upland fields converted paddy (especially in Japan).

Furthermore, the yield declines under continuous monocropping may be accompanied by quality deterioration of crops and high value plants. The seed yield of soybean decreased by 22% after four seasons of continuous monocropping system accompanied by significant decrease in seed crude protein, phosphorous (P) and iron (Fe) contents compared to rotation cropping with corn during a three year field experiment in the USA (Bellaloui et al., 2010). Bellaloui et al. (2010) attributed the decrease in seed mineral contents to unfavorable soil nutrient conditions indicated by decrease in boron (B) that affected crude protein, and a decrease phosphorous (P) and iron (Fe) including overall nutrient uptake in continuous soybean compared to rotation suggesting continuous monocropping could affect nutritionally quality of crops. They also reported a decrease in oleic acid content in continuous monocropping of soybean whereas linoleic acid tended to increase suggesting continuous monocropping could alter seed fatty acid compositions and overall quality. Furthermore, it was reported that continuously monocropping soils decreased *Angelica sinensis* root yield and essential oil by 49.63% and 28.75% respectively in addition to growth inhibition compared to a non-continuous monocropping treatment in a pot experiment (Xin-Hui et al., 2015). Kaye et al. (2007) also compared continuous monocropping of sorghum with a rotation cropping using soybean in a two years (2003 to 2004) field experiment in Nebraska, USA and reported significant decrease in grain yield, kernel weight and seed quality in terms of low N content that was attributed to decrease in N availability. They observed sorghum grain yield in continuous monocropping decreasing by 143% and 79% in 2003 and 2004 respectively compared to a non-nodulating soybean rotation without soil amendment whereas seed N content decreased by 9% in continuous monocropping sorghum compared to that in the non-nodulating soybean in 2004. These studies suggested decrease in nutritional quality under continuous monocropping systems could occur in croplands. Seed quality is one of the most important aspect considered in oilseed crop production since it determines the marketability of the produce. Poor seed quality under continuous monocropping systems would result into significant economic losses to producers of oilseed crops including sesame. However, there is lack of information on the effect of continuous monocropping on the mineral and fatty acid quality of sesame seeds including yield on upland field converted paddy.

Several factors have been implicated for causing continuous monocropping obstacles. These include changes in mineral nutrient availability, deterioration of soil physicochemical and biological characteristics, allelochemical accumulation, and soil-borne pathogens (Ventura and Watanabe, 1978; Gentry et al., 2013). Deterioration in soil chemical properties are associated with both depletion of a specific mineral nutrient or poor absorption of nutrients despite its availability due to allelopathy, autotoxicity and soil-borne pathogens (Wang et al., 2014; Ye et al., 2014; Zhou and Wu, 2015; Li et al., 2016). For instance, Wang et al. (2014) compared continuous monocropping and relay intercropping in a field experiment from 2010 to 2012 in Northwest China and observed significant decline in eggplant yield due to the depletion in the available N, P and K in continuous monocropping evidenced by decrease in the activities of soil enzymes such as urease and phosphatase. The decrease in the available soil mineral nutrients consequently resulted into significant decrease the nutrient uptake in the plant tissues negatively affecting growth and yield. Furthermore, a significant decrease in maize grain yield under continuous monocropping on an Alfisol soils in a field experiment that was conducted from 1984 to 1987 in Ghana was reported and attributed to severe N deficiency due to allelopathic effects and deterioration of soil physical properties such as bulk density (Horst and Hardter, 1994). N, P, K, Mg, Ca and Cu were also significantly decreased due to the low organic matter content in the soils with continuous monocropping history of 21 years in Argentina that led to soybean yield decline compared to rotation cropping system (Perez-Brandan et al., 2014). Moreover, it was reported that a 30-year continuous monocropping of sugarcane on a Mexico Fluvisol soil caused significant decrease in soil organic carbon, total N and P contents that could negatively influence sugarcane yield (Naranjo de la et al., 2006). Continuous monocropping could lead to depletion of soil mineral nutrients also indicating a loss in soil quality (Reeves, 1997). Decrease in the soil mineral nutrients not only affect growth and yield but also lower the mineral nutrient contents such as seed N, P, Fe among others of crops (Riedell et al., 2009; Bellaloui et al., 2010). Therefore, it is important to monitor and manage soil mineral nutrient status in continuous monocropping systems while ensuring their availability to crops. However, there is still lack of information on the changes in soil mineral nutrient availability directly linked to continuous monocropping obstacles of sesame on upland field converted paddy.

Furthermore, several factors could interact in continuous monocropping systems leading to the overall crop yield, growth and quality declines. These could range from depletion of soil mineral nutrients and imbalances, to increase in allelochemicals from decomposing residues and root exudates and increase in diseases all interacting at the same time and increasing the magnitude of continuous monocropping obstacles (Bennett et al., 2012). For instance, the decline in sugarcane yield with increase in ratooning time was reported in continuous monocropping fields in India attributed due to the depletion of soil N, P and K indication loss in soil fertility loss, increase in allelochemicals (or chemical interferences) and accumulation of phytotoxic soil microflora (Ghayal et al., 2011). This indicates that the existence of allelopathy and autotoxicity caused by allelochemicals is an important aspect in continuous monocropping systems (Tsuchiya et al., 1994; Huang et al., 2010).

Allelopathy refers to the inhibition of one plant by another due to inhibitory substances released through root exudation, and leaching, volatilization and through decomposition of plant residues in soil (Batish et al., 2007). On the other hand, autotoxicity refers to the release of allelochemicals including organic acids into the soil from decomposing residues that inhibit the germination and growth of the same plant (Putnam, 1985). Both allelopathy and autotoxicity play significant roles in continuous monocropping obstacles. For instance, decomposing maize residues release soil phytotoxins (allelochemicals) which inhibit the growth of maize leading to the yield decline in continuous monocropping (Stanger and Lauer, 2008; Rogovska et al., 2012). Moreover, the autotoxicity potentials of continuously monocropped soils have been reported to increase with increase in duration of continuous monocropping (Hao et al., 2006). They demonstrated in a glasshouse experiment with rhizobox and bioassays that continuous monocropping soils of watermelons cropped for 3 and 30 consecutive seasons exhibit autotoxicity due to phenolic compounds including *p*-hydroxybenzoic and *p*-coumaric acids decreasing the plant dry weight more than in soils of rotation cropping. Hence, continuous monocropping obstacle could increase with increase in the cropping that are released from exudation and decomposing residues. For instance, Yeasmin et al. (2013) also reported that decomposing residues of asparagus released allelochemicals that inhibited the growth of the subsequent cropping asparagus crop causing continuous monocropping obstacles. However, allelopathy and autotoxicity depends on the crop species or cultivars thereby leading to variation in tolerance to continuous monocropping obstacles depends on the cultivars (Liu and Herbert, 2002; Peng et al., 2006; Yeasmin et al., 2014). Therefore, it is important to identify cultivars with high growth and yield performances despite continuous monocropping obstacles.

Although sesame roots release allelochemicals which could include terpenes, esterols, hydrocarbons, fatty acids, phenolic, alkaloids and nitrogen compounds that inhibit growth of other crops and weeds (Kumar and Varshney, 2008; Soleymani and Shahrajaban, 2012), continuous monocropping obstacles of sesame related to allelochemicals has not yet been fully studied (Wang et al., 2010). Allelochemicals released from the roots could increase soil borne pathogen that could aggravate the problems of continuous monocropping. Earlier researches indicate that continuous monocropping obstacles of sesame are cause by increase in disease incidences caused by *Fusarium oxysporum* (Nam et al., 2007; Hua et al., 2012). Nam et al., reported sesame yield as low as 489 kg ha^{-1} in addition to poor growth under continuous mono cropping compared to 650 kg ha^{-1} under rotation cropping in Korea. The build-up of soil microorganisms such as *Fusarium oxysporum* f. sp. *sesami* (*fusarium* wilt) and *Ralstonia solanacearum* (bacterial wilt) in continuous monocropping also contributed to yield declines (Hua et al., 2012). Therefore, continuous monocropping diseases could also negatively affect sesame cultivars that release large quantity of allelochemicals. Hence, it is important to study autotoxicity of sesame understand continuous monocropping obstacles on upland field converted paddy.

Although growth, yield and quality declines is still major challenges in continuous monocropping systems, several mitigation strategies have been recommended. These include the use of intercropping to counteract effects of root exudates, additional fertilizers to supply more nutrients, soil amendments such as activated carbon and biochar to add more nutrients and adsorb allelochemicals, cattle manure increasing nutrients, and use of resistant cultivars (Motoki et al., 2012; Bennett et al., 2012; Wang et al., 2015; Yang et al., 2016; Cao et al., 2017). However, the selection of methods to mitigate continuous monocropping obstacle could depend on the associated cause of the growth and yield declines. For instance, the decline of aerobic rice yield due to low availability of N could be mitigated through addition of the right sources of N fertilizers to increase its uptake from the soil resulting into high growth and yields under continuous monocropping (Nie et al, 2008; 2009). Five sources of N including ammonium sulfate, urea, ammonium chloride, ammonium nitrate and potassium nitrate were compared at different rates on the growth, N uptake and grain yield of aerobic rice and was found that ammonium sulphate increased N uptake, growth and yield more than the other N sources, also suggesting more N could mitigate continuous monocropping obstacle of rice (Nie et al., 2009). However, since the nutrients limiting growth and yield in continuous monocropping may not be the same across all crops, it is important to first identify the limiting nutrient and supply the right sources of fertilizers. Addition of K and P fertilizers into soils where these nutrients have been depleted were proposed, including trace elements inform of foliar sprays could overcome and mitigate soil nutrient imbalances in continuous monocropping systems (Liu and Herbert , 2002; Ye et al., 2014). Therefore, the supply of additional nutrients that are limiting yield in continuous monocropping soils could increase nutrient availability while preventing nutrient imbalances in crops to mitigate continuous monocropping obstacles. However, there is lack of information on the changes of nutrients under continuous monocropping soils of sesame and how potential soil nutrient imbalances could be restored.

Soil amendments such as biochar has potential to mitigate continuous monocropping obstacle (Elmer and Pignatello, 2011). Biochar is a material produced from thermal degradation of organic materials in the absence of air and is used as a soil amendment to improve soil quality and nutrient availability increasing crop yields (Lehmann and Joseph, 2009; Jones et al., 2012). Apart from increasing soil mineral nutrients, biochar addition have been used as soil amendment to adsorb phytotoxins in the soil alleviating growth inhibitory effects due to allelopathy and other toxic compounds (Keech et al., 2005; Elmer and Pignatello, 2011; Rogovska et al., 2012; Ebeheakey, 2014). For instance, in a field study to investigate the effect of rice straw biochar addition at rate of 6 t ha^{-1} in combination with compost at 15 t ha^{-1} including inorganic fertilizers, on *Fusarium* wilt disease occurrence and soil chemical properties of continuous monocropping watermelon, it was found that watermelon yield increased attributed to increase in availability of N, P and K improving soil fertility although no significant effect was observed on *Fusarium* wilt disease incidences (Cao et al., 2017).

Furthermore, Elmer and Pignatello (2011) reported decrease in fungal diseases as biochar added at 1.5 and 3.0 % (wt./wt.) increased dry weight of asparagus on despite the addition of allelopathic compounds suggesting biochar adsorbed phytotoxic compounds mitigating continuous obstacle. These studies showed that, where soil mineral nutrients limiting crop growth and yield in continuous monocropping, nutrients could be replenished at the same time detoxifying allelochemicals in soil through biochar addition. However, there are no researches on the influence of biochar addition to increase sesame productivity on upland field converted paddy under continuous monocropping.

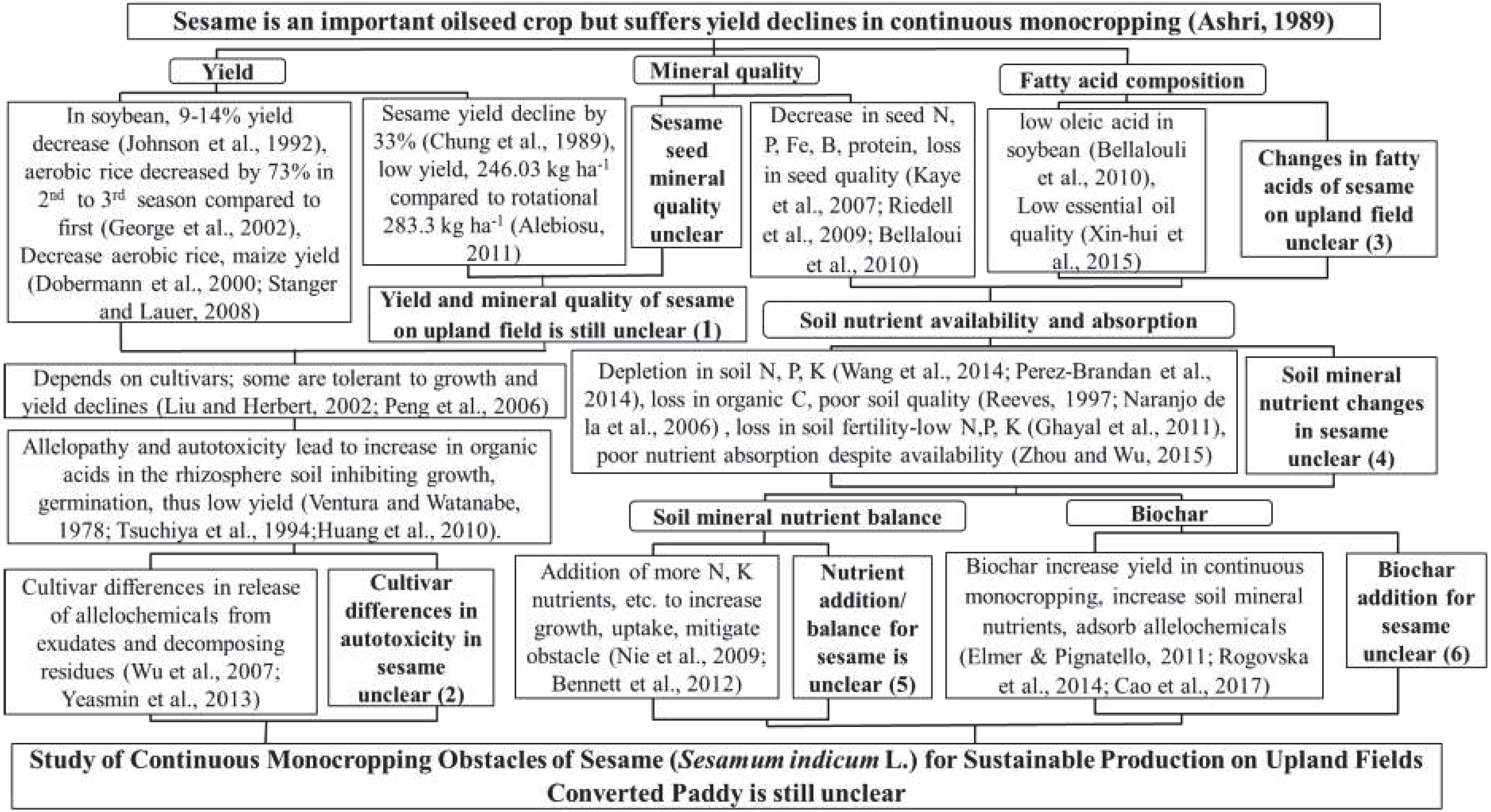


Figure 5. Summarized literature review flowchart.

1.2. Overall scope and study objectives

The overall goal of this study was to generate information on continuous monocropping obstacles of sesame and strategies to maintain high growth, yield and quality despite potentially negative influences of continuous monocropping on upland fields converted paddy in Japan.

1.2.1. Specific objectives

1. To determine the influence of continuous monocropping (obstacles) of sesame on upland field converted paddy on seed yield and mineral nutrient contents of sesame cultivars.
2. To determine the autotoxicity potentials of sesame cultivars.
3. To determine the fatty acid compositions in relation to yield of sesame.
4. To identify potentially limiting mineral nutrients.
5. To determine influence of additional nutrients on the seedling growth of sesame on continuously monocropped soils.
6. To determine influence of rice husk biochar addition on the growth, seed yield, and seed mineral nutrient contents of sesame.

1.3. Hypotheses

1. Sesame cultivars comprise of a large variation with respect to yield and mineral nutrients in response to continuous monocropping that could be exploited for sustainable sesame production on upland field converted paddy.
2. Sesame cultivars differ in their resistance towards autotoxicity relating to their differences in responses to continuous monocropping obstacles.
3. Continuous monocropping alters the fatty acid compositions in relation to yield of sesame on upland field converted paddy.
4. Continuous monocropping of sesame causes nutrient imbalances resulting into growth and yield declines on upland fields converted paddy.
5. Additional nutrients improves sesame growth on continuously monocropped soils from on upland fields converted paddy.
6. Rice husk biochar addition improves sesame growth, yield, and seed mineral nutrient contents of sesame under continuous monocropping on upland fields converted paddy.

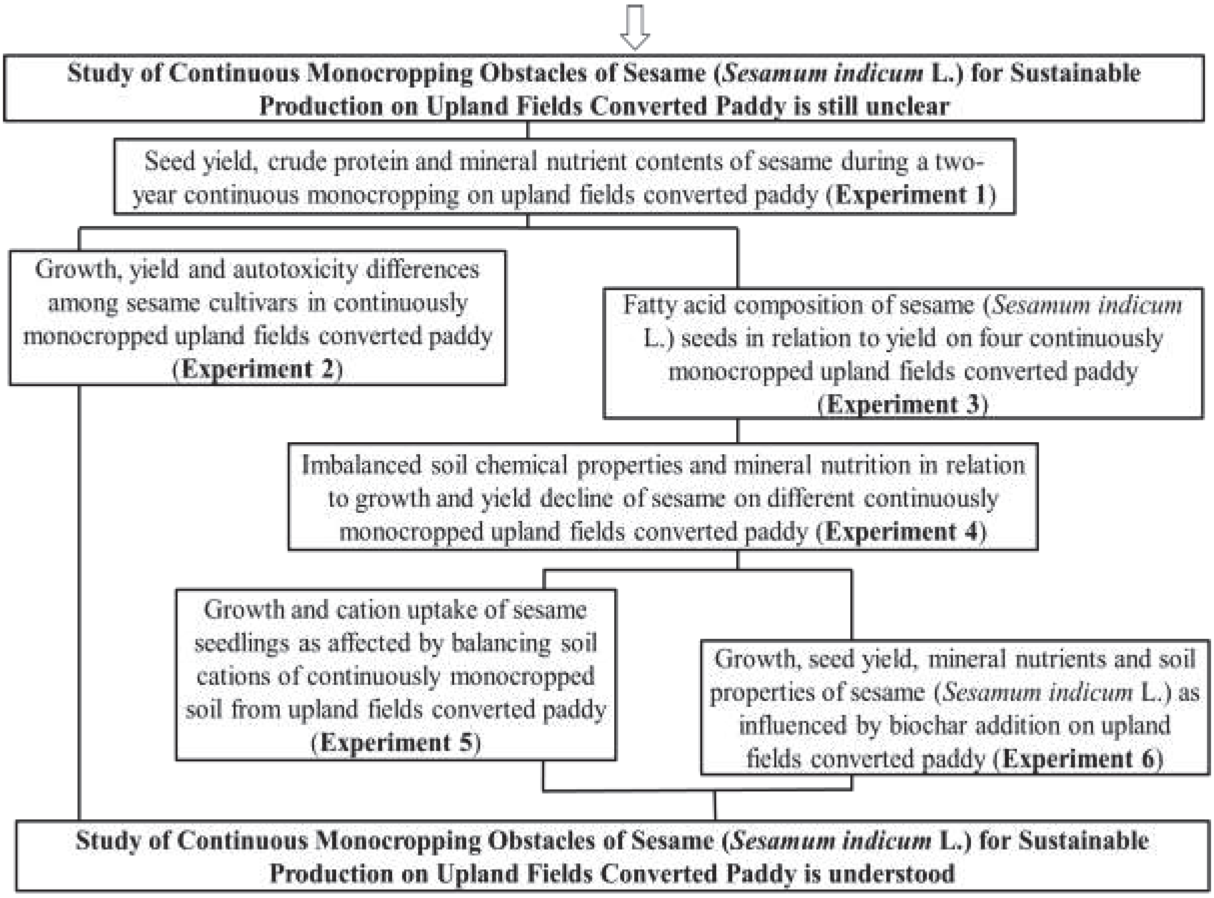


Figure 6. Summarized experiment flowchart.

CHAPTER TWO

Seed yield, crude protein and mineral nutrient contents of sesame during a two-year continuous monocropping on upland fields converted paddy

2.1. Introduction

Sesame production in Japan is negligible, although it is increasing because it is considered a local specialty crop (Yasumoto and Katsuta, 2006). However, earlier research that continuous monocropping of sesame often negatively affects yield (Ashri, 1989). Most researches have mainly focused on soil borne diseases (Paik et al., 1988; Hua et al., 2012) and unbalanced soil chemical properties (Chung et al., 1989). Chung et al. (1989) reported a decrease in seed yield by 33% after 4 years of continuous monocropping compared with the first partly attributed to low soil pH including exchangeable Ca and Mg whereas soil exchangeable K and available P increased. In a comparison between continuous monocropping and rotational cropping, sesame yield increased by 33% (650 kg ha^{-1}) in rotation with green manure crops than in continuous monocropping which yielded 489 kg ha^{-1} (Nam et al., 2007). However, these studies had little focus on upland field converted paddy cultivation or sesame production. Although upland crop cultivation is encouraged on converted paddy fields, yield decreases haves been noticed (Matsumoto and Yoshikawa, 2010; Hattori et al., 2013). However, there is no information on sesame cultivation including continuous monocropping on upland fields converted paddy in Japan. Hence, a better understanding of the influence of continuous monocropping on sesame productivity will be necessary to learn the most appropriate number of consecutive crops.

Sesame seeds also contain approximately 25% protein with a range of 17–31% and about 50% oil content (Hwang, 2005). In addition, sesame seed is composed of high calcium, phosphorous, potassium, magnesium, iron, copper, zinc and manganese (Nzikou et al., 2009; Alyemeni et al., 2011). These are important for mineral nutritional benefits and different continuous monocropping years are likely to influence their contents for seed quality since seed yield is affected by the cropping years. In addition, sesame yield depends on cultivars, environmental factors such as temperature, humidity and management practices (Nath et al., 2001; Diouf et al., 2010). However, there is lack of knowledge on how continuous monocropping affects seed yield and quality of different sesame cultivars under consecutive years of cropping in Japan.

Until now, there has been no research on the changes in seed yield, crude protein and seed mineral nutrients of different sesame cultivars in Japan on upland field converted from rice paddy under different continuous monocropping years. Generating information on effect of continuous monocropping will enable selection of suitable cultivars with high performance in continuous monocropping. The objectives of this study were to; i) determine the effect of continuous monocropping on the seed yield, crude protein and mineral nutrient contents in different sesame cultivars under continuous monocropping and ii) identify cultivars adaptable to continuous monocropping obstacle while maintaining seed yield and quality under continuous monocropping of sesame.

2.2. Materials and Methods

2.2.1. Location and site description

The study was conducted on upland field converted from rice paddy during the summer seasons of 2012, 2013, and 2014 from June to September at Tottori Prefecture, Japan (35°29′N, 134°07′E). Since the experiment was conducted in upland field converted from a paddy, the soil type was clay–loam containing 42.3% sand, 30.8% silt and 26.9% clay for rice production. The initial soil physical and chemical properties of 0–15 cm soil layer were as follows: pH 5.4 (1:5, soil/water), EC 0.05 dS m^{-1} (1:5, soil/water), total N (TN) 2.14 g kg^{-1}, total C (TC), 22.4 g kg^{-1}, C/N ratio 10.5, available P 29 mg kg^{-1}, exchangeable K 248 mg kg^{-1}, exchangeable Mg 107 mg kg^{-1},exchangeable Ca 918 mg kg^{-1}, CEC 10.5 $cmol_c\,kg^{-1}$,Fe 349.1 ppm, Mn 39.9 ppm, Zn 6.1 ppm, Cu 8.5 ppm, bulk density 1.2 g cm^{-3} and porosity 56.2%.

2.2.2. Field experimental design

The experiment was conducted for three consecutive years from 2012 to 2014. Four sesame cultivars (‘Maruhime’, ‘Nishikimaru’, ‘Gomazou’, and ‘Masekin’) bred by The Institute of Crop Sciences, National Agricultural and Food Organization (NARO), Japan were continuously monocropped in an upland field converted from a rice paddy. These four cultivars were selected based on their high lignan contents and adaptability for commercial production in Japan.

In 2012, one experimental plot was established to cultivate sesame for first time (first cropping) and in 2013, another new field of the same size adjacent to the old field was established for first cropping cultivation, while cultivation was done for the second time on the previous field of 2012 (second cropping of 2013). In 2014, cultivation was done for the second cropping on the first cropping field of 2013. The first field established in 2012 and the second established in 2013 are therein referred to as field A and field B cultivated for two consecutive times from 2012-2013 and 2013-2014 respectively. Averages of first and second cropping were obtained from both fields A and B. In addition, each field measured 14.4 m x 6.5 m (93.6 m^2) of each year was divided into 12 small plots, each 3 m x 2.3 m (6.9 m^2). We grew one cultivar in each small plot in a completely randomized blocked design with three replicates per cultivar. According to the cultivation manual from NARO, the field was plowed and harrowed to a fine tilth, to a depth of 15 cm, using a power tiller before each sowing. Basal inorganic fertilizer was applied at rates of 70 kg ha^{-1} N, 105 kg ha^{-1} P_2O_5-equivalent, and 70 kg ha^{-1} K_2O-equivalent, and dolomite was applied at 1000 kg ha^{-1}.

The plants were cultivated on raised ridges that were 75 cm wide and separated by 40 cm. On each ridge, double rows of each sesame cultivar were planted at a spacing of 45 cm × 15 cm. Five seeds were sown on each hill and later thinned to two plants per hill at 2 weeks after sowing, then to one plant per hill at 3 weeks. Sesame was sown on 10 June 2012, 23 June 2013, and 26 June 2014. Weeding was done by hand whenever necessary to keep the field free of weeds. Harvesting was done on 23 September 2012, 27 September 2013, and 28 to 29 September 2014. Harvested plants for samples in each treatment were tied up in bags to allow air-drying; when the material was adequately dry, seeds and chaff were separated by winnowing after harvest. The total seed weight per replicate of each cultivar was used to determine the mean seed yield (kg ha^{-1}) and the weight of 100 seeds was used to extrapolate the 1000-seed weight (g). Ten plants per replicates were selected for analysis.

2.2.3. Seed mineral nutrient and crude protein analysis after harvesting

Seed mineral nutrient concentrations were determined by means of dry-ashing, as described by Estefan et al. (2013). Ground sesame seed samples (1.0 g) was placed in a crucible, ignited, and burnt to ash in a muffle furnace at 550°C for 5 h. The ash was cooled and dissolved into 5 mL of 2 N HCl, diluted to 50 mL in a volumetric flask with reverse-osmosis water, and filtered through Advantec 110-mm filter paper. Phosphorous (P) concentration was determined from the filtrates by means of the vanado-molybdate yellow colorimetric method using a spectrophotometer (Model U-5100, Hitachi Co., Japan), with absorbance measured at 420 nm. The seed K, Mg, Ca, Fe, Cu, Mn and Zn concentrations were determined from the filtrates by means of atomic absorption spectrophotometry (Model Z-2300, Hitachi Co., Japan). The seed N concentration was measured by means of the dry combustion method using a CN-corder (Macro corder JM 1000CN, J-Science Co., Ltd., Japan) and the values of total N values (%) were multiplied by the N factor 6.25 to obtain crude protein values (N x 6.25%) (Mitchell et al., 1974).

2.2.4. Soil chemical analysis

Soil samples were collected at 0-15 cm depth from the sesame field after each harvest, air-dried, crushed, sieved through 2 mm mesh before analysis. Soil pH, and electrical conductivity (EC) were measured in a solution of 1:5 (Soil: water) extracts. Total C and N were analysed by dry combustion method on a CN corder (Macro corder JM 1000CN, J-Science Co., Ltd., Japan) from which C/N ratio was calculated. Exchangeable cations K, Ca and Mg were extracted with 1 N ammonium acetate pH 7.1 and the cations determined using atomic absorption spectrophotometer (Model Z-2300, Hitachi Co., Japan). Available phosphorous was determined using Truog method (Truog, 1930) on absorption spectrophotometer at 710 nm (Model U-5100, Hitachi Co., Japan). Soil micronutrients Fe, Zn, Cu, and Mn were determined by Inductively Coupled Plasma Atomic Emission Spectrometry (Model ICP-AES, SPECTRO CIROS CCD, Germany).

2.2.5. Statistical analysis

Data were analyzed using a three-way analysis of variance (ANOVA) to examine the main effects and interactions of field, cropping and cultivars as they relate to measured variables using SPSS 22.0 for Windows; pairs of means were compared on significant ANOVA tests using Tukey's honestly significance difference (HSD) test ($p < 0.05$). Unless otherwise noted, differences were considered significant at $p < 0.05$. Results are presented as the mean ± SE (standard errors) of the three replicates for each cultivar.

2.3. Results

2.3.1. Field weather conditions

The experimental site was characterized by lower rainfall in July as compared with August and September 2012 to 2014 (**Figure 7a**). However, the rainfall was significantly low in September 2014 compared with 2012 and 2013. In addition, the rainfall received in August and September 2013 was above the normal amount for the area. Total rainfall during the growing seasons were 555, 806 and 635 mm in 2012, 2013 and 2014, respectively indicating higher rainfall received in 2013 as compared with 2012 and 2014.

The average daily temperature range was between 23 and 25°C with an average of 25°C during the cultivation period from 2012 to 2014 (**Figure 7b**). The growing season of 2014 was cooler than 2012 and 2013.

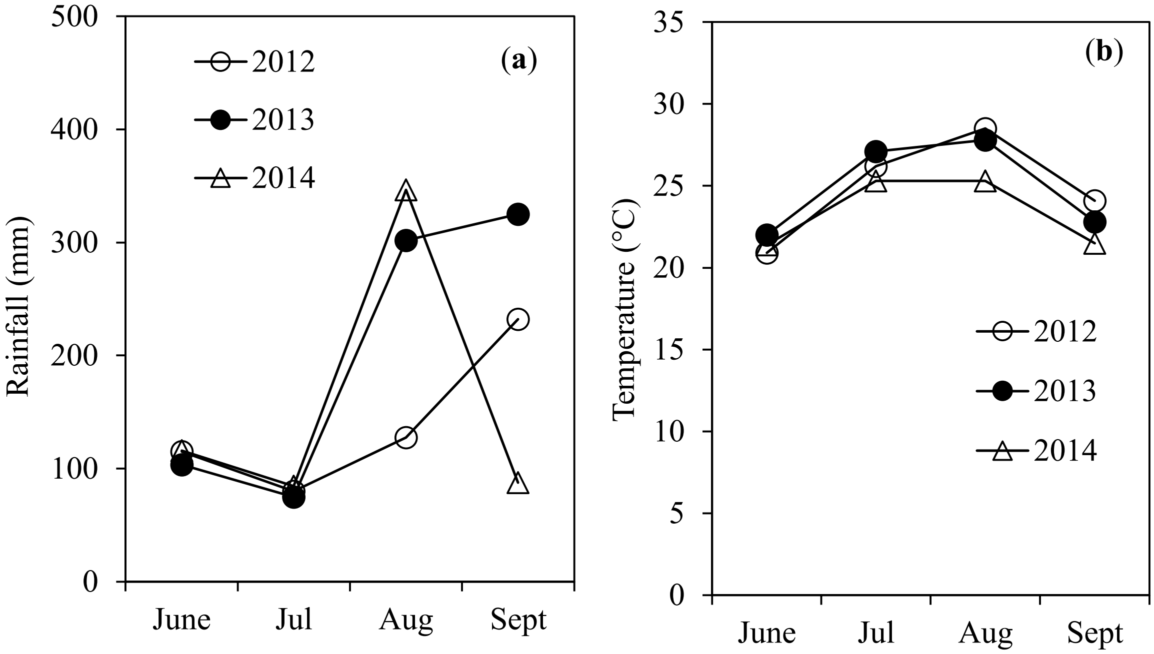


Figure 7. (a) Mean monthly rainfall (mm) and (b) mean monthly temperature (°C) in Koyama during the growing season in 2012, 2013 and 2014. Data obtained from Japan Meteorological Agency.

2.3.2. Yield parameters

Analysis of variance showed that the seed yield and 1000-seed was affected by the field x cropping interaction. In addition, there was a field x cultivar effect on the 1000-seed weight (**Table 1**). Neither cropping x cultivar nor field x cropping x cultivar interactions affected seed yield and 1000-seed weight.

Both seed yield and 1000-seed weight did not show significant differences between cultivars in 2012 in field A while in 2013, significant differences occurred between cultivars in the seed yield which was the highest in 'Gomazou' (700.2 kg ha^{-1}) and lowest in 'Maruhime' (135.4 kg ha^{-1}) and 'Nishikimaru' (236.7 kg ha^{-1}) **(Figure 8)**. In addition, seed yield and 1000-seed weight averaged across cultivars decreased in 2013 by 56.5 and 24.1% respectively compared with 2012. In field B, significant differences in seed yield and 1000-seed weight occurred between cultivars in 2013 and 2014 respectively. The 1000-seed weight of 'Maruhime' (1.65 g) was significantly lowest among the cultivars. Averaged across cultivars, the effect of cropping years was not significant on both yield and 1000-seed weight in field B whereas field A showed significant decreases in the seed yield and 1000-seed weight.

Table 1. Analysis of variance (ANOVA) of seed yield and 1000-seed weight of sesame cultivars after harvesting as influenced by continuous monocropping.

Source	Seed yield	1000-seed weight
Field	**	**
Cropping	**	***
Cultivar	***	**
Field x cropping	***	***
Field x cultivar	ns	*
Cropping x cultivar	ns	ns
Field x cropping x cultivar	ns	ns

* Indicates significant differences at $p < 0.05$.

** Indicates significant differences at $p < 0.01$.

*** Indicates significant differences at $p < 0.001$.

ns - Indicates non-significant difference at 0.05 level of probability.

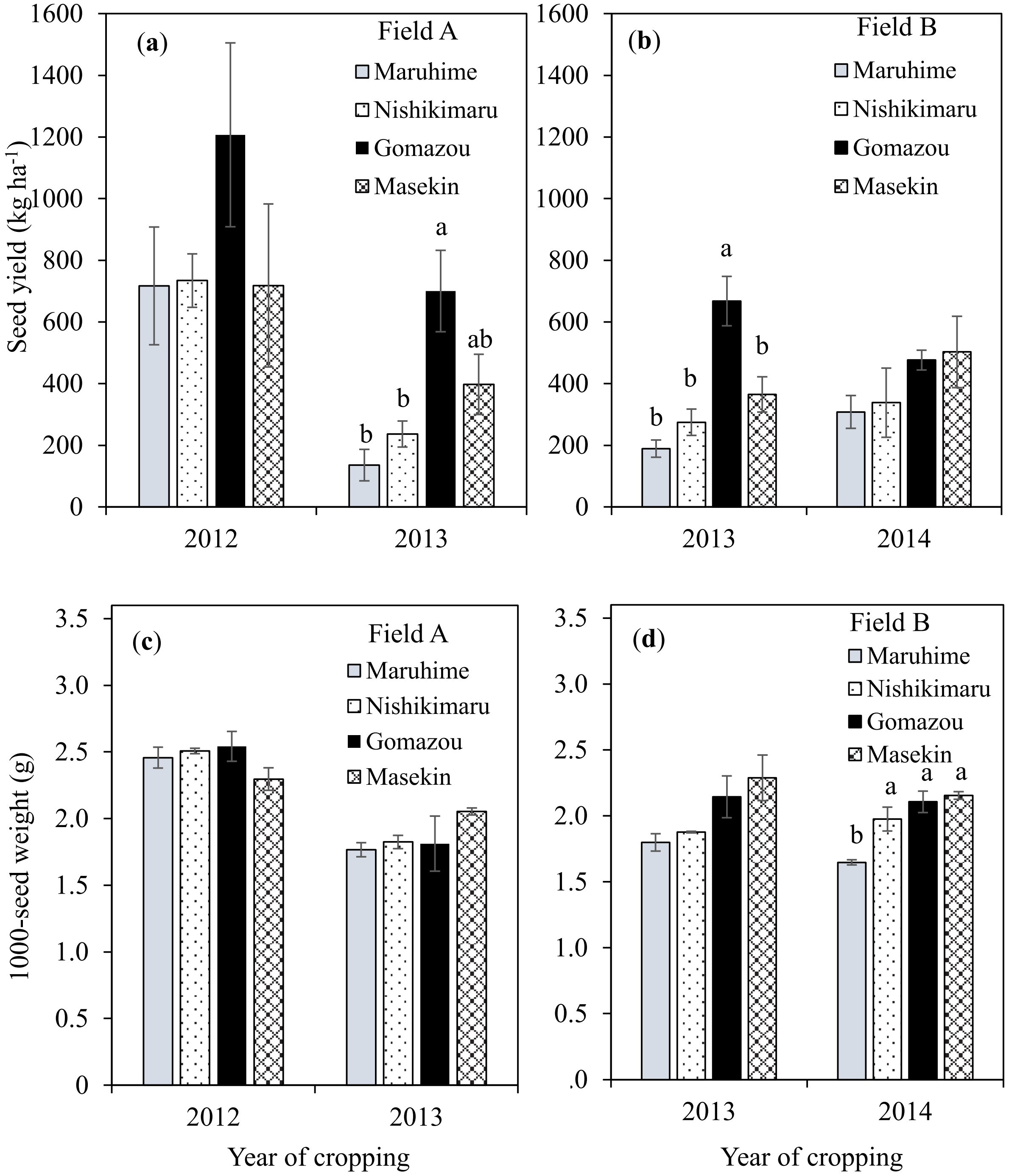


Figure 8. (a) Seed yield of the four sesame cultivars in fields A and (b) B; (c) 1000-seed weight in fields A and (d) B. Values are means ± standard errors (n = 3). In a given number of continuous monocropping, cultivar means labelled with different lower-case letters differ significantly (Tukey's HSD test at $p < 0.05$).

Continuous monocropping significantly decreased both seed yield and 1000-seed weight in the second cropping regardless of cultivars (**Figure 9**). Averaged over fields and cropping years, the seed yield was in the range of 453.1–937.3 kg ha^{-1} in first cropping with the lowest seed yield in cultivar 'Maruhime' and the highest in 'Gomazou' although there was no significant differences among the four cultivars (**Figure 9a**). In the second cropping, seed yield showed significant differences between the cultivars. Seed yield was in the range of 221.7–588.3 kg ha^{-1} in second cropping and significantly lowest in 'Maruhime' and 'Nishikimaru' compared with 'Gomazou'.

Compared with the first cropping, the percentage of the seed yield reduction of the second cropping were highest in 'Maruhime' (51.1%), followed by 'Nishikimaru' (43.0%), 'Masekin' (37.2%) and lowest in 'Gomazou' (16.8%). However, a significant difference in seed yield was found only between 'Gomazou' and 'Maruhime' as well as 'Nishikimaru'. Overall, the mean seed yield averaged across cultivars was significantly decreased by 36.5% in the second cropping compared with first cropping indicating decrease in yield of sesame by continuous monocropping. Similarly, averaged over fields and cropping years the 1000-seed weight was in the range of 2.19–2.34 g in first cropping with lowest mean in 'Nishikimaru' although there were no significant differences between the four cultivars (**Figure 9b**). In the second cropping, the 1000-seed weight showed significant differences between the cultivars and significantly decreased by 19.8, 13.3, 16.4 and 8.2% in 'Maruhime', 'Nishikimaru', 'Gomazou' and 'Masekin' respectively compared to first cropping. However, a significant difference was found only 'Maruhime' and 'Masekin'. Overall, the mean 1000-seed weight averaged over cultivars significantly decreased by 14.3% in the second cropping as compared with first cropping.

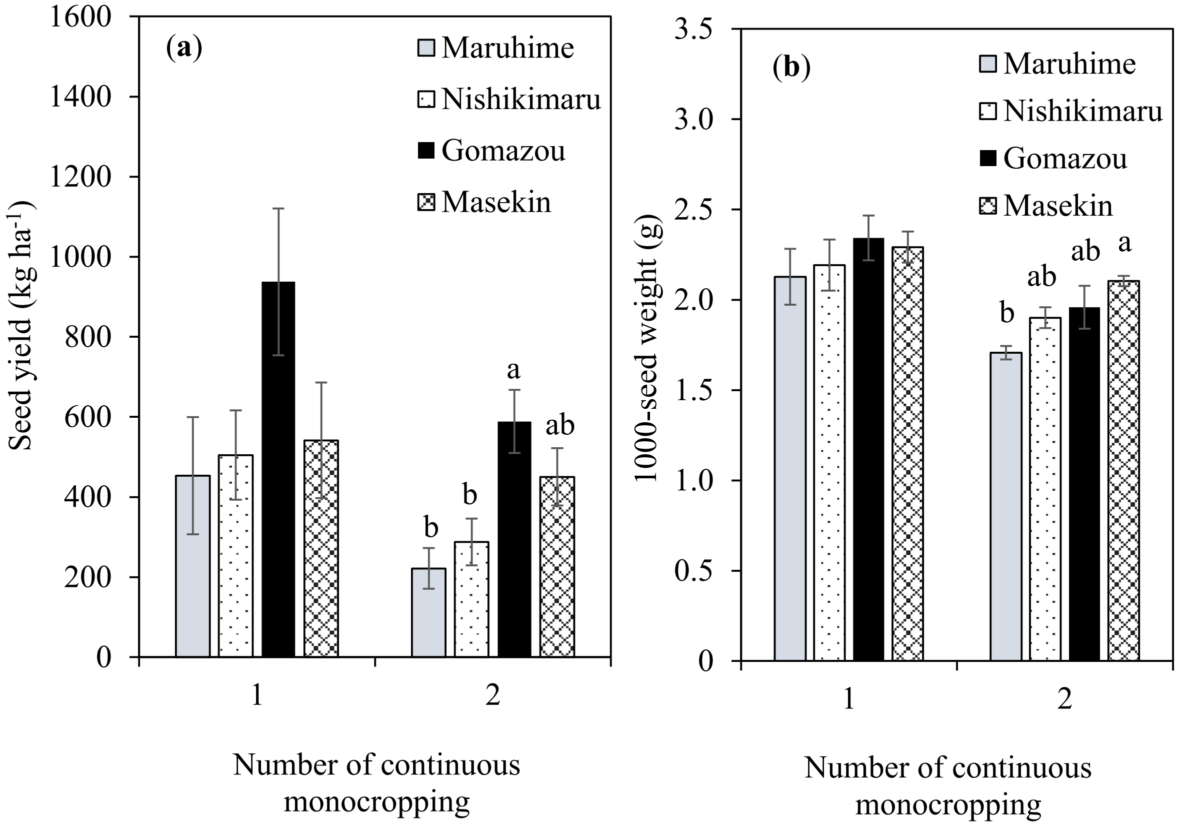


Figure 9. (a) Seed yield and (b) 1000-seed weight of the four sesame cultivars averaged over fields A and B in first and second cropping. Values are means ± standard errors ($n = 3$). In a given number of continuous monocropping, cultivar means labelled with different lower-case letters differ significantly (Tukey's HSD test at $p < 0.05$).

2.3.3 Crude protein and mineral nutrient concentrations of seeds of sesame cultivars

In the first cropping, the seed mineral nutrients except seed K showed higher values than in the second cropping. Analysis of variance showed significant effect of continuous monocropping on the seed crude protein, N, P, Fe, Zn and Mn (**Table 2**). The seed crude protein, N, P, K, Mg, Fe, Cu, Zn and Mn was affected by the field x cropping interaction. In addition, there was a cropping x cultivar effect on the seed K and Ca content whereas a field x cropping x cultivar effect on the seed P, Ca and Cu occurred.

Averaged across cultivars, field A showed significant decreases in the seed crude protein, N, P, Zn and Mn in 2013 compared with 2012 while field B did not significantly decrease crude protein, N and Mn instead of significantly decreasing P, Mg and Fe content in 2014 as compared with 2013 (**Table 3**). Both fields showed significant decreases in seed P and Zn content in the 2013 and 2014 for fields A and B, respectively. Seed P decreased more on field B (14.3%) than in field A (5.7%). The seed P content of 'Maruhime' (562.0 mg/100 g) on field A did not decrease despite continuous monocropping effect on all cultivars in 2013. Averaged over cultivars in field A, the crude protein and N contents decreased by 19.7% in 2013 as compared with that in 2012. Among the cultivars, 'Masekin' showed highest crude protein, N and Zn contents though no significant differences from 'Nishikimaru' and 'Maruhime' for crude protein and N in the 2013 on field A.

Table 2. Analysis of variance (ANOVA) of seed crude protein, seed N, P, K, Ca, Mg, Fe, Cu, Zn and Mn of the four sesame cultivars as influenced by continuous monocropping.

Source	Crude protein	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn
Field	**	**	ns	***	ns	ns	ns	*	**	ns
Cropping	***	***	***	ns	ns	ns	***	ns	***	***
Cultivar	***	***	***	**	***	***	*	ns	ns	***
Field x cropping	***	***	**	*	ns	**	*	*	**	**
Field x cultivar	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cropping x Cultivar	ns	ns	ns	*	*	ns	ns	ns	ns	ns
Field x cropping x cultivar	ns	ns	*	ns	*	ns	ns	***	ns	ns

* Indicates significant differences at $p < 0.05$.

** Indicates significant differences at $p < 0.01$.

*** Indicates significant differences at $p < 0.001$.

NS - Indicates non-significant difference at 0.05 level of probability.

Table 3. Seed crude protein and mineral nutrient concentration in seeds of sesame cultivars in fields A and B.

Field	Year of cropping	Cultivar	Crude protein (%)	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn
				(mg/100g of seed)								
A	2012	Maruhime	24.0	384.4	549.7	706.2	506.3b[z]	468.0ab	9.63	1.50	7.26	2.67ab
		Nishikimaru	26.1	417.4	534.0	677.7	668.8b	458.1ab	8.22	1.41	7.75	2.54ab
		Gomazou	23.1	375.3	557.4	696.1	1440.8a	503.6a	9.93	1.26	7.60	3.31a
		Masekin	26.0	416.6	518.2	742.2	483.0b	435.2b	9.67	1.52	7.47	2.22b
		Mean value	24.9A	398.4A	539.8A	705.6	774.7	466.2	9.36	1.42	7.52A	2.69A
	2013	Maruhime	20.6ab	329.6ab	562.0a	659.7	592.3b	478.6	10.3	1.08b	5.50b	1.92ab
		Nishikimaru	19.4ab	309.5ab	499.5b	728.9	670.8b	482.5	11.1	1.30ab	5.64b	2.17ab
		Gomazou	17.7b	282.8b	483.1b	635.9	1129.2a	470.8	6.82	1.45a	5.46b	2.34a
		Masekin	22.4a	357.8a	492.6b	694.4	448.4c	465.7	7.23	1.39ab	7.17a	1.64b
		Mean value	20.0B	319.9B	509.3B	679.7	710.2	474.4	8.85	1.31	5.94B	2.02B
B	2013	Maruhime	21.4ab	341.5ab	604.7a	778.9	604.4b	514.1a	12.3	1.38	6.29b	2.33ab
		Nishikimaru	19.5bc	311.2bc	535.6b	702.6	629.0b	466.1ab	10.8	1.31	6.47ab	2.44a
		Gomazou	17.8c	284.8c	548.7b	701.4	1168.3a	502.2a	9.38	1.47	6.58ab	2.97c
		Masekin	23.1a	370.3a	519.4b	765.4	429.3c	443.9b	9.07	1.58	6.74a	2.09a
		Mean value	20.4	327.0	552.10A	737.1	707.8	481.6A	10.4A	1.44	6.52A	2.46
	2014	Maruhime	24.3a	389.0a	510.0a	863.1a	614.5b	459.9	10.39a	1.72	6.09	2.15ab
		Nishikimaru	22.3ab	356.2ab	491.6a	853.2a	584.0b	452.3	7.15ab	1.55	6.49	2.46ab
		Gomazou	18.1b	288.8b	475.6a	674.9b	1165.6a	464.7	5.45b	1.30	5.54	3.14a
		Masekin	23.1a	369.7a	416.1b	784.0ab	461.9b	408.5	5.24b	1.44	6.02	1.98b
		Mean value	21.9	350.9	473.3B	793.8	706.5	446.4B	7.06B	1.50	6.04B	2.43

[z] Within each column, means labelled with different letters differ significantly (Tukey's HSD test at $p < 0.05$).

Results also showed that the second cropping significantly decreased crude protein and N by 7.5%, whereas P was decreased by 10.0% compared with the first cropping. In addition, seed micronutrients such as Fe, Zn and Mn were decreased by 19.4, 14.7, and 13.6% respectively compared with first cropping. (**Table 4**). The main effect of cropping on seed K, Ca, Mg and Cu were not significant. Averaged over fields and cropping years, both crude protein, N and P contents were decreased by continuous monocropping. In the second cropping, seed crude protein and N showed significant differences between the cultivars and was in the range of 17.9–22.7% and 285.8– 363.8 mg/100g crude protein and N, respectively. However, a significant difference was found only 'Maruhime' and 'Gomazou'. The seed P content showed significant differences between the cultivars in both first and second cropping. Seed P was in the range of 518.8–577.2 mg/100 g in the first cropping and lowest in 'Masekin' that was significantly different from 'Maruhime'. Similarly, seed P content was significantly the lowest in 'Masekin' (454.4 mg/100 g) and the highest in 'Maruhime' (536.0 mg/100 g). Results also showed that both seed Fe and Zn showed significant differences between the cultivars in the second cropping. Seed Fe was in the range of 6.13–10.33mg/100 g with lowest content in 'Gomazou' although not significantly different from 'Masekin' and 'Nishikimaru'. Seed Zn was in the range of 5.50– 6.60 mg/100 g with lowest in 'Gomazou' although not significantly different from 'Maruhime' and 'Nishikimaru'. On the other hand, the seed Mn content was significantly lowest in 'Masekin' in both first and second cropping. Seed Mn was in the range of 2.16–3.14 mg/100 g in the first cropping and 1.81–2.74 mg/100g in the second cropping.

Table 4. The seed crude protein, seed N, P, K, Ca, Mg, Fe, Cu, Zn and Mn contents of the four sesame averaged over fields A and B in first and second cropping.

Cropping	Cultivar	Crude protein (%)	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn
			(mg/100g of seed)								
1	Maruhime	22.7	363.0	577.2a[z]	742.5	555.4bc	491.0ab	10.9	1.44	6.78	2.50b
	Nishikimaru	22.8	364.3	534.8ab	690.1	648.9b	462.1bc	9.50	1.36	7.11	2.49b
	Gomazou	20.6	330.0	553.0ab	698.8	1304.5a	502.9a	9.65	1.36	7.09	3.14a
	Masekin	24.6	393.4	518.83b	753.8	456.1c	439.5c	9.37	1.55	7.10	2.16b
	Mean value	22.7A	362.7A	546.0A	721.3	741.2	473.9	9.87A	1.43	7.02A	2.57A
2	Maruhime	22.5a	359.3a	536.0a	761.4	603.4bc	469.3	10.3a	1.40	5.80ab	2.03b
	Nishikimaru	20.8ab	332.9ab	495.5ab	791.1	627.4b	467.4	9.12ab	1.43	6.07ab	2.31ab
	Gomazou	17.9b	285.8b	479.3b	655.4	1147.4a	467.7	6.13b	1.38	5.50b	2.74a
	Masekin	22.7a	363.8a	454.4b	739.2	455.1c	437.1	6.23b	1.41	6.60a	1.81b
	Mean value	21.0B	335.44B	491.30B	736.8	708.3	460.4	7.95B	1.40	5.99B	2.22B

[z] Within each column, means labelled with different letters differ significantly (Tukey's HSD test at $p < 0.05$).

2.3.3. Soil chemical properties under sesame cropping

Analysis of variance showed significant effect of continuous monocropping on the soil pH, TN, C/N, available P, exchangeable Ca and Cu that showed significant differences between the first and second cropping regardless of the cultivars (**Table 5**). The soil pH, EC, TN, C/N, K, Ca, Mg, Cu and Zn was affected by the field x cropping interaction. The field x cropping interaction was caused by the high levels of pH, EC, C/N, Ca and Mg in the first field compared with the second field. Neither field x cultivar, cropping x cultivar nor field x cropping x cultivar affected all the soil chemical properties.

Averaged across fields, cropping years and cultivars, the soil pH increased from 5.26 in the first cropping to 5.47 in the second cropping (**Table 6**). Total N, was also increased by 0.05 g kg^{-1} in the second cropping compared with first cropping whereas the C/N decreased by 0.39 in the second cropping compared the first cropping. The field x cropping interaction effect was caused by the significantly low pH and TN in 2013 cropping in all fields. The field x cropping interaction also had significant effect on the soil exchangeable K and the mean exchangeable K tended to decrease in the second cropping compared with first cropping by 10.3 mg kg^{-1}. Conversely, continuous monocropping significantly increased soil available P from 18.0 mg kg^{-1} in the first cropping to 25.8 mg kg^{-1} in the second cropping. Similarly, soil exchangeable Ca and Cu increased in the second cropping by 146.8 mg kg^{-1} and 0.53 ppm respectively compared with first cropping. Overall, soil EC tended to decrease, exchangeable Mg and micronutrients Fe, Zn and Mn increased with continuous monocropping though no significant differences between first and second cropping was observed.

Table 5. Analysis of variance (ANOVA) of soil chemical properties as influenced by continuous monocropping.

Source	pH	EC	TN	C/N	P	K	Ca	Mg	Fe	Cu	Zn	Mn
Field	**	ns	*	ns	***	**	***	***	**	***	ns	***
Cropping	**	ns	*	***	**	ns	*	ns	ns	***	ns	ns
Cultivar	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Field x cropping	***	***	***	***	ns	*	**	***	ns	*	***	ns
Field x cultivar	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cropping x Cultivar	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Field x cropping x cultivar	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

* Indicates significant differences at $p < 0.05$.

** Indicates significant differences at $p < 0.01$.

*** Indicates significant differences at $p < 0.001$.

NS - Indicates non-significant difference at 0.05 level of probability.

Table 6. The soil chemical properties averaged across cultivars in each field and cropping year.

							Exchangeable cations			Fe	Cu	Zn	Mn
Field	Year of cropping	pH (H_2O)	EC (dS m^{-1})	TN (g kg^{-1})	C/N	Available P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})	(ppm)			
A	2012	5.69a^z	0.07b	2.80a	8.3b	9.50b	79.5	1701.7b	358.5a	311.0	8.51b	6.98b	78.2
	2013	5.21b	0.20a	2.29b	9.9a	18.3a	110.2	2018.8a	298.7b	339.8	9.26a	7.81a	75.8
B	2013	4.84b	0.22a	2.30b	10.3a	26.0b	152.0	1655.7	230.5b	399.5	9.31b	7.58a	55.4
	2014	5.73a	0.04b	2.90a	7.8b	33.0a	115.5	1632.3	306.9a	388.2	9.62a	6.78b	56.0
Cropping	1	5.26	0.14	2.55	9.3	18.0b	117.5	1678.7b	294.5	355.3	8.91b	7.28	66.8
	2	5.47	0.12	2.59	8.9	25.8a	107.2	1825.5a	302.8	364.0	9.44a	7.30	65.9

^{z}Within each column, means labelled with different letters differ significantly (Tukey's HSD test at $p < 0.05$). Cropping 1 represents average of first croppings on field A (2012) and field B (2013) whereas cropping 2, the average of second croppings on field A (2013) and B (2014).

2.4. Discussion

This study found that a two-year consecutive cropping decreased seed yield, 1000-seed weight and the content of most seed mineral nutrients of some cultivars. Both seed yield and 1000-seed weight decreased significantly in the second cropping as compared with the first cropping (**Figure 9**). The seed yield decreased by 36.5% in the second cropping accompanied by 14.3% decrease in the 1000-seed weight, indicating that the negative effect of continuous monocropping is more on seed yield than seed weight regardless of the cultivar. These results were consistent with those previous reported by Chung et al. (1989) which in continuous monocropping of sesame for 4 years, seed yield initially decreased by 33% when compared to the first cropping. A similar significant decline in seed weights was reported in soybean grown in continuous monocropping, which accompanied the yield decline of soybean (Kelly et al., 2003). Several researches also showed yield decline in continuous monocropping in other crop species (Crookston et al., 1991; George et al., 2002; Peng et al., 2006; Stanger and Lauer, 2008). These have been attributed to decreases in soil mineral nutrients, accumulation of phytotoxins from decomposing root residues, allelochemicals exuded from root, soil borne diseases (Fageria and Baligar, 2003; Rogovska et al., 2012).

The field x cropping interaction on the seed yield and 1000-seed weight was caused by the low productivity of sesame on the first field in 2013 compared with the second field in 2014 (**Table 1**). The field x cropping interaction effect showed that the two-year consecutive cropping of sesame influenced the soil nutrient status depending on field indicating that the location of a field could be one of the factors determining sesame yield of under continuous monocropping. In addition, the fields affected the seed weight depending on the cultivar type implying that sesame cultivars respond differently to similar soil types. In this study, soil total N content did not decrease in field B, whereas it decreased in the field A significantly influencing yield and seed crude protein content (**Tables 4** and **6**). The decrease in the total N content was indicated by the increase in the soil C/N ratio that showed that organic carbon partly remained high in field A in the second cropping. Both total N and organic carbon content decreased due to continuous monocropping that depletes organic matter content. This agrees with the findings that continuous monocropping decreases soil total organic carbon and nitrogen pools thus lowering the soil quality and crop yields (Al-Kaisi et al., 2005; Van Eerd et al., 2014; Buchi et al., 2017). Previous research reported that when soil available N declined in continuous monocropping of eggplants, yield decline occurred (Wang et al., 2014). Soil N has also been reported to cause yield decline in continuous aerobic rice cropping (Nie et al, 2008). Although this study did not determine available N, decrease in the total N possibly decrease sesame yield through limiting N availability.

The lower soil pH in 2013 than in 2012 and 2014 possibly indicated soil acidification by nitrate leaching evident by the high soil EC values in 2013. A previous research indicated that soil EC values predict the concentration of soil nitrate because a positive linear regression exists between the two parameters (Miyamoto et al., 2015). Furthermore, rapid nitrification may occur at low pH leading to accumulation of soil NO_3^--N and consequently nitrate leaching contributing to low pH values (Zebarth et al., 2015). This could also have affected the total N content in 2013 since nitrate was leached in drainage water reducing the content of total N in that year since total rainfall from August to September in 2013 was the highest compared to 2012 and 2014 (**Figure 7a**). It could be speculated that soil N status determines sesame yield on upland field converted paddy but the available N is prone to leaching.

This study also found that the magnitude of the yield decline varied among the cultivars, indicating differences among the cultivars in their response to changes in the soil conditions under from the continuous monocropping. Although the cropping x cultivar interaction effect was not significant on yield and 1000-seed weight, tolerant cultivars could possibly exist since the percentage decreases in seed yield in the second cropping were low in ‘Gomazou’ and ‘Masekin’, indicating these cultivars may be sparingly tolerant to continuous monocropping obstacle. The reduction of seed yield could result from low seed weight. However, ‘Masekin’ significantly showed higher seed weight than ‘Maruhime’, suggesting resistance to the cropping obstacle through maintaining a higher seed weight. The variation in the seed yield in the second cropping among the cultivars suggests that morphological differences such as branching ability, number of capsules and plant heights could have been influenced by continuous monocropping. For instance, the direct effect of number of branches on seed yield has been reported in sesame (Uzun et al., 2002). Resistant cultivars possibly had less influence on their morphological characters since ‘Gomazou’ has taller branches on the lower stem than that of ‘Masekin’ that could have led to its higher yield as compared with other cultivars (Yasumoto and Katsuta, 2006).

‘Gomazou’ had the highest seed yield in the second cropping compared with ‘Maruhime’ suggesting it could partially overcome any obstacles that result from continuous monocropping. This suggests that sesame cultivars that are resistant to these obstacles can maintain considerable yields. In contrast, susceptible cultivars such as ‘Maruhime’ showed significantly decreased yield in the second cropping. Although there was lack of information on response of sesame cultivars to continuous monocropping before conducting this study, several crops such as rice and wheat were reported to vary in yield with cultivar type (Peng et al., 2006; Zhou et al., 2014). This might be attributed to the differences in exudation of root exudates responsible for autotoxicity in continuous monocropping (Li et al., 2014). In addition, allelochemicals from decomposing residues or root exudates of different cultivars might directly or indirectly decrease crop yields in continuous monocropping through increasing soil pathogenic fungi and bacteria (Chen et al., 2011; Zhou et al., 2012; Hua et al., 2012). Actually, sesame roots exudated allelochemicals (Hussain et al., 2017) and its plant residues inhibited its own growth (Shah et al., 2016). However, the allelopathic activities could differ among these cultivars. Hence screening and adoption of resistant cultivars have been suggested as a way to overcome negative effects of continuous monocropping (Liu and Herbert, 2002; Nie et al., 2012). Sesame cultivars tolerant to allelopathic growth inhibition under continuous monocropping could sparingly maintain high yield.

The variation in the seed yield between the years could have been caused by changing environmental conditions. Although the total rainfall received in all cropping years were adequate for sesame production (Uçan et al., 2007), rainfall received in 2013 was higher than 2012 and 2014 accompanied by typhoon winds at the physiological maturity stage of sesame. Whereas the wind caused lodging of sesame, the excessive rainfall in August and September 2014 during the reproductive and physiological maturity of sesame caused water-logged conditions on the upland field converted paddy soils that affected yield (**Figure 7**). Sesame is susceptible to waterlogging and yield losses of up to 50% can be caused by even a short duration of waterlogging conditions (Sarkar et al., 2016). Although the study did not determine the soil moisture content of the abandoned paddy field, increased waterlogged conditions could have decreased productivity in 2014.

The yield decline was accompanied by decrease in seed nutrient contents except K and Ca regardless of the cultivars suggesting continuous monocropping is detrimental to yield, crude protein and mineral quality such as N, P, Fe, Zn and Mn of sesame cultivars. Similar studies show that continuous monocropping alters seed mineral composition, decreasing the quality attributed to decrease in soil mineral nutrients such as P and N (Bellaloui et al., 2010; Stepien et al., 2017). In this study, the decrease in soil total N indicated low available N in the second cropping that counted for the low seed crude protein and N (**Table 6**). A similar reduction in the seed N content of maize due to low soil N in continuous monocropping was also reported (Riedell et al., 2009). On the other hand, the significant increase in the soil available P could possibly indicate decreased absorption of P from soil in the second cropping. This study is consistent with Zhou and Wu (2015) who reported accumulation of available P in continuous monocropping due to its poor absorption attributed to continuous monocropping obstacle arising from soil-borne pathogen and autotoxic substances in the soil. Hence, could be speculated that continuous monocropping obstacles arising from autotoxic substances (allelochemicals) in continuous monocropping could prevent absorption of P. In addition, increase in the soil available P could result from P fertilizer application (Mazarura and Chisango, 2012). This suggests P utilization by sesame in continuous monocropping could be inhibited by factors other than P fertilization. Poor absorption of P by sesame cultivars despite its availability in the second cropping could result in low seed P. In this study, the decrease in seed P content is consistent with other studies although not in sesame (Bellaloui et al. 2010; Sebetha et al., 2015). Bellaloui et al. (2010) reported lower seed P in continuous monocropping of soybean attributed to low P content in the soil contrary to this study where P increased in soil. The low seed P implied that sesame cultivars had low uptake of P that could consequently result into low 1000-seed weights. P is a component of the nucleic acid and plays important role in plant reproduction including seed production (Douglas and Phillips, 2008). Therefore, poor uptake and storage of P resulted into low seed P depending on cultivar. For instance, seed P was highest in 'Maruhime' compared with other cultivars suggesting sesame cultivars exhibit different abilities to uptake and utilize soil P under similar condition.

Soil micronutrients tended to increase in the second cropping possibly due to the slight increase in soil pH attributed to dolomite lime (Anderson et al., 2013). However, this did not increase the seed micronutrient contents that could be attributed to the poor nutrition of macronutrients N and P. Adequate N nutrition normally increases uptake of micronutrients in plant tissues (Hamilton et al., 1998; Ai et al., 2017). The low N and crude protein in the second cropping resulted into the low seed Fe, Zn and Mn contents in sesame cultivars. In addition, the decrease in the crude protein content and P in the seeds also suggested synergistic interaction between N and P in sesame seed nutrient accumulation (Aulakh and Malhi, 2005). The overall decreased micronutrient accumulation in the sesame seeds also suggested that the decreased macronutrients N and P had a synergistic effect with micronutrients (Rietra et al., 2017). The significant decrease in soil Zn on field B in 2014 contributed to the mean Zn content in second cropping that negatively affected the seed Zn content in continuous monocropping. Usually, excessive P in soils can affect the Zn content because of antagonistic interaction on field B (Mousavi, 2011). The accumulation of the micronutrients is also due to the differences in the genotypes of the sesame cultivars (Pandey et al., 2017). Until now, there are no reports on the effects of continuous monocropping on seed mineral nutrient contents that could be used for comparison. However, a study of mineral nutrient in sesame seeds reported high contents of mineral components in Congo-Brazzaville cultivars (Nzikou et al., 2009). In this study, ‘Maruhime’ had a higher content of most mineral nutrients than the other cultivars, but despite this, had a lower yield than ‘Gomazou’ in the second cropping. This demonstrated that although ‘Gomazou’ had a lower mineral content, it nonetheless produced the highest yield in continuous monocropping.

2.5. Conclusions

This study demonstrated that a–two year continuous monocropping decreased seed yield and most seed mineral nutrient contents of sesame cultivars in the second cropping suggesting occurrence of continuous monocropping obstacle on the abandoned paddy field in Japan. The variation in the seed yield, crude protein and N, P, Fe, Zn and

Mn contents in the second cropping reflected differences in the cultivar response to continuous monocropping that influence the seed composition. The changes in the soil total N and available P partially explain the cause in the reduction in yield and seed mineral contents although factors other than soil mineral nutrients could have contributed to the overall variation in cropping among the sesame cultivars. To minimize yield decreases, adoption of two sesame cultivars ‘Gomazou’ and ‘Masekin’ could be recommended to overcome continuous monocropping obstacle on upland fields converted paddy since they maintained considerably high seed yield and quality under continuous monocropping of sesame. It could be suggested that crop management strategies to be developed to reverse the yield reduction in the second cropping after the main causes of the yield reduction are identified in a long-term continuous monocropping on upland fields converted paddy.

CHAPTER THREE

Growth, yield and autotoxicity differences among sesame cultivars in continuously monocropped upland fields converted paddy

3.1. Introduction

Continuous monocropping is known to negatively influence crop growth and yield a term referred to as soil sickness, or continuous monocropping obstacle (Rice, 1984; Bennett et al., 2012; Huang et al., 2013). In continuous monocropping systems, decreases in both yield and growth have been attributed to biotic and abiotic factors, including changes in the soil nutrient balance, accumulation of phytotoxins from decomposing residues and root exudates causing autotoxicity and allelopathy, changes in the soil microbial diversity, and accumulation of fungal pathogens and nematodes (Ventura and Watanabe, 1978;Fageria and Baligar, 2003; Rogovska et al., 2012).

Several compounds have been implicated for allelopathic activities in croplands including phenolic compounds, esters, alkaloids, fatty acids etc. that are released through root exudation, and leaching, volatilization and through decomposition of plant residues in soil (Rice, 1984 ; Bertin et al., 2003; Lee et al., 2006; Batish et al., 2007). For instance, several phenolic compounds have been reported as major autotoxicity compounds in alfalfa (*Medicago sativa* L.) that affect the radicle growth more than germination thus a soil sickness problem in continuous monocropping of alfalfa (Sampietro et al., 2006). These compounds include phenolic, esters, alkaloids among others that affect growth. Previous researches also show that phenolic compounds are produced by plants and responsible for affecting growth in cropland and also continuous monocropping systems. Huang et al. (2000) reported that decomposing roots including stumps from Chinese fir trees significantly decreased seed germination and growth of its own at increasing concentrations thus exhibiting autotoxicity activity. They identified several phenolic compounds including *p*-hydroxybenzoic, vanillic, *p*-coumaric and ferulic acids. Furthermore, it has been reported that organic acids such as 4-hydroxybenozic acids, cinnamic acids and pthalic acids concentrations in the rhizosphere soils increased with increase in duration of continuous monocropping from 0, 4 and 8-year fields of cowpeas (Huang et al., 2010). These compounds were implicated for causing soil sickness through autotoxicity in continuous monocropping of cowpeas. The bioassay results of the cowpea continuous monocropping rhizosphere soils showed decrease in the seedling growth of cowpea, hence implicated for causing autotoxicity in cowpeas. Furthermore phenolic compounds haven identified as allelochemicals in continuous monocropping rhizosphere soils under cucumber cropping (Zhou et al., 2012). In that study, cucumber for nine consecutive cropping gradually increased benzoic and ferulic acids identified by methanol extracts in soil under continuous cucumber cropping year associated with poor growth. Song et al. (2016) also reported that phenolic compounds from rhizosphere soils of adzuki beans showed autotoxicity potential by inhibiting the seedling growth but without effect on germination. Both plant extracts and rhizosphere soils of adzuki beans contained allelochemicals released from the root exudates and decomposing plant residues that inhibited the growth of adzuki bean itself. These studies showed that chemicals released from plants accumulated in soil and could hinder the growth of the subsequent crop. Continuous monocropping obstacle has also been reported in flue-cured tobacco caused by autotoxicity of phytotoxic compounds in the rhizosphere as one of the major factor of soil sickness (Ren et al., 2015). Rhizosphere soils collected from tobacco field had significant inhibitory effect on the growth of tobacco and lettuce seedlings at higher concentrations that had more effect on the root growth than stem length. Furthermore, it has been reported that allelochemicals including phenolic compounds released from plant roots into rhizosphere soils have influence on microbial characterizes (Bais et al., 2004; Zhou et al., 2012). Hence, increase in allelochemicals in soils could alter the microbial population that in turn increases the disease incidence and severity (Cao et al., 2016). It is well known that soil borne disease such as *Fusarium* wilt are associated with compounds released into soils from plant roots.

Although the role of allelopathy in continuous sesame cropping has not yet been fully explored, sesame is known to release autotoxic allelochemicals (Shah et al., 2016) that are toxic to numerous crop and weed species (Kumar and Varshney, 2008; Soleymani and Shahrajaban, 2012). Several chemicals including flavonoids, saponins, alkaloids, tannins, and phenols were identified from sesame (Fasola and Ogunsola, 2014), and lignans in the form of sesamin and sesaminol glucosides (Noguchi et al., 2008); sesamin and sesamolin are the two most important allelochemicals in sesame plants that are contained in residues, and inhibit the growth of rice, sorghum, and sesame itself (Shah et al., 2016). In addition, it has been reported that root exudates from sesame significantly reduced growth of purple nut sedge and it was presumed that allelochemicals released naturally from roots of sesame as exudates have greater ability to suppress and degrade roots of purple nut sedge confirming the presence of active but unknown molecules in sesame root exudates which is still unclear (Kumar and Varshney, 2008). In addition, sesame yield decline has been associated also to *Fusarium* wilt disease in continuous monocropping (Hua et al., 2012). However, under continuous monocropping of sesame on upland converted paddies, disease incidences and severity have not been quantified. Disease incidences and allelochemicals could increase under continuous monocropping of sesame. Moreover, autotoxicity in in plants depends on the cultivar and genotypes since some cultivars release more compounds than others exhibiting different autotoxicity and allelopathy activities (Wu et al., 2007; Yeasmin et al., 2014). Therefore, it could be important to evaluate allelochemicals with autotoxicity potentials from different sesame cultivars in order to select suitable cultivars and to analyze and identify the phenolic in the decomposing root residues and the rhizosphere soils in order to further explain the phenomenon of continuous monocropping obstacle.

The objective of this study was to: i) analyze and identify phenolic compounds in rhizosphere soils and decomposing roots of four sesame cultivars in order to understand the mechanisms of cultivar differences in responses towards continuous cropping obstacle, and ii) to determine the phytotoxicity of identified phenolic compounds towards sesame germination and radicle growth in a bioassay.

3.2. Materials and methods

3.2.1. Location and site description

The experiment was conducted on fields converted from paddy at Tottori, Japan (35°29′N, 134°07′E). The soil chemical properties of the upland field converted from paddy before sesame cultivation in 2012 were characterized by pH 5.4 (1:5, soil/water), EC 0.05 dS m^{-1} (1:5, soil/water), total N (TN) 2.14 g kg^{-1}, total C (TC), 22.37 g kg^{-1}, available P 29 mg kg^{-1}, exchangeable K 248 mg kg^{-1}, exchangeable Mg 107 mg kg^{-1},exchangeable Ca 918 mg kg^{-1}, CEC 10.4 $cmol_c$ kg^{-1}.

Sesame was cultivated during the warm summer from June to September of 2018.

The total monthly rainfall received in June, July, August and September were 109.5, 321.0, 19.5 and 609.5 mm, respectively. The average daily maximum temperatures in June, July, August and September were 24.8, 30.7, 32.4 and 25.8°C respectively.

3.2.2. Field experimental design

The long-term continuous sesame cropping experiment began in 2012 with one plot measuring 14 m x 6.5 m, divided into micro plots of 3 m x 2.3 m (6.9 m^2) to cultivate four sesame cultivars. In 2013, another plot of similar dimension was established to cultivate sesame for the first time (first year) while the previous plot became the second year field. The technique was repeated in the following years until the seventh year field was established in 2018. Therefore, in 2018, a total of seven fields of 0, 1, 2, 3, 4, 5 and 6-yr (years) of continuous monocropping were already established. All fields were adjacent to each other and with relatively similar initial soil chemical properties before sesame cultivation. Each year, same agronomic practice of sesame as recommended by the National Agriculture and Food Research Organization (NARO, Japan) was followed from planting methods to inorganic fertilizer application. Inorganic fertilizer $N–P_2O_5–K_2O$ at a rate of 70-105-70 kg ha^{-1} in the form of cyclo-diurea (CDU) compound fertilizer (15%–15%–15%) and triple superphosphate (34%). In addition, CaO and MgO in the form of dolomite (Total alkali, 53%; CaO 39.1% and MgO 10%) were applied at 1000 kg ha^{-1} before sowing. During each harvest, plants were cut down from the base, and all crop residues were removed from the fields to minimize fungal and bacterial disease and release of allelopathic compounds exuded from the residues. Sesame cultivars were cultivated on raised ridges (75 cm in width; 40 cm between ridges), onto which double rows of plants were sown at spacing of 45 cm x 15 cm (**Figure 10**). All ridges were covered with black plastic mulch to maintain soil moisture, temperature and to reduce weed growth. Sowing was done on 9 July 2018 and seedlings thinned to two plants per hole on 16 July 2018 (one week after sowing), then finally to one plant per hole on 24 July 2018 (two weeks after sowing). Weeding in between the ridges was done, at 15 and 38 DAS and with a two-stroke engine brush cutter around the fields and between ridges whenever necessary (**Figure 11**). Due to limited rainfall in July, seedlings were watered by a watering can every after two days for three weeks before August rainfall set in to allow proper seedling growth.

Figure 10. (a) Land preparation by power tiller, (b) ridge preparation and laying down black plastic mulch on to ridges, (c) planting ridges with sowing holes prepared, (d) sesame plants at seedling stage, (e) plants at 50% flowering stage and (f) plants at physiological maturity prior harvesting.

Figure 11. (a) Weed growth before 50% flowering stage, (b) hand hoe weeding between ridges and (c) weed-free ridges.

3.2.3. Sesame disease incidence and severity and determination of fungal disease on potatoes dextrose agar (PDA) media

Data were collected on sesame wilt and root rot disease incidence and severity at 51 DAS (mid reproductive stage) in all the continuous monocropping fields. The disease incidence was determined based on symptoms on diseased plants.

$$\text{Disease incidence (\%)} = \left(\frac{\text{Number of diseased plants}}{\text{Total number of plants assessed}}\right) \text{x } 100$$

The diseased severity index was determined on sesame shoots according to a linear scale from 0 to 5 according to Ziedan et al. (2011) as showing in **Figure 12**. The linear scale based on disease symptom used to score the diseased plants was as follows: 0 = no disease symptoms, 1 = stem necrosis, 2 = a third of the plant wilted, 3 = two third of the plant wilted, 4 = the whole plant wilted, 5 = dead plant. The disease severity index (%) was calculated on the basis of the observations using the formula below (Kim et al., 2000);

$$\text{Disease severity index (\%)} = \left(\frac{(0 \text{ x S0}) + (1 \text{ x S1}) + (2 \text{ x S2}) + (3 \text{ x S3}) + (4 \text{ x S4}) + (5 \text{ x S5})}{(\text{N x 5})}\right) \text{x100}$$

Where S0 to S5 = Total number of observed plants in each disease symptom grading per continuous monocropping year; N = Total number of plants observed and 5 = Maximum disease grading scale i.e. 5.

Fungal disease was selected based on the reports of *Fusarium wilt* disease in continuous monocropping of sesame (Hua et al., 2012). To determine the possible fungal diseases, pathogen from infected sesame was isolated on potato dextrose agar (PDA) media. Infected sesame plant tissue was collected from the field on 10 September 2018 from cultivar ‘Maruhime’ with scale of 4 severity upon observing high severity in this cultivar, and was taken to laboratory. PDA media was prepared by peeling and cooking 300 g of potato in 1 L of reverse osmosis water (RO) for 30 min. After boiling, the potato was crushed and mixed well. Then, 250 mL of the paste was collected into an autoclave bottle and to it was added 5 g of dextrose and 4 g of agar before autoclaving at 121°C for 15 min. The PDA solution was then carefully poured into 10 cm diameter petri dishes and allowed to cool before transferring infected tissues.

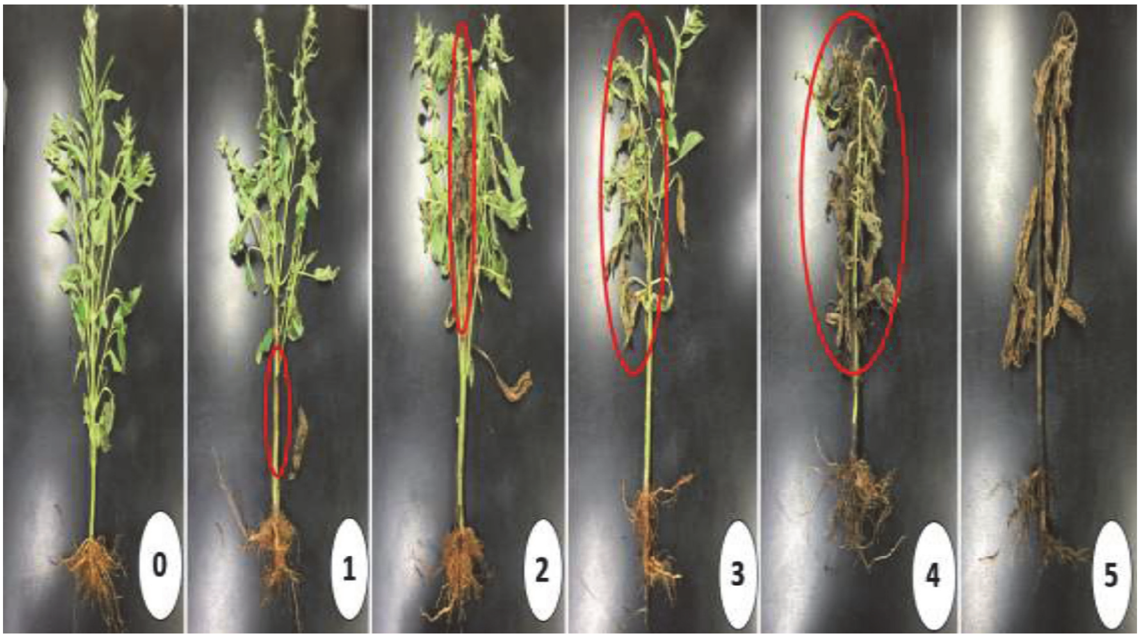


Figure 12. Disease scoring at 51 DAS.

Lesions from stem base, upper part of the root and leaf were cut into small pieces of about 1 cm long. The fragments were then disinfected in 75% ethanol, followed by 1% dimethyl sulfoxide (DMSO) and then rinsed in sterile water prior to transferring onto separate petri dishes labelled as root, leaf and stem. All procedures were performed under laminar flow hood and petri dishes were sealed before incubation. The petri dishes were incubated at 25°C in the dark for 10 days. All petri dishes were checked after 10 days. From each petri dish, a clear and dominant colony was isolated and inoculated on a new petri dishes and incubated at same conditions for another 10 days.

3.2.4. Growth and yield analysis

Sesame growth was determined at full maturity stage from the field. Growth was determined by measuring plant height, height of the first capsule and counting the number of branches per plant of ten representative plants per replication on 24 September 2018 (**Figure 13**). Harvesting from years 1, 2, 3, 4, 5 and 6 fields was done on 28 September 2018 due to early drying of sesame plants while year 0 field was harvested a week later (5 October 2018). All plants per micro-plot per replication were cut down and taken to dry in a greenhouse at Tottori University. After drying, the samples were threshed to release seeds. Total seed weight harvested per micro plot was then obtained to calculate yield in kg ha^{-1} whereas 1000-seed weight was determined by weighing 100 mature seeds in triplicate for each replication to extrapolate the weight of 1000 seeds.

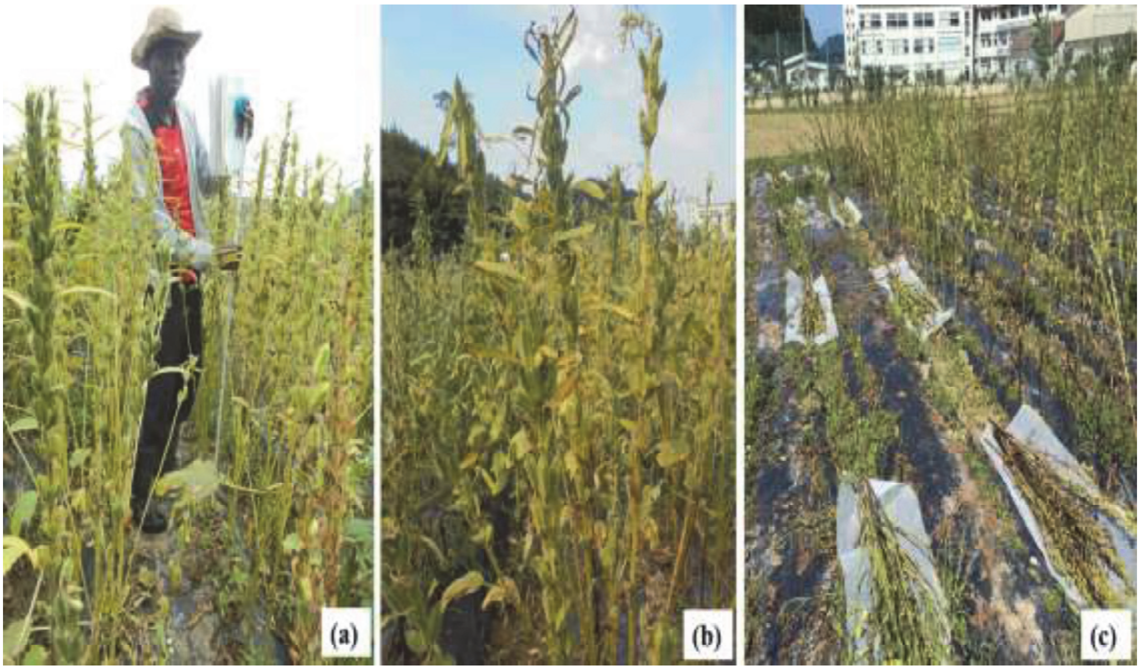


Figure 13. (a) Measuring plant height, (b) sesame plant at physiological maturity and (c) during harvesting.

3.2.5. Sampling of rhizosphere soil and decomposing root from continuously monocropped fields

Decomposing sesame roots with rhizosphere soils intact were collected using a trowel from the fields under continuous monocropping of sesame at Tottori, on 15 December 2018 after harvesting all sesame cultivars in different continuous monocropping years in October 2018. The collected samples included soils and root of continuous monocropping years of 0, (soil used to crop sesame for the last one year) , 1 years (soil used to crop sesame for the last two years), 2 years (soil used to crop sesame for the last three years), 3 years (soil used to crop sesame for the last four years), 4 years (soil used to crop sesame for the last five years), 5 years (soil used to crop sesame for the last six years), and 6 (soil used to crop sesame for the last seven years). Rhizosphere soils were gently shaken from the roots into clean polythene bags and stored at -80 and later transferred to dry freezer unit (Model FDU-1110, EYELA, Tokyo, Japan) for one week prior to analysis. The freeze-dried soils were then crushed in a mortar, sieved through 0.5 mm sieve to remove all tiny root debris prior to analysis. All root samples from years 0, 1, 2, 3, 4, 5 and 6 were combined into one representative sample for allelochemicals analysis. The roots were thoroughly washed, air dried and ground into small particles before analysis.

3.2.6. Extraction and determination of phenolic compounds

3.2.6.1. Phenolic compounds from decomposing roots of sesame cultivars

Phenolic compounds were extracted using the method described by Dalton et al. (1987). Briefly, 0.5 g of finely ground root sample was mixed with 40 mL of 2 M sodium hydroxide (NaOH) and shaken on a mechanical shaker for 24 h at 25°C (**Figure 14**). The samples were then centrifuged at 15,000 g for 15 minutes at 12°C. Then, 30 mL of the supernatant was transferred into a beaker and the pH adjusted to 2.5 with 4 M hydrochloric acid (HCl). The solution was then extracted twice with ethyl acetate, centrifuging at 5,000 g for 15 minutes 12°C upon each extraction. The extraction with ethyl acetate was performed twice to extract as much phenolic compounds as possible. The resulting extracts were then pooled and evaporated to dryness on a rotary evaporator (Model Rotavapor RII, BUCHI, Flawil, Switzerland).

Figure 14. (a) Phenolic compound extraction with 2 M NaOH, (b) evaporation to dryness on rotary evaporator, (c) and (d) dissolving evaporated sample in 80% CH_3OH, (e) and (f), HPLC analysis of phenolic compounds.

The residue was then dissolved in 5 mL of 80% methanol and stored at -20°C prior to detection of phenolic compounds on High Liquid performance chromatography (HPLC).

Phenolic compounds in the decomposing root samples were determined with a HPLC system (Model ELITE LaCrom, Hitachi Co., Tokyo, Japan). The HPLC components were; pump HITACHI L-2130, column oven HITACHI L-2350 and UV detector HITACHI L-2400, Column GL Science Inestsil ODS-3 (5 µm), 4.6 mm x 150 mm). The mobile phase consisted of solvent A made of only 100% methanol CH_3OH) and solvent B, a mixture containing 2.67 g of ammonium acetate (CH_3COONH_4) and 3.47 mL of acetic acid CH_3COOH and 500 mL of water (MilliQ). The mobile phase solvents A and B was set in a linear gradient program as follows: 0 min, A: B of 6:94; 3 min, A: B of 6:94; 5 min, A: B of 15:85; 7 min, A: B of 15:85; 10 min, A: B of 20:80; 20 min, A: B of 20:80; 30 min, A: B of 40:60; 40 min, A: B of 80:20; 45 min, A: B of 80:20; 47 min, A: B of 6:94; and 55 min, A: B of 6:94.

The flow rate was kept constant at 1.0 mL per minute and the injection volume was 10 µL per sample. The detection was performed at wavelength of 280 nm and the column temperature maintained at 25°C. Identification and quantification of phenolic compounds were confirmed by comparing retention times and areas with pure standards. The content of each identified phenolic compound in the soil was expressed as µg per gram of root dry mass.

3.2.6.2. Phenolic compounds from rhizosphere soils of continuously monocropped fields

Soil phenolic compounds were extracted using the method described by Dalton et al. (1987) as used for decomposing roots. Briefly, 5 g of soil was mixed with 30 mL of 2 M sodium hydroxide (NaOH) and shaken on a mechanical shaker for 24 h at 25°C. The samples were then centrifuged at 15,000 g for 15 minutes at 12°C. Then, 20 mL of the supernatant was transferred into a beaker and pH adjusted to 2.5 with 4 M hydrochloric acid (HCl). The solution was then transferred into centrifuge tube (two tubes per sample) and to each tube, was added about 15 mL of ethyl acetate as extraction reagent shaking each sample vigorously before centrifuging at 5,000 g for 15 minutes 12°C. The extraction with ethyl acetate was performed twice to extract as much phenolic compounds as possible. The resulting extracts were then pooled and evaporated to dryness on a rotary evaporator machine (Model Rotavapor RII, BUCHI, Flawil, Switzerland). The residue was then dissolved in 5 mL of 80% methanol and stored at -20°C prior to detection on High Liquid performance chromatography (HPLC).

Phenolic compounds from the rhizosphere soil samples were determined with a HPLC system at similar conditions described for decomposing roots. However, the column temperature was maintained at 30°C during detection. The content of each identified phenolic compound in the rhizosphere soil was expressed as μg per gram of soil dry mass.

3.2.6.3. Total phenolic compound contents in the decomposing roots and rhizosphere soil

The total phenolic compounds content in the decomposing roots and rhizosphere soil was determined by Folin-Ciocalteau method as described by the international Organization for Standardization (ISO 14502–1, 2005). Briefly, 5 mL of Folin-Ciocalteau reagents for total phenol analysis was added to 1 mL of diluted soil or root extract samples in a test tube. Immediately (within 3 minutes), 4 mL of 7.5% (w/v) anhydrous sodium bicarbonate (Na_2CO_3) was added vortexed to mix uniformly. The mixture was allowed to stand for 1 h and absorbance measured on spectrophotometer at 765 nm (Model U-5100, Hitachi Co., Tokyo, Japan). A calibration curve was constructed with different concentrations of gallic acid. The total phenolic compounds contents were expressed as μg of gallic acid equivalents (GAE) per gram dry weight (DW) of root for the decomposing roots and GAE μg g^{-1} DW soil for the rhizosphere soil.

3.2.7. Bioassay with identified compounds on sesame germination

The bioassay test solution consisted of pure grade laboratory chemicals of the identified phenolic compounds *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, and ferulic acids were tested in the concentrations of 0, 1, 5, 10 and 20 mM prepared with 100% methanol solution. The control solution was 100% methanol solution. Filter paper was cut (circular) to fit in a 3 cm diameter petri dish. To each petri dish was then added 0.5 mL of each phenolic compound at varying concentrations was added into separate. The petri dishes were left open to allow methanol to evaporate until filter paper was dry. Then all bioassay petri dishes received 1 mL of RO water.

Sesame seeds of four cultivars; 'Gomazou', 'Nishikimaru', 'Masekin' and 'Maruhime' were used for the bioassay against phytotoxicity of phenolic compounds. Seeds were sterilized with 4% (w/v) solution of sodium hypochlorite solution for 1 min and rinsed in RO water. Five seeds were sown on the filter paper in the petri dishes and allowed to germinate in the dark at 25°C for 4 days. Each treatment was replicated three times. The germinated seeds were then counted and radicle lengths (mm) of germinated seeds were measured. The percentage germination and radicle growth inhibition was calculated as shown in equation (1).

$$\text{Inhibition (\%)} = \left(\frac{Xc-Xt}{Xc}\right) \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots.. (1)$$

Xc and Xt represents germination or radicle length in the control and treatment respectively.

3.2.8. Statistical analysis

All experimental data presented are the means of three replicates. All data were analyzed using one-way analysis of variance (ANOVA) using SPSS version 22.0 (SPSS for windows Inc., Chicago, Illinois, USA). Tukey's multiple comparison test at $p < 0.05$ was used to compare means. Results are presented as the mean ± SE (standard errors) of the three replicates. When considering the differences between the cultivars, a two-way analysis of variance was used with the different cultivars and fields as two fixed factors.

3.3. Results

3.3.1. Growth and yield components among cultivars under different continuously monocropped fields

Continuous monocropping year significantly decreased plant height, height of first capsule and number of branches per plant (**Table 7**). The plant height of all cultivars were significantly higher in the year 0 than years 1 to 6. For instance, the plant height of 'Gomazou' was significantly decreased from 139.3 cm in the year 0 to 109.9 cm in year 3. In addition, 'Gomazou' branch number per plant was significantly highest in the year 1 (3.2) and decreased in the year 3 without significant differences among years 3, 4, 5 and 6. On the other hand, the plant height of 'Masekin' significantly decreased from 133.4 cm in the year 0 to 110.4 cm in year 3 whereas the plant height of 'Maruhime' decreased from 124.1 cm in the year 0 to 94.1 cm in year 3. In ‘Nishikimaru’, the plant height significantly decreased from 124.8 cm in the year 0 to 103.9 cm in year 3 and 104.7 cm in year 4. Analysis of variance showed that the decrease in the plant height, height of the first capsule and number of branches per plant depended on the cultivars with significant interactions between cultivars and cropping on the number of branches per plant observed. For instance, there was significant differences in plant height in the year 1 field in whereby plant height was significantly highest in ‘Gomazou’ without significant differences from ‘Masekin’ and significantly lowest in ‘Maruhime’ and ‘Nishikimaru’. In year 3 field, plant height was significantly highest in ‘Masekin’ and ‘Gomazou’ whereas lowest in ‘Maruhime’ and height of first capsule was highest in ‘Gomazou’, being lowest in ‘Maruhime’. Furthermore, in year 4, ‘Masekin’ and ‘Gomazou’ has the highest plant height and lowest in ‘Maruhime’ whereas ‘Nishikimaru’ showed the highest height of first capsule but without significant differences from ‘Masekin’ and ‘Gomazou’. In addition, the number of branches per plant was significantly highest in ‘Maruhime’ despite its low yield in year 4. In the year 5, there was no significant differences in plant height and height of the first capsule among the cultivars. However, ‘Maruhime’ had the highest number of branches compared to all cultivars. In year 6, the height of first capsule was significantly highest in ‘Gomazou’ and ‘Nishikimaru’ and lowest in ‘Maruhime’ and ‘Masekin’. In addition, there was higher number of braches per plant in ‘Maruhime’ than all the cultivars.

Table 7. Growth parameters among cultivars under different continuous monocropping years.

Cultivar	Year	Plant height (cm)	Height of first capsule (cm)	Branches/plant
Gomazou	0	139.3a	61.3ab	2.37bc
	1	131.7ab	61.9ab	3.23a
	2	129.0ab	64.4a	2.80ab
	3	109.9c	57.9b	2.13bc
	4	113.1c	51.0c	1.77c
	5	118.1bc	56.4bc	1.70c
	6	120.3bc	62.2ab	1.90c
Masekin	0	133.4a	51.23	0.30
	1	124.5abc	52.37	0.07
	2	131.9ab	54.77	0.17
	3	110.4c	48.47	0.07
	4	115.1bc	43.80	0.27
	5	110.5c	48.37	0.03
	6	114.2c	47.87	0.03
Maruhime	0	124.1a	61.3a	3.97
	1	113.4abd	58.2a	3.80
	2	114.2ab	57.0ab	3.37
	3	94.1c	46.4ab	3.16
	4	101.0cd	47.4ab	3.87
	5	107.9bd	46.6ab	3.33
	6	106.5bcd	42.4b	2.80
Nishikimaru	0	124.8a	60.43	2.97ab
	1	115.1ab	53.97	2.93ab
	2	123.4a	61.67	3.33a
	3	103.9b	53.62	2.31ab
	4	104.7b	53.10	2.70ab
	5	110.1ab	54.03	1.8b
	6	110.1ab	57.93	2.07ab
Source of variation				
Cultivar		***	***	***
Cropping		***	***	***
Cultivar x Cropping		ns	ns	**

Different letters within each column are significantly different at $p < 0.05$ (Tukey's test).

***, **, * and ns represent $p < 0.001$, $p < 0.01$; $p < 0.05$ and non-significant, respectively.

Results also indicates significant decrease in the seed yield and 1000-seed weight of sesame in long duration continuous monocropping fields compared to year 0 ,non-continuous monocropping (**Figure 15a** and **b**). In all cultivars, seed yield was significantly highest in the year 0 field with no significant differences among years 1-6. Although there was no significant differences among cultivars, the seed yield decreases in year 1 compared to year 0 were 59.9%, 58.4%, 66.6 and 66.7% for ‘Gomazou’, ‘Masekin’, ‘Maruhime’ and ‘Nishikimaru’ respectively. On the other hand, significant differences in the 1000-seed weight was observed in the different continuous monocropping fields. For instance, in ‘Gomazou’, the 1000-seed weigh decreased in years 1, 2, 3, 4, 5 and 6 were 5.48, 6.68, 10.2, 8.69, 12.2 and 8.17% respectively compared to year 0. However, there was no significant differences between years 1 and 2, among 3, 4 and 6, and among years 4, 5 and 6. In ‘Masekin’, the 1000-seed weight decreased by -1.62, 0.93, 12., 9.97, 9.53, and 6.93% in years 1, 2, 3, 4, 5 and 6 respectively compared to year 0. However, there was no significant differences among years 4, 5 and 6. There was a tendency of 1000-seed weight to increase in the year 6 compared to year 4 in ‘Masekin’. In ‘Maruhime’, 1000-seed weight decreased by 12.0, 12.6, 19.8, 18.7, 19.1 and 14.6% in years 1, 2, 3, 4, 5 and 6 respectively compared to year 0. There was also a tendency of 1000-seed weight to increase in the year 6 compared to year 4 in ‘Maruhime’. In ‘Nishikimaru’, 1000-seed weight decreased by 5.27, 9.22, 7.56, 12.5, 9.90 and 8.88% in years 1, 2, 3, 4, 5 and 6 respectively compared to year 0. However, there was no significant differences among years 2, 3, 5 and 6.

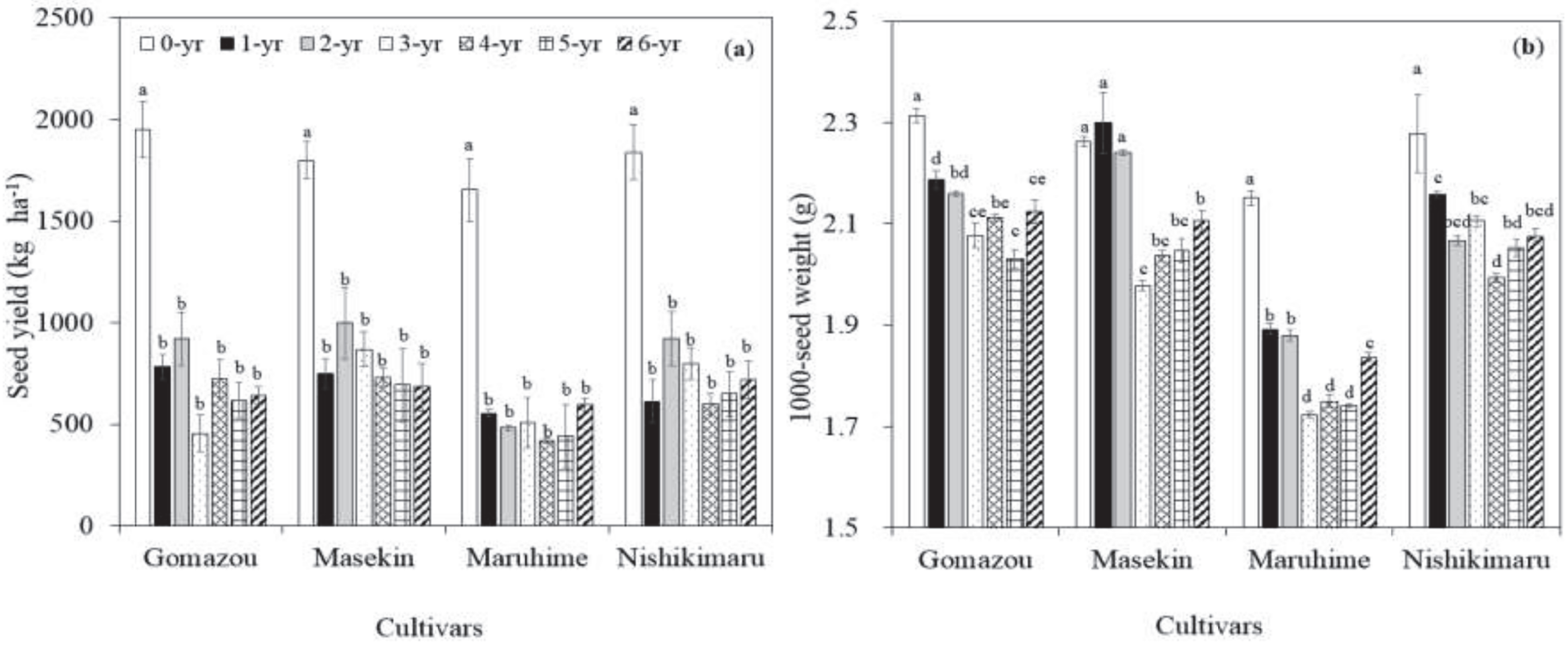


Figure 15. (a) The seed yield and (b) 1000-seed weight of sesame cultivars as affected by continuous monocropping on different continuously monocropped fields. The error bars represent standard errors ($n = 3$). Different letters within each column are significantly different at $p < 0.05$ (Tukey's test).

The analysis of variance showed that the seed yield and 1000-seed weight decreased in the long duration cropping depending on the cultivar. There was also significant interaction between the cultivar and cropping on 1000-seed weight. Moreover, the percentage decreases in 1000-seed weight was more pronounced on ‘Maruhime’ seeds than the other cultivars. For instance, in the year 2 field, the 1000-seed weight was highest in ‘Masekin’ (2.24 g), followed by ‘Gomazou’ (2.16 g), ‘Nishikimaru’ (2.07 g) and ‘Maruhime’ (1.88 g). However, all cultivars sowed tendency to increase 1000-seed weight in the year 6 field although still lower than year 0 values.

3.3.2. Disease incidence and severity index under different continuously monocropped fields and pathogen culture on potato dextrose agar (PDA) medium

The disease symptoms scored for severity index are shown in **Figure 16**. Overall, disease incidence and severity index gradually increased from year 1 through years 2, 3, 4 and 5, then decreased in year 6 (**Figure 17**). In ‘Gomazou’, disease incidence significantly increased in year 2 (30.8%) and year 3 (34.3%) compared to year 0 (6.98%) whereas in ‘Masekin’, disease incidence significantly increased in year 1 (23.89%) and year 4 (26.0%) compared to year 0 (2.06%). In ‘Maruhime’, the disease incidence significantly increased in year 2 (53.0%) and year 3 (54.4%) compared to year 0 (9.37%) whereas in the disease incidence in ‘Nishikimaru’, significantly increased in year 1 (39.3%), year 3 (34.5%) and year 4 (37.2%) compared to year 0 (2.08%).

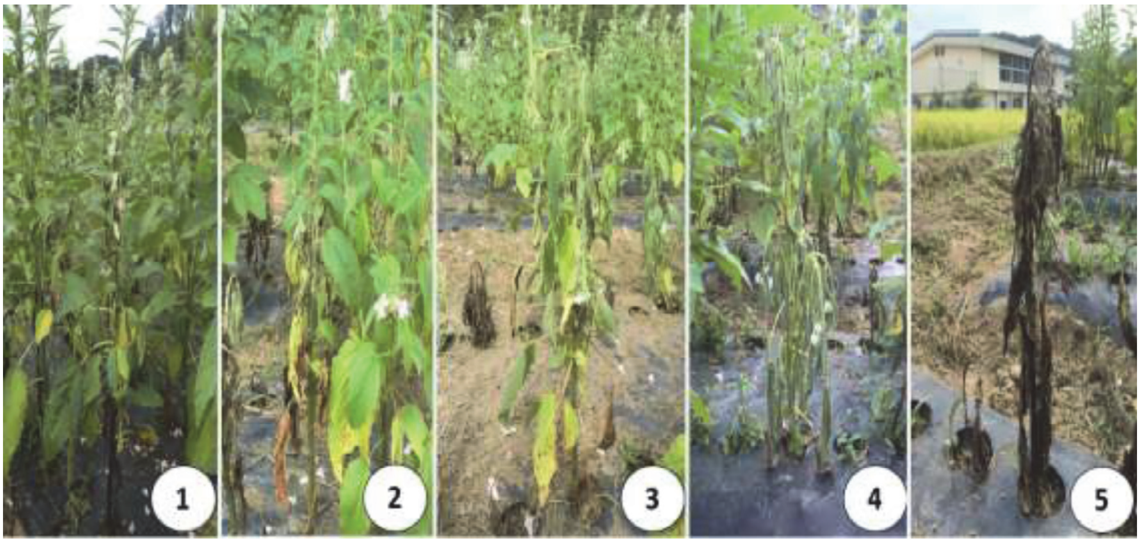


Figure 16. The varying disease signs and symptoms scored in the different continuously monocropped fields.

Furthermore, analysis of variance showed that disease incidences and severity index significantly ($p < 0.001$) increased in continuous monocropping depending on the cultivars. Significant interaction between cultivar and cropping effect on disease incidence and severity index was observed. For instance, results showed that in the year 2 field, disease incidence was significantly highest (53.0%) in 'Maruhime' with severity index of 39.93% compared to all cultivars. There was significantly highest disease severity (38.9%) was observed in 'Maruhime' in year 5. On the other hand, the disease severity index was lowest in 'Masekin' that was not significantly different from 'Gomazou'. Furthermore, disease incidence was highest in 'Maruhime' and 'Gomazou' whereas low in 'Masekin' and 'Nishikimaru'. Generally, disease incidence and severity index decreased in the year 6 compared to years, 1, 2, 3, 4 and 5 and 'Maruhime' showed the most susceptibility to disease incidence and severity under continuous monocropping.

Although further identification of the specific pathogen was not performed, the disease pathogen isolated on the potato dextrose agar medium indicated colonies related to fungal pathogen including *Fusarium oxysporum* f.sp. *sesami* and *Macrophomina phaseolina* (**Figure 18**).

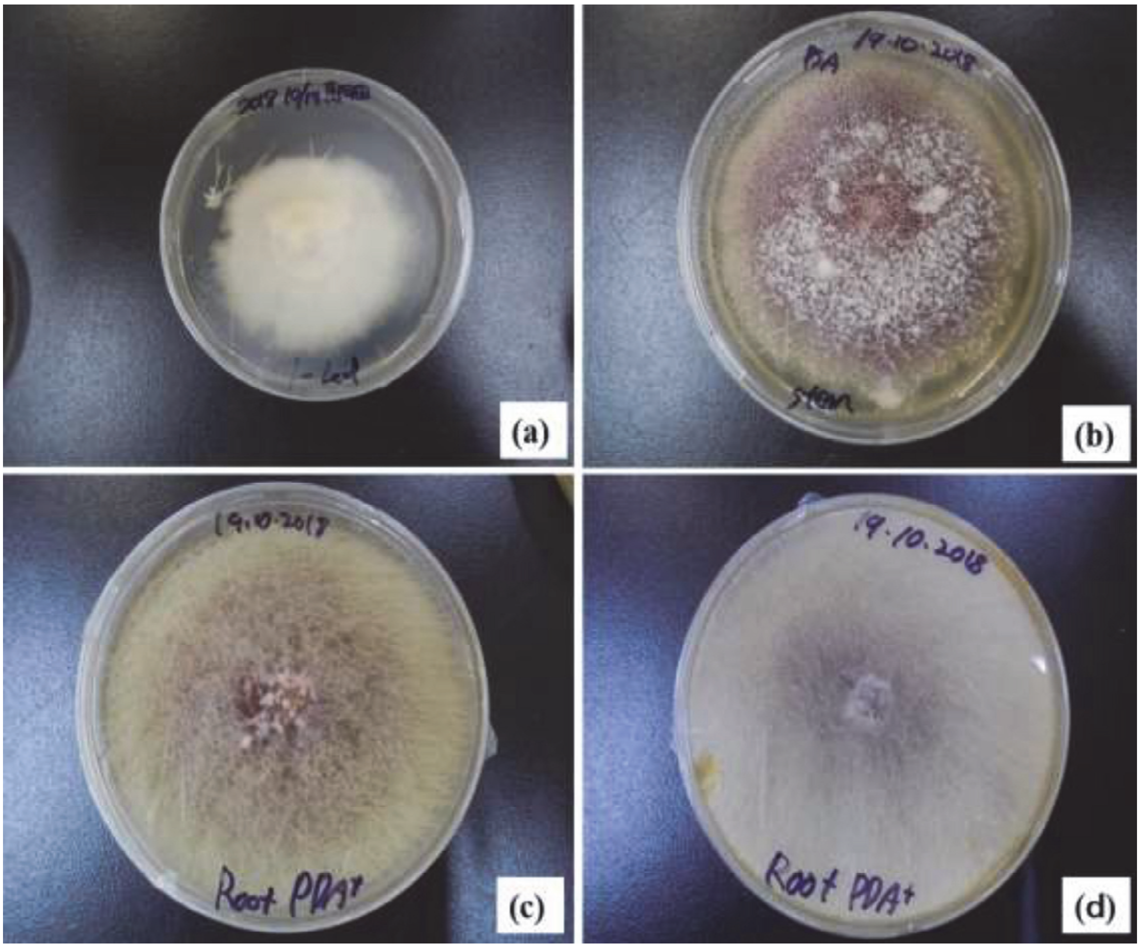


Figure 17. (a) The leaf, (b) stem, (c and d) root tissue samples showing white colony of fungal spores after incubation.

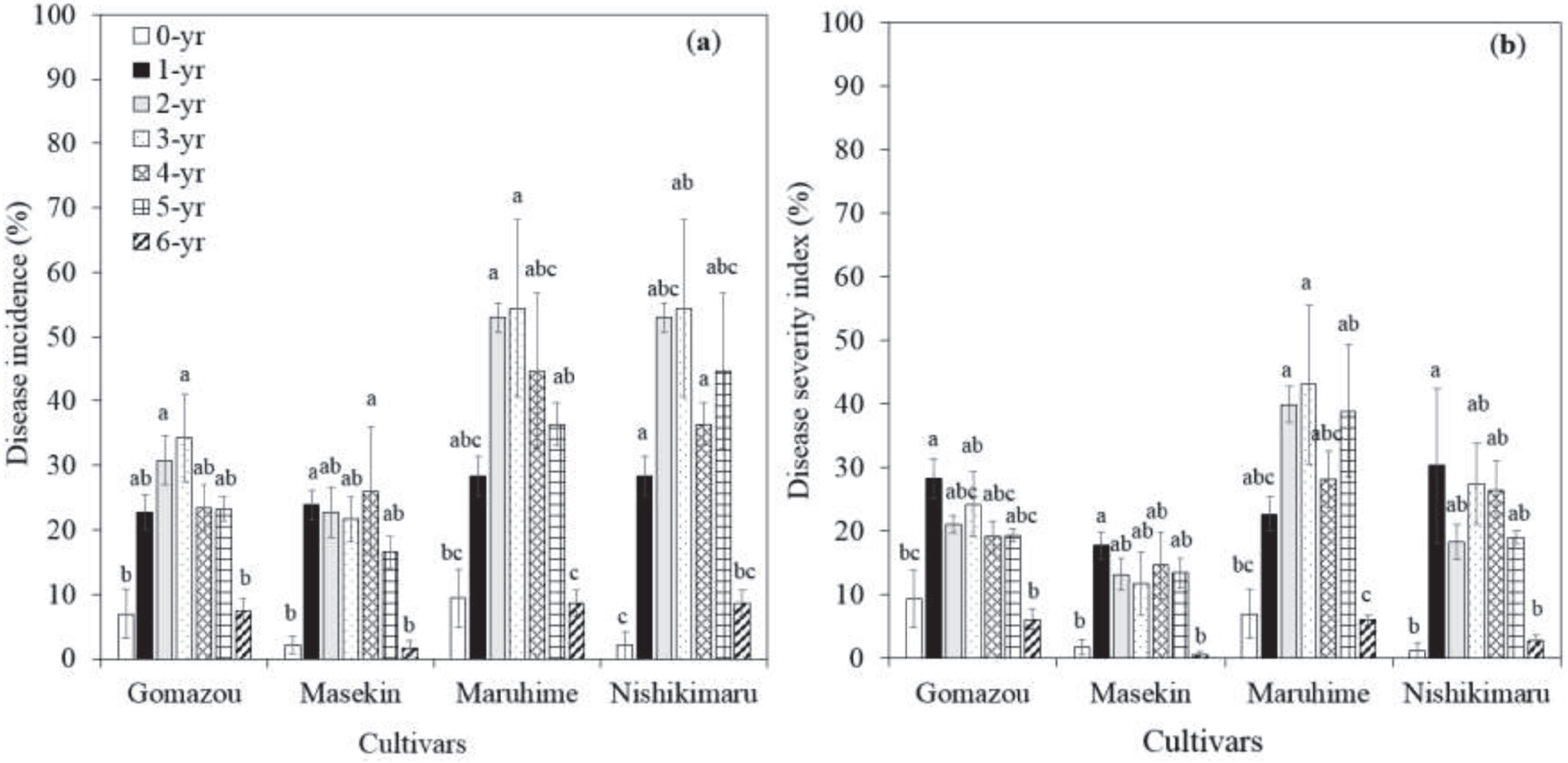


Figure 18. (a) Disease incidence and (b) severity index among sesame cultivars under the different continuously monocropped fields. The error bars represent standard errors ($n = 3$). Different letters within each column are significantly different at $p < 0.05$ (Tukey's test).

3.3.3. Phenolic compound contents in decomposing root residues from continuously cropped fields

Results indicated that decomposing sesame roots contained ferulic, *p*-hydroxybenzoic, caffeic, *p*-coumaric and vanillic acids as the dominant phenolic compounds in the methanol extract of roots detected by the HPLC. Among these compounds, only caffeic acid and the total phenolic compounds contents were significantly ($p < 0.001$) different among the four sesame cultivars (**Table 8**). 'Maruhime' had significantly highest (226.5 μg g^{-1} DW root) caffeic acid content compared to all cultivars whereas no significant differences in the contents of caffeic acid was observed among 'Gomazou', 'Masekin' and 'Nishikimaru'. The total phenolic compounds content in the decomposing root was significantly different ($p < 0.05$) among the cultivars. Although the total phenolic compounds content of cultivar 'Nishikimaru' showed significantly higher content (2.07 GAE μg g^{-1} DW root) than that of 'Gomazou' (1.68 GAE μg g^{-1} DW root), there was no significant difference between 'Nishikimaru' and all cultivars.

Table 8. Cultivar differences in phenolic and total phenolic compounds content in the decomposing sesame root residues.

	Content (μg g^{-1} DW root)					Total phenolic compounds
Cultivar	Ferulic acid	*p*-Hydroxybenzoic acid	Caffeic acid	*p*-Coumaric acid	Vanillic acid	(GAE μg g^{-1} DW root)
Gomazou	155.9	18.1	80.0b	22.9	27.3	1.68b
Masekin	213.0	34.4	42.8b	30.5	27.7	1.82ab
Maruhime	200.6	20.4	226.5a	28.3	26.3	1.84ab
Nishikimaru	205.8	30.0	7.31b	24.7	30.8	2.07a
ANOVA (*p*-values)	ns	ns	***	ns	ns	*

Different letters within each column are significantly different at $p < 0.05$ (Tukey's test). ***, **, * and ns represent $p < 0.001$, $p < 0.01$; $p < 0.05$ and non-significant, respectively.

3.3.4. Phenolic compounds in rhizosphere soil of four sesame cultivars from continuously cropped fields

Results showed that the soil phenolic compounds tended to decreased with increase in the duration of continuous monocropping (**Figure 19**). In the rhizosphere soil of 'Gomazou', ferulic acid decreased from 7.63 µg g^{-1} DW soil in the year 0 to 5.37 µg g^{-1} DW soil in the year 3 and 5.23 µg g^{-1} DW soil in the year 5. In the rhizosphere soil of 'Gomazou', caffeic acid content also tended to decrease but without significant differences among year 0 and all other fields. Similarly, *p*-coumaric acid content significantly decreased from 31.3 µg g^{-1} DW soil in the year 0 to 14.6 µg g^{-1} DW soil in year 3, 13.7 µg g^{-1} DW soil in year 4, and 13.0 µg g^{-1} DW soil in the year 5. Furthermore, vanillic acid content significantly decreased from 6.70 µg g^{-1} DW soil in the year 0 to 2.84 µg g^{-1} DW soil in the year 6 µg g^{-1} DW soil of 'Gomazou'. In 'Masekin', *p*-hydroxybenzoic acid content was significantly highest in the year 2 (12.0 µg g^{-1} DW soil) as compared to those of year 4 (7.52 µg g^{-1} DW soil) and year 5 (7.19 µg g^{-1} DW soil) soils. Caffeic acid significantly decreased from 4.17 µg g^{-1} DW soil in the year 0 soil and to 1.84 µg g^{-1} DW soil in the year 4 soil in 'Masekin' soil whereas *p*-coumaric acid significantly decreased from 30.0 µg g^{-1} DW soil in the year 0 soil and to 12.2 µg g^{-1} DW soil in the year 4 soil. Vanillic acid content showed no significant differences among years 0, 1 and 2 but significantly decreased to 3.25 µg g^{-1} DW soil in the year 4 compared to year 2 soil (6.24 µg g^{-1} DW soil). In 'Maruhime', ferulic acid content significantly decreased from 6.13 µg g^{-1} DW soil in the year 0 soil to 3.68 µg g^{-1} DW soil in the year 5 whereas caffeic acid content decreased from 4.62 µg g^{-1} DW soil in the year 0 to 1 1.87 µg g^{-1} DW soil in the year 3.

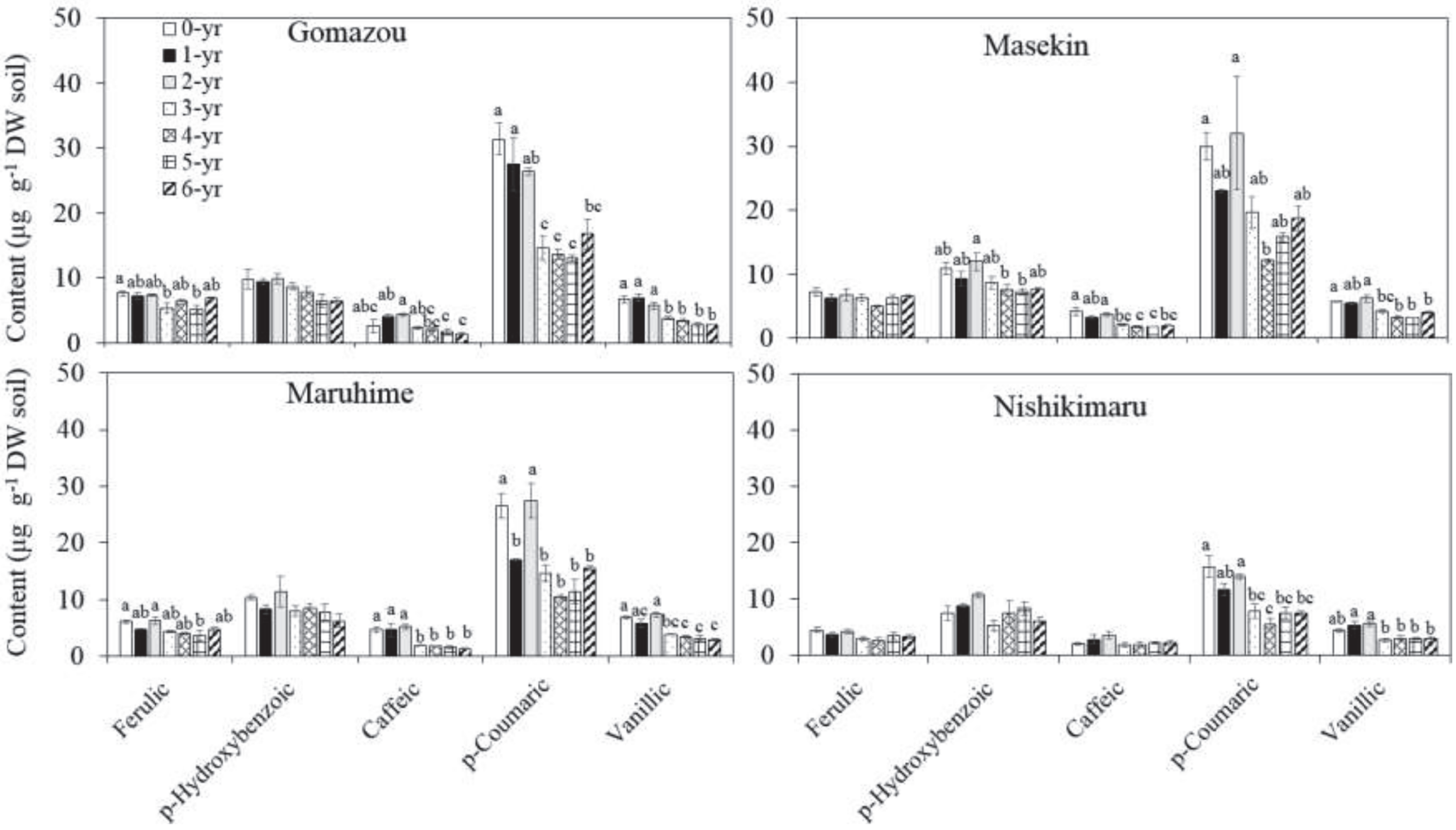


Figure 19. Soil phenolic compounds and their contents in continuously monocropped soils from four sesame cultivars. The error bars represent standard errors ($n = 3$). Different letters within each column are significantly different at $p < 0.05$ (Tukey's test).

p-coumaric acid significantly decreased from 26.6 μg g^{-1} DW soil in the year 0 and to 17.0 μg g^{-1} DW soil in the year 1 whereas vanillic acid content significantly decreased from 6.83 μg g^{-1} DW soil in the year 0 to 3.88 μg g^{-1} DW soil in the year 3 with no significant differences among years 3, 4, 5 and 6. In 'Nishikimaru', *p*-coumaric acid content significantly decreased from 15.7 μg g^{-1} DW soil in the year 0 and to 7.89 in the year 3. Although there was no significant differences in the vanillic acid contents among years 0, 1 and 2, vanillic acid content significantly decreased to 2.74 μg g^{-1} DW soil in the year 3 compared to year 2 (5.63 μg g^{-1} DW soil).

The total phenolic compound content in 'Gomazou' decreased from 0.14 μg GAE g^{-1} DW soil in the year 0 to 0.06 μg GAE g^{-1} DW soil in the year 4 (**Table 9**). Similarly, in 'Masekin', total phenolic compounds content decreased from 0.13 μg GAE g^{-1} DW soil in the year 0 to 0.05 μg GAE g^{-1} DW soil in the year 4. Similar decreases in the year 4 when compared to year 0 were also observed in 'Maruhime' and 'Nishikimaru'. Furthermore, results showed that total phenolic compound content depended on the cultivar indicating cultivar differences in concentrations of phenolic acids. Moreover, 'Gomazou' and 'Maruhime' showed higher content of mean total phenolic compounds than those of 'Masekin' and 'Nishikimaru'.

Analysis of variance indicated that in the rhizosphere soil, ferulic acid, *p*-coumaric acid, vanillic acid and the total phenolic compounds significantly decreased with increase in continuous monocropping ($p < 0.001$) depending on the cultivars ($p < 0.001$). On the other hand, *p*-hydroxybenzoic and caffeic contents decreased with increase in duration of continuous monocropping regardless of cultivar type. Furthermore, the analysis of variance showed significant interactions between cropping year and cultivar for caffeic acid ($p < 0.05$) and vanillic acid in the soils under continuous monocropping.

Results indicated that ferulic acid was significantly highest in the rhizosphere soils of 'Gomazou' and 'Masekin' in Years 0, 1, 2, 3, 4 and 6 whereas in year 2, both 'Gomazou' (7.34 μg g^{-1} DW soil) and 'Nishikimaru' (4.34 μg g^{-1} DW soil) showed high ferulic acid contents among all cultivars. The *p*-coumaric acid was significantly lowest in the rhizosphere soil of 'Nishikimaru' (15.7 μg g^{-1} DW soil) in years 0 compared to all cultivars. In addition, *p*-coumaric acid was significantly higher in 'Gomazou' and 'Masekin', in the years 3, 4, 5 and 6 than that in 'Nishikimaru' and 'Maruhime'. In year

3, there was significantly lowest vanillic acid content in rhizosphere soil of ‘Nishikimaru’ (2.74 μg g^{-1} DW soil) when compared to all cultivars. On the other hand, in year 6, vanillic acid was highest in ‘Masekin’ (4.01 μg g^{-1} DW soil) compared to all cultivars. There was also significant differences in the total phenolic compounds among the cultivars. For instance, among all cultivars, the total phenolic compounds was significantly lowest in the rhizosphere soils of ‘Nishikimaru’ (0.08 μg g^{-1} DW soil) in year 0. Furthermore, results showed that in year 2, soil total phenolic compound content was higher in ‘Masekin’ (0.15 μg GAE g^{-1} DW soil) ‘Gomazou’, (0.13 μg GAE g^{-1} DW soil) and ‘Maruhime’ (0.13 μg GAE g^{-1} DW soil), than in ‘Nishikimaru’ (0.08 μg GAE g^{-1} DW soil) whereas the highest soil total phenolic compound content in years 4 and 6 was observed in Maruhime’ (0.08 μg GAE g^{-1} DW soil) and ‘Gomazou’ (0.09 μg GAE g^{-1} DW soil) respectively. Overall, results showed that ‘Nishikimaru’ had lower content of individual and total phenolic compounds in rhizosphere soils compared to all cultivars whereas ‘Gomazou’ and ‘Masekin’ showed higher contents than ‘Maruhime’.

Table 9. Differences in the total phenolic compounds contents among cultivars in the rhizosphere soils from different continuous monocropping years.

	Soil total phenolic compounds content (GAE µg g^{-1} DW soil)			
Year	Gomazou	Masekin	Maruhime	Nishikimaru
0	0.14a	0.13a	0.14a	0.08abc
1	0.11a	0.11abc	0.14a	0.09a
2	0.13ac	0.15ac	0.13a	0.08ac
3	0.09b	0.07bcd	0.07b	0.06bd
4	0.06b	0.05d	0.08b	0.05d
5	0.07b	0.04bd	0.06b	0.06bcd
6	0.09bc	0.05bcd	0.08b	0.06bcd
ANOVA (*p*-values)	***	***	***	**
Mean	0.1	0.09	0.1	0.07

Different letters within each column are significantly different at $p < 0.05$ (Tukey's test). ***, **, * and ns represent $p < 0.001$, $p < 0.01$; $p < 0.05$ and non-significant, respectively.

3.3.5. Differences in germination and radicle growth inhibition of phenolic compounds among cultivars

The bioassay results with identified phenolic compounds indicated significant germination inhibition of sesame cultivars with increase in the concentrations of acids (**Table 10**). The germination inhibition percentage by ferulic and vanillic acids depended on the cultivar and significant interactions observed between cultivar and concentration for *p*-hydroxybenzoic, *p*-coumaric and vanillic acids in germination inhibition percentage. Results showed no significant differences in the germination inhibition by 1 mM concentration of all the phenolic compounds among cultivars. In addition, 1 mM concentration had the least germination inhibition percentage compared to 10 mM and 20 mM. There was significant differences in the 5 mM *p*-hydroxybenzoic acid concentration among the cultivars with highest germination inhibition observed in 'Maruhime' (26.67%) and lowest in 'Masekin' (0.0%).

Furthermore, 5 mM vanillic acid showed significantly highest germination inhibition on 'Gomazou' (50.0%) and 'Maruhime' (33.3%) and no inhibition of 'Masekin' and 'Nishikimaru' germination. There was also significant differences among cultivars in the germination inhibition by 20 mM vanillic acid with highest inhibition observed in 'Gomazou' (100%) and 'Maskin' (100%) and 'Maruhime' (80.0%). On the other hand, 'Gomazou' (93.3%) showed significantly highest germination inhibition by 20 mM ferulic acid and lowest in 'Nishikimaru' (40.0%). Hence, it could be observed that ferulic acid inhibited the germination 'Gomazou' more than all cultivars at highest concentrations. In addition, 'Gomazou' followed by 'Masekin' and 'Maruhime' showed highest germination inhibition by phenolic compounds compared to 'Nishikimaru'.

Table 10. The germination inhibition (%) of seeds of different cultivars as affected by phenolic compounds.

		Germination inhibition (%)				
Cultivar	Concentration (mM)	Ferulic acid	*p*-Hydroxybenzoic acid	Caffeic acid	*p*-Coumaric acid	Vanillic acid
Gomazou	1	-1.67b	6.7c	6.67	23.3b	11.7b
	5	33.3ab	6.7c	13.3	50.0ab	50.0ab
	10	83.3a	55.0b	40.0	63.3ab	78.3a
	20	93.3a	100a	40.0	85.0a	100a
Masekin	1	0b	0b	0b	0c	0b
	5	53.3ab	0b	0b	60.0b	0b
	10	53.3ab	60.0a	0b	73.3ab	60.0a
	20	80.0a	60.0a	46.7a	100.0a	100a
Maruhime	1	6.67b	0b	0.0b	0b	0b
	5	0b	26.7ab	6.7b	26.7b	33.3b
	10	66.7a	73.3a	13.3b	80.0a	80.0a
	20	73.3a	53.3ab	73.3a	86.7a	80.0a
Nishikimaru	1	0	0b	0b	0b	0.0
	5	20.0	13.3b	0b	13.3b	0.0
	10	26.7	33.3ab	0b	86.7a	33.3
	20	40.0	80.0a	66.7a	100a	33.3
Source of variation						
Cultivar		*	ns	ns	ns	***
Concentration		***	***	***	***	***
Cultivar x Concentration		ns	*	ns	*	*

Different letters within each column are significantly different at $p < 0.05$ (Tukey's test). ***, **, * and ns represent $p < 0.001$, $p < 0.01$; $p < 0.05$ and non-significant, respectively.

The concentrations required to cause 50% germination inhibition (IC_{50}) was extrapolated using exponential regression curve (**Table 11**). Across all cultivars, the IC_{50} values were highest for caffeic acid indicating more than 20 mM of caffeic acid was required to cause 50% germination inhibition. Conversely, ferulic, *p*-hydroxybenzoic and *p*-coumaric at concentrations below 20 mM caused significant 50% germination reduction for 'Gomazou', 'Masekin', and 'Maruhime'. However, ferulic and vanillic acids were required in concentrations above 50 mM to cause 50% germination inhibition of cultivar 'Nishikimaru'.

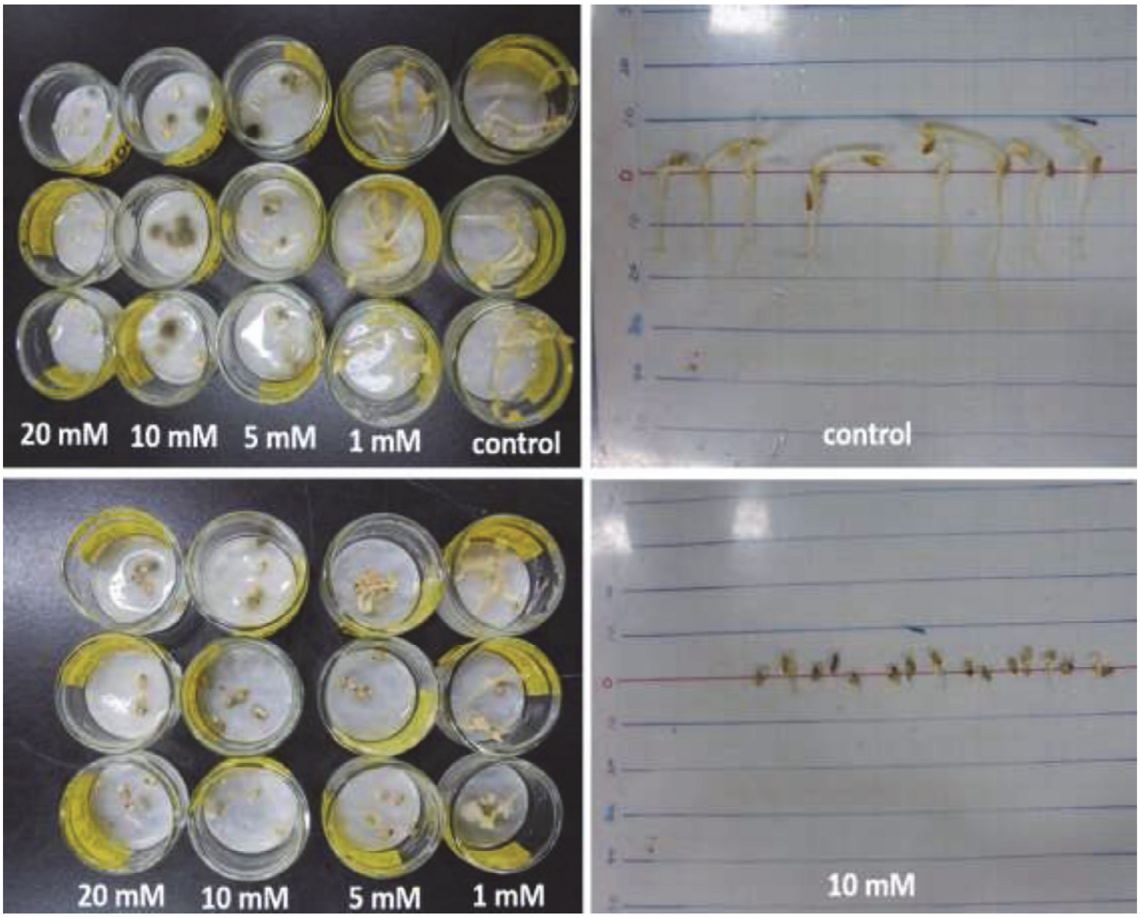


Figure 20. Bioassay petri showing germination and radicle growth inhibition at varying concentrations of phenolic compounds and measurement of radicle length after 4 days of incubation.

Table 11. The regression equations and concentrations of phenolic compounds required to cause 50% germination inhibition (%) of seeds of different cultivars.

Cultivar	Phenolic compound	Regression equation	R^2	IC_{50} (mM) for germination
Gomazou	Ferulic	y = 33.49ln(x) - 5.7524	0.94	5.28
	p-Hydroxybenzoic	y = 29.62ln(x) - 9.0686	0.72	7.35
	Caffeic	y = 12.294ln(x) + 3.7696	0.81	42.97
	p-Coumaric	y = 19.876ln(x) + 21.092	0.98	4.28
	Vanillic	y = 29.56ln(x) + 8.9512	0.99	4.01
Masekin	Ferulic	y = 25.507ln(x) + 2.6171	0.95	6.41
	p-Hydroxybenzoic	y = 22.414ln(x) - 8.708	0.69	13.7
	Caffeic	y = 11.992ln(x) - 9.0434	0.43	137.5
	p-Coumaric	y = 32.82ln(x) + 1.6551	0.99	4.36
	Vanillic	y = 32.693ln(x) - 16.46	0.73	7.64
Maruhime	Ferulic	y = 24.286ln(x) - 5.2739	0.65	9.74
	p-Hydroxybenzoic	y = 21.621ln(x) + 0.9953	0.76	9.65
	Caffeic	y = 20.241ln(x) - 11.622	0.59	21.0
	p-Coumaric	y = 30.964ln(x) - 5.1399	0.90	5.93
	Vanillic	y = 29.092ln(x) - 1.9074	0.92	5.96
Nishikimaru	Ferulic	y = 12.912ln(x) - 0.6321	0.99	50.5
	p-Hydroxybenzoic	y = 24.127ln(x) - 9.9999	0.78	12.0
	Caffeic	y = 17.132ln(x) - 12.919	0.43	39.4
	p-Coumaric	y = 35.485ln(x) - 11.281	0.81	5.62
	Vanillic	y = 12.452ln(x) - 4.8378	0.69	81.8

The bioassay results also showed significant inhibition of radicle growth by phenolic compounds identified (**Figure 20**).

Radicle growth inhibition significantly increased with increase in the concentrations of all phenolic acids (**Figure 21**). Compared to the 1 mM, 10 mM concentration of all phenolic acids significantly increased radicle growth inhibition of 'Gomazou'. The highest radicle inhibition of over 90% was observed in the 20 mM concentrations of all acids. In 'Masekin', the radicle growth inhibition was significantly highest in the 5 mM compared to the 1 mM of ferulic and *p*-coumaric acids without significant differences among 5 mM, 10 mM and 20 mM. However, radicle growth inhibition by each concentration of *p*-hydrpoxybenzoic increased with increase of concentration such as 1 mM (19.8%), 5 mM (79.9%), 10 mM (96.2%) and 20 mM (100%). There was no radicle growth inhibition of 'Masekin' by 1 mM caffeic acid. However, significant radicle growth inhibition rate of 74.9% was observed in the 5 mM (in caffeic while significantly increasing to 91.3% in the 10 mM without significant differences between 10 mM and 20 mM concentrations. Furthermore, a significant difference in the radicle growth inhibition was observed between 1 and 10 mM vanillic acid on growth of 'Masekin' without differences among 5 mM, 10 mM and 20 mM treatments. The radicle growth inhibition of 'Maruhime' was significantly increased in the 5 mM concentration compared to the 1 mM. However, there were no significant differences among 5 mM, 10 mM and 20 mM concentrations of all phenolic compounds. Similarly, the radicle growth inhibition of 'Nishikimaru' showed increase in inhibition with concentrations higher than 1 mM without significant differences among the 5, 10 and 20 mM concentrations of ferulic, *p*-hyrdoxybenzoic, caffeic and *p*-coumaric acids. Furthermore, the radicle growth inhibition of 'Nishikimaru' by 1 mM concentration was significantly highest in the vanillic acid when compared to the rest of the acids. In addition, vanillic acid at 5 mM (70.5%) was significantly different from 1 mM (43.3%) and 10 mM (95.1%) in radicle growth inhibition without significant differences between 10 mM and 20 mM.

The analysis of variance showed that radicle growth inhibition depended on cultivar ($p < 0.001$) for both caffeic and vanillic acids with significant interactions between cultivar and concentrations of phenolic compounds ($p < .01$) for both caffeic and vanillic acids. Results showed that the significantly highest radicle growth inhibition by

1 mM caffeic acid was observed in ‘Gomazou’ (36.9%) and ‘Maruhime’ (8.70%) and there was no significant differences in radicle growth inhibition in 5 mM, 10 mM and 20 mM among cultivars. On the other hand, the significantly highest radicle growth inhibition by 5 mM vanillic acid was observed in ‘Gomazou’ (94.9%) and ‘Maruhime’ (88.3%) whereas the 20 mM vanillic acid significantly inhibited radicle growth of ‘Gomazou’ (100%), ‘Masekin’ (100%) and ‘Maruhime’ (98.7%) more than ‘Nishikimaru’ (94.9%). Therefore, radicle growth inhibition of ‘Nishikimaru’ was the lowest in compared to other cultivars.

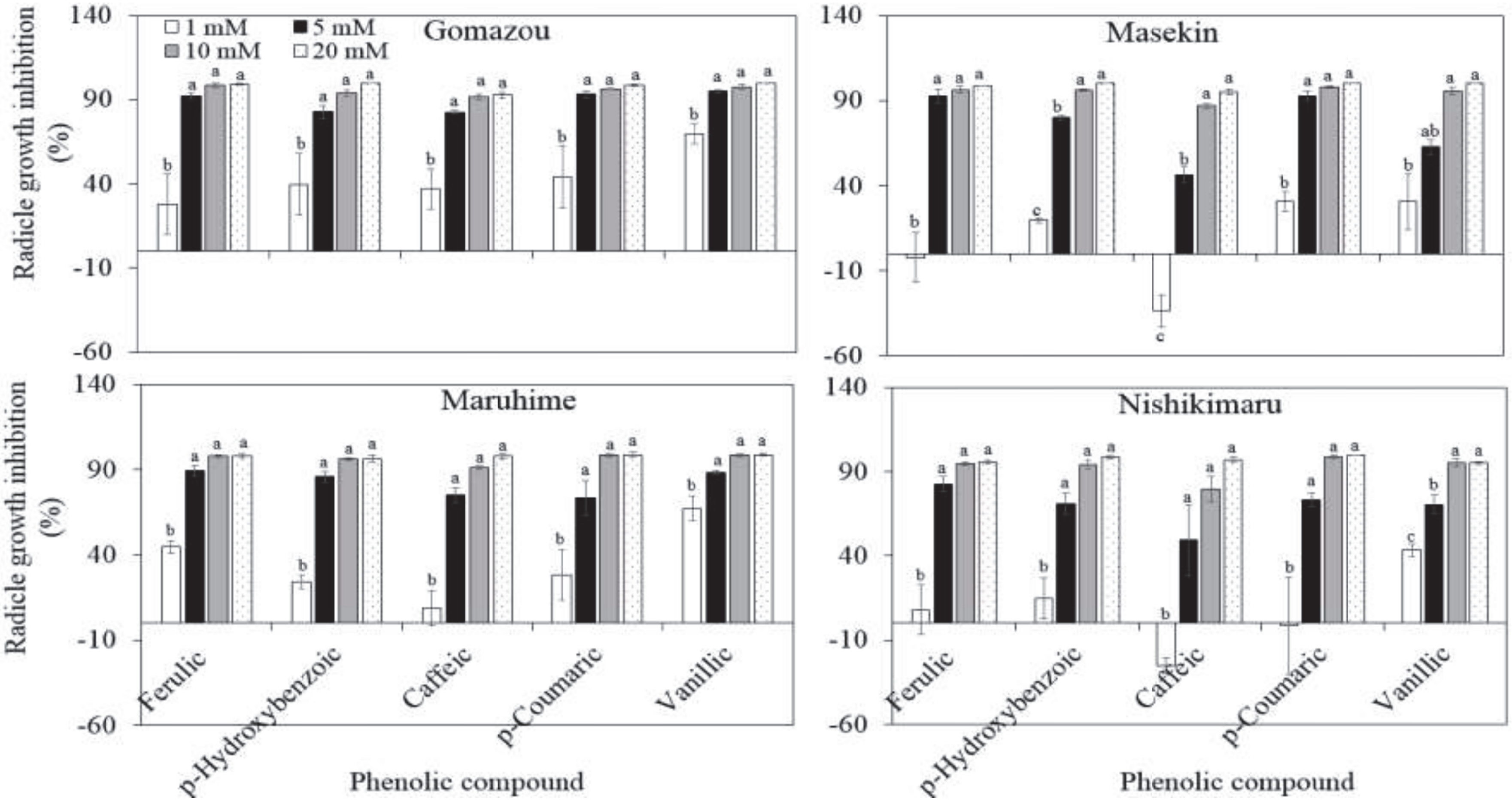


Figure 21. Radicle growth inhibition (%) among sesame cultivars at varying concentrations of phenolic compounds. The error bars represent standard errors ($n = 3$). Different letters within each column are significantly different at $p < 0.05$ (Tukey's test).

The percentage of radicle growth increased with increase in the concentrations and the concentrations required to cause 50% growth inhibition (IC_{50}) was extrapolated using exponential regression curve (**Table 12**). The concentration of all phenolic compounds required as IC_{50} radicle growth inhibition was lower than 5 mM with exception of caffeic acid on radicle growth of 'Masekin' and 'Nishikimaru' showing values slightly over 5 mM. Moreover, caffeic, *p*-hydroxybenzoic and ferulic acids showed higher IC_{50} values than *p*-coumaric and vanillic acids.

Table 12. The regression equations and concentrations of phenolic compounds required to cause 50% radicle growth inhibition (%) of seeds of different cultivars.

Cultivar	Phenolic compound	Regression equation	R^2	IC_{50} (mM) for radicle growth
Gomazou	Ferulic	$y = 25.019\ln(x) + 36.385$	0.87	1.72
	p-Hydroxybenzoic	$y = 20.847\ln(x) + 43.021$	0.96	1.40
	Caffeic	$y = 19.691\ln(x) + 42.014$	0.91	1.50
	p-Coumaric	$y = 18.945\ln(x) + 50.459$	0.87	0.98
	Vanillic	$y = 10.557\ln(x) + 72.254$	0.91	0.12
Masekin	Ferulic	$y = 35.083\ln(x) + 10.75$	0.84	3.06
	p-Hydroxybenzoic	$y = 28.064\ln(x) + 25.512$	0.94	2.39
	Caffeic	$y = 45.273\ln(x) - 29.542$	0.97	5.79
	p-Coumaric	$y = 24.076\ln(x) + 38.827$	0.87	1.59
	Vanillic	$y = 24.641\ln(x) + 29.718$	0.96	2.28
Maruhime	Ferulic	$y = 18.839\ln(x) + 49.852$	0.90	1.01
	p-hydroxybenzoic	$y = 25.46\ln(x) + 31.647$	0.89	2.06
	Caffeic	$y = 30.955\ln(x) + 14.708$	0.95	3.13
	p-Coumaric	$y = 25.258\ln(x) + 31.081$	0.95	2.11
	Vanillic	$y = 11.234\ln(x) + 68.8$	0.95	0.19
Nishikimaru	Ferulic	$y = 30.83\ln(x) + 16.97$	0.89	2.92
	p-hydroxybenzoic	$y = 29.466\ln(x) + 18.59$	0.96	2.90
	Caffeic	$y = 41.815\ln(x) - 22.166$	0.99	5.62
	p-Coumaric	$y = 36.063\ln(x) + 5.3004$	0.94	3.45
	Vanillic	$y = 18.653\ln(x) + 43.765$	0.94	1.40

3.4. Discussion

In this study, continuous monocropping significantly decreased sesame plant heights, height of first capsule, number branches, seed yield and 1000-seed weight indicating occurrence of continuous monocropping obstacle (**Table 7**, **Figure 15**). The result of this study is consistent with previous researches of continuous monocropping that growth and yield decreases suggests continuous monocropping obstacles (George et al., 2002; Peng et al., 2006; Stanger and Lauer, 2008; Bennett et al., 2012). Although there was no significant differences in the seed yield among the sesame cultivars under continuous monocropping years, 'Maruhime' showed the lowest seed yield suggesting it is less tolerant to continuous monocropping obstacle. However, the 1000-seed weight was significantly lowest in 'Maruhime' in the years 1-6 whereas the highest 1000-seed weight was observed in the 'Masekin' in year 2, field, 'Nishikimaru' in year 3, 'Gomazou' in years 4, 5 and 6 indicating that tolerant cultivars exist, especially 'Gomazou' and 'Masekin' could be desirable for continuous monocropping. Growth was highest in the year 0 field indicated by the high values of plant height, number of branches per plant and height of the first capsule while growth decreased in the long duration of continuous monocropping reflecting negative influence of continuous monocropping on cultivars. The high 1000-seed observed in cultivars that showed high yield despite continuous monocropping obstacle could be attributed to maintaining high growth. For instance, the significantly higher plant height in the year 1 field for 'Gomazou' and 'Masekin' than other cultivars could suggests that high yield in terms of high 1000-seed weight is attributed to high growth despite continuous monocropping obstacles. The decreases in the growth was more pronounced in 'Maruhime' than the other cultivars suggesting 'Maruhime' could be less tolerant cultivar to continuous monocropping obstacle. Furthermore, the significant interaction between cultivars and cropping on the number of branches per plant and plant height could be due to differences in the branching characteristics of the sesame cultivars related to their genotypes and the overall effect of growth reduction by continuous monocropping. 'Nishikimaru' was bred as a cross between 'Masekin' and 'Gomazou' with shorter plant height than 'Masekin' and 'Gomazou' and 'Nishikimaru' yield is lower than 'Gomazou' but not significantly different from 'Masekin' (Kato et al., 2017). In addition, 'Masekin' has few or no branches compared to 'Gomazou' in addition to its lower yield than 'Gomazou' (Yasumoto and Katsuta, 2006).

The significantly highest disease incidence and severity index in ‘Maruhime’ observed especially in the year 2 and 5 fields suggested that ‘Maruhime’ is most susceptible to continuous monocropping diseases among the cultivars (**Figure 17**). On the other hand, the low disease incidence and severity in ‘Gomazou’ and ‘Masekin’ indicated their resistance to continuous monocropping obstacles as reflected by their sparingly higher growth and yield than ‘Maruhime’. Furthermore, this study also showed that disease incidence and severity significantly increased in in the years 1, 2, 3, 4 and 5 indicating deterioration of soil health (Hua et al., 2012). The build-up of soil microorganisms such as *Fusarium oxysporum* f. sp. *sesami* (*Fusarium* wilt) and *Ralstonia solanacearum* (bacterial wilt) in continuous monocropping contributed to significant yield declines and that the number of rhizosphere soil bacteria and actinomycetes became significantly lower than the number of fungi that cause disease. The culture of pathogens from infected sesame plant tissues on the PDA media further revealed fungal disease pathogens could be the most prevalent under continuous monocropping of sesame on upland field converted paddy (**Figure 18**). It could be speculated that sesame wilt and root rot diseases caused by *Fusarium oxysporum* f.sp. *sesami* and *Macrophomina phaseolina* could be the major diseases affecting sesame yield (Ziedan et al., 2011). The low disease incidence and severity in year 6 could suggest that continuous monocropping obstacle of sesame related to diseases may temporarily decrease in the long-term continuous monocropping allowing possible recovery in growth and yield. Results showed that 1000-seed weight slightly increased in the year 6 field suggesting tendency in yield recovery although less than year 0. An earlier research indicated that occasionally yield declines of wheat can be recovered to some extent if the continuous monocropping is continued (Cook, 2003). He observed wheat yield decline in in continuous monocropping was observed due to buildup of soil pathogens that cause take-all wheat (*Gaeumannomyces graminis* var *tritici*) but with increase in the number of continuous monocropping, the biocontrol agents naturally developed against the take-all pathogens allowing the yield to recover to some extent and lower than in a non-continuous monocropping. In our study with sesame, such soil borne pathogens were not quantified and this warrant further research to confirm this hypothesis.

Results showed that decomposing root residues and rhizosphere soils contained allelochemicals. Similar findings have been shown that allelochemicals such as phenolic compounds exist in continuous monocropping soils (Hao et al., 2006; Zhou et al., 2012; Song et al., 2016). This study also showed that soil phenolic compounds significantly decreased in years 5 and 6 compared to year 0 and 1 suggesting low accumulation of allelochemicals with increase in the duration of continuous monocropping under this management practice on upland field converted paddy (**Figure 19**). This study is contrary to several researches that indicate allelochemicals including phenolic compounds such as could accumulate and increase with increase in the duration of continuous monocropping of cucumber, peanuts, cowpeas, strawberry and watermelon (Hao et al., 2006; Li et al., 2010; Huang et al., 2010; Zhou et al., 2012; Li et al., 2016). The decrease in the allelochemicals including phenolic compounds could be explained low build-up of decomposing crop residues since continuous monocropping was performed without residue return. Crop residues and root exudates are the major sources of allelochemicals (Rice, 1984). However, sesame residues could contain more allelochemicals than the build up from root exudates resulting into low quantity of phenolic compounds when residues are not in cooperated back into the soil. It is reported that phenolic compounds have positive correlation with decomposing organic materials (Martens, 2002). Hence, little residues imply low quantity of phenolic compounds released into the soil.

The decrease in the ferulic acid, *p*-coumaric acid, vanillic acid, and total phenolic contents in the rhizosphere soil depended on the cultivars suggesting differences in the release and contents of these allelochemicals. On the other hand, *p*-hydroxybenzoic acid and caffeic acid content decreased with increase in duration of continuous monocropping regardless of cultivar type indicating no variation in the amount and release of these compounds. Furthermore, the decomposing root residues of ‘Maruhime’ contained the highest caffeic acid compared to all cultivars and moderately high total phenolic compounds contents suggesting ‘Maruhime’ could produce high allelochemicals in the plant tissue. However, the release of the compounds could be low as indicated by the low rhizosphere phenolic compounds in ‘Maruhime’ soil. Conversely, ‘Gomazou’ and ‘Masekin’ showed higher contents whereas ‘Maruhime’ showed moderate content of phenolic compounds in the rhizosphere soil.

In this study, the phenolic compounds were identified in the decomposing residues and rhizosphere soils inhibited both germination and radicle growth suggesting allelochemicals could cause continuous monocropping obstacle of sesame. Several researches also confirmed that phenolic compounds in rhizosphere soils inhibit germination and radicle growth (Miller et al., 1991; Li et al., 2010; Iannucci et al., 2013). The phenolic compounds inhibited radicle growth more than germination inhibition and the results of bioassay suggested that caffeic, *p*-hydroxybenzoic and ferulic acids could affect germination of sesame whereas *p*-coumaric and vanillic acids have more pronounced effects on radicle growth than germination. Results also showed that *p*-coumaric acid was the major soil phenolic with highest concentrations detected in rhizosphere soils of all cultivars. It could be of the dominant allelochemicals in phenolic compounds released by sesame roots or decomposing root root residue. Hao et al. (2006) reported phenolic compounds including *p*-hydroxybenzoic and *p*-coumaric acids in methanol extracts that were implicated for continuous monocropping obstacles of watermelon. In their study, allelopathic activity increased with increase in the duration of continuous monocropping suggesting that duration of cropping is an important factor affecting the structure and dynamics of allelochemicals in soils. This is contrary to this study since with increase in continuous monocropping duration; soil phenolic compounds were significantly decreased in sesame cultivation. This could also suggest that depending on the management strategies applied in the continuous monocropping systems, autotoxicity could be managed differently and occurrence of soil sicknesses depends on the level of management adopted in the crop production. However, the concentration of *p*-coumaric acid was significantly lowest in 'Nishikimaru' as compared to all cultivars. Furthermore, significant radicle growth inhibition by 1 mM of caffeic acid was the highest in 'Gomazou' and the lowest in 'Masekin' and 'Nishikimaru' despite the fact that 'Maruhime' showed high caffeic acid in the decomposing root residues suggesting that although decomposing residues contain high allelochemicals, the compounds may not directly affect the germination and radicle growth of the same cultivar but could affect another indicating a possible varietal allelopathy. Yeasmin et al. (2014) reported that some cultivars have significant negative effects on the growth of others through release of compounds that affect growth of different species. Furthermore, allelochemicals from barely have autotoxicity and has been found that there is genotypic variation with some cultivars showing more autotoxicity potential than others (Ben-Hammouda et al., 2002; Bouhaouel et al., 2015).

This study also indicated that the radicle growth inhibition of cultivars significantly increased with increase in the concentrations of phenolic compounds suggesting phytotoxicity of allelochemicals could only occur at high concentrations (**Figure 21**). Furthermore, the concentrations required to cause 50% germination inhibition (IC_{50} values) were highest for caffeic acid indicating more than 20 mM of caffeic acid was required to cause over 50% germination inhibition suggesting caffeic acid could be weak in autotoxicity activity of sesame germination (**Table 10**). On the other hand, ferulic, *p*-hydroxybenzoic and *p*-coumaric could cause significant decrease in the germination rate of 'Gomazou', 'Masekin', and 'Maruhime' at concentrations below 20 mM suggesting a strong ability to cause continuous monocropping obstacles of sesame. However, cultivar 'Nishikimaru' required ferulic and vanillic acid concentrations above 50 mM in IC_{50} value. It could be highly resistant to allelopathy and autotoxicity in terms of germination in continuous monocropping. Results also showed that the concentrations of all phenolic compounds required to IC_{50} for radicle growth inhibition was lower than 5 mM with exception of caffeic acid on radicle growth of 'Masekin' and 'Nishikimaru' showing values slightly over 5 mM (**Table 12**). Moreover, caffeic, p-hydroxybenzoic and ferulic acids showed higher IC_{50} values than *p*-coumaric and vanillic acids suggesting *p*-coumaric and vannilic acids could be the most important acids influencing sesame growth because of direct seedling growth inhibition. It has also been reported that *p*-hyrdozxybenzoic acid cause significant plant height, root length and fresh weight reduction in *Pogostemon cablin* exhibiting autotoxicity at concentration as low as 0.2 mM (200 μM) (Xu et al., 2015). Furthermore, *p*-coumaric acid has been isolated from asparagus plant and have been shown that it caused 50% inhibition of root and shoot growth of tests plants at range of 0.36 to 0.85 mM and could be associated with continuous monocropping obstacle of asparagus after residues of asparagus decompose in the soil accumulating with increase on duration of continuous monocropping (Kato-Noguchi et al., 2017). In addition, several phenolic compounds have been reported to have bioactivity ranfe between 0.1 and 1 mM (Muscolo and Sidari, 2006). In this study, the phenolic compound ranges that could affect 50% inhibition on both germination and radicle growth were less than 20 mM slightly higher than this reported. Overall, the high content of phenolic compounds in year 0, 1 and 2 or short duration of continuous monocropping in the rhizosphere soils of 'Gomazou' and 'Masekin' despite the high yield of these two cultivars suggested these compounds did not directly affect growth and yield. This could be attributed to the fact that the concentrations in the soil including in year 0 was not adequate to cause phytotoxicity under field condition. For allelochemicals to cause significant inhibition, the compounds must be accumulated in the rhizosphere soil and persist to toxic level that allowing significant damages on the plant (Inderjit, 2005). For example, vanillic acid exhibited strong autotoxicity effect on 'Gomaozu', 'Masekin' and 'Maruhime' whereas 'Nishikimaru' showed tolerance to vanillic acid at high concentrations up to 20 mM whereas caffeic acid significantly inhibited both the germination and radicle growth of ‘Gomazou’ compared to all cultivars. Hence, ‘Gomazou’ and ‘Masekin’ could therefore be tolerant to these compounds in terms of autotoxicity. The results indicated that the concentration of phenolic compounds in the bioassay could be more than that existing in the soil to cause significant germination and growth inhibition. Zhou et al. (2012) reported low phenolic compounds in the seventh cropping as compared to that in the ninth cropping and attributed this to poor cucumber growth leading to low production of phenolic other than phytotoxicity.

On the other hand, ‘Maruhime’ and ‘Nishikimaru’ although tolerant to phenolic compounds autotoxicity in bioassay, could be highly susceptible to the indirect effect of these compounds under field conditions. The high contents of the total phenolic compound from ‘Maruhime’ could explain the significant increase in the disease incidence and severity in the year 2 and 3 compared to all cultivars. Several researches have shown that phenolic compounds from decomposing residues and root exudates were related to increasing disease causing microorganisms apart from causing autotoxicity (Bonanomi et al., 2007; Zhou et al., 2012; Qi et al., 2015; Xin-hui et al., 2015). Li et al. (2014) reported that both benzoic acid and *p*-coumaric acids were the dominant phenolic acids in the root exudates of peanut cultivars and that release and accumulation of phenolic compounds differed depending on the cultivars. Moreover, the phenolic compounds directly influenced the soil-borne pathogens influenced peanut growth. The resistance towards continuous monocropping obstacles was attributed to the differences in the quantities of phenolic compounds released by the peanut cultivars suggesting the mechanism of resistance was due to differences in the release of phenolic compounds that directly proliferated the increase in the soil borne pathogens. Allelochemicals were also exuded into rhizosphere soils and then accumulated. These allelochemicals have been shown to increase the disease index in verticillium wilt of eggplants at high concentrations apart from decreasing germination and seedling growth (Chen et al., 2011). Chen et al. concluded that allelochemicals exuded in high concentrations could inhibit eggplant growth while promoting soil-borne disease incidences causing continuous monocropping obstacles. Zhou et al. (2012) also found that *p*-hydroxybenzoic, vanillic, *p*-coumaric and ferulic acids were significantly higher in the continuously monocropped cucumber soil than non-cropped field soil. *p*-coumaric acid was the most abundant followed by *p*-hyroxybenzoic acid and each phenolic compound tended to be low in the seventh as compared to the ninth cropping. Furthermore, Zhou and Wu (2012) assessed the effect of *p*-coumaric acid isolated from rhizosphere soils on seedling growth, rhizosphere microbial communities including *Fusarium oxysporium* f.s.p. *cucumerinum*. Usually phenolic compounds are used as carbon sources for microorganisms in soil and partly they are utilized by the micrograms upon release (Souto et al., 2000). Therefore, the high content of caffeic acid in decomposing root residues of sesame such as 'Maruhime' observed in this study could show its susceptibility to autotoxicity and disease infections. For instance, earlier research showed that caffeic acid was identified as one of the most important autotoxicity allelochemicals in asparagus inhibiting germination and it was found that caffeic acid showed synergistic action in increasing *Fusarium oxysporum* f. Sp. *Asparagi* (Miller et al, 1991). The allelochemicals identified in the rhizosphere soils could therefore predispose sesame to diseases such as *Fusarium* infections. Conversely, in this study, 'Gomazou' and 'Masekin' are tolerant to disease occurrence under continuous monocropping whereas the 'Nishikimaru' and 'Maruhime' are highly susceptible leading to their low yields under continuous monocropping. Hence, an indirect way of continuous monocropping obstacle in sesame could be speculated arising from increased soil phenolic compounds promoting disease occurrences although this hypothesis needs to be tested in sesame.

3.5. Conclusions

This study indicated that the extent of continuous monocropping obstacle was depending on cultivar characteristics regardless of being tolerant to autotoxicity that is directly linked with disease incidence and severity. Under this management of continuous monocropping, soil phenolic compounds exuded from roots and decomposing root residues account for increase in ferulic, caffeic, *p*-hydroxybenzoic, *p*-coumaric and vanillic acids that significantly inhibit germination and radicle growth of sesame cultivars. However, phenolic compounds are significantly highest in short duration of continuous monocropping and decrease in the long duration suggesting decrease in autotoxicity from allelochemicals. The high content of phenolic compounds could possibly promote occurrence of diseases under continuous monocropping evidenced by increase in disease incidence and severity. 'Gomazou' and 'Masekin' were tolerant whereas the 'Nishikimaru' and 'Maruhime' are highly susceptible to continuous monocropping obstacle evidenced by decrease in 1000-seed weight and plant height under continuous monocropping. Although 'Gomazou' and 'Masekin' showed high phenolic contents in the rhizosphere soils and high inhibition of germination and radicle growth in bioassay, their growth and yield are high under continuous monocropping in the field, suggesting the allelochemical concentrations in the field were not sufficient to cause autotoxicity or autotoxicity is not directly linked to sesame continuous monocropping. Hence, phenolic compounds could play a significant role in modifying microbial population leading to increased sesame diseases under continuous monocropping although more research is need to verify this hypothesis.

CHAPTER FOUR

Fatty acid composition of sesame (*Sesamum indicum* L.) seeds in relation to yield on four continuously monocropped upland fields converted paddy

4.1. Introduction

Sesame (*Sesamum indicum* L.) is one of the oldest oilseed cultivated throughout the world for its edible oil and use as food (Ashri, 1989). The seeds contain several minerals, lignans, high oil, saturated and unsaturated fatty acids which contribute to nutritional and health benefits when included in diet. The fatty acid composition in sesame seeds consist of abundant polyunsaturated fatty acids: oleic (35.9-42.3%) and linoleic (41.5-47.9%) acids form 80% of total fatty acids and less than 20% are saturated fatty acids mainly palmitic (7.9-12%) and stearic acids (4.8-6.1%) (Hwang, 2005). The high abundance of essential fatty acids such as linoleic that cannot be synthesized by the body makes sesame seeds paramount in the human diet. For instance, the fatty acids in oil prevent cardiovascular diseases, cancer, brain and liver damage, and hypertension (Erol et al., 2011; Kaur et al., 2014).

Despite the importance of sesame seeds, its fatty acid compositions of sesame varies with the type of species (Kamal-Eldin and Appelqvist, 1994). For instance, the fatty acid composition of sesame has been reported to vary with the type of cultivar which is also affected by the amount of the oil content due to differences in genetic background, and those accessions with high oil content also show high linoleic acid (Uzun et al., 2008). In addition, cultivation factors such as type and concentration of fertilizer, climatic conditions or soil type may also influence the chemical composition of crops (Jorgensen et al., 2012). Hence, the fertilizer application and changes in soil nutrient availability under continuous monocropping could play important role in determining fatty acid composition as well as yield.

Soil macronutrients such as N, P, K, Ca and Mg play important role in plant growth and development including controlling seed quality. For instance, nitrogen (N) is generally required for synthesis of fat, which requires both N and carbon skeletons during seed development (Patil et al., 1996). Recent research indicate that adequate soil N increased linoleic acid and linolenic concentrations in sesame seeds (Ali et al., 2016; Bellaloui et al., 2018). In the other macronutrients, adequate soil P and K have been reported to increase oleic and linolenic acids respectively that are important unsaturated fatty acids. Furthermore, saturated fatty acids such as lauric and palmitic acids, and oleic acid were reported to slightly increase in response to adequate soil K since K plays a role in fatty acid and lipid metabolism (Aytac et al., 2017). In addition to N, P and K, Mg is also used a major plant nutrient in oil synthesis in oilseed crops (Hodges, 2010). Therefore, maintaining adequate supplies of these macronutrients in the soil would improve crop productivity and quality.

Several researches indicate yield decreases in continuous monocropping attributed to changes in nutrient status that also affect seed compositions (Crookston et al., 1991; Riedell et al., 2009; Bellaloui et al., 2010). For instance, soybean yield decreased in continuous monocropping attributed to the imbalance in chemical and physical properties and then consequently low seed quality (Crookston et al., 1991; Bellaloui et al., 2010). In addition to low mineral quality, Bellaloui et al. (2010) reported decrease in the oleic acid (C18:1) content whereas linoleic (C18:2) acid increased in continuous monocropping. Furthermore, a higher linoleic acid in continuous monocropping of canola than that in rotation was reported (Harker et al., 2013). As a result, although continuous monocropping may negatively influence a crop yield, the changes in the fatty acid compositions were largely influenced. Yield decline of rapeseed have also been reported in a fifth year of continuous monocropping whereas fatty acid composition was greatly improved through increasing oleic, linoleic and linolenic acids under different input technologies (Stepien et al., 2017). The detrimental effect of continuous monocropping may not reflect decrease in seed quality depending on the management practices adopted.

Although sesame yield decline under continuous monocropping on upland fields converted paddy have been reported (see previous chapters), there is lack of information on the fatty acid compositions of sesame seeds produced under such conditions. Moreover, one of the key factors that determines the quality of sesame seed is the fatty acid composition. Hence, it is important to understand how the fatty acid contents is influenced in continuous monocropping of sesame as seed yield is negatively influenced.

The objective of this study was to evaluate the quality or composition of the fatty acid in sesame seeds produced from four continuously monocropped fields while comparing seed fatty acid contents with sesame yield.

4.2. Materials and methods

4.2.1. Location and site description

A field experiment was conducted on upland field converted from abandoned paddy field during summer season in 2015 and 2016 at Tottori, Japan (35° 29′ 15″ N 134° 07′47″ E). Four different adjacent fields A, B, C and D with sesame cropping history of 0, 1, 2 and 3 continuous monocropping years respectively were selected (**Figure 22**). Briefly, prior to the start of this experiment, fields B, C and D had been under continuous monocropping of sesame; field B had previously been cropped with sesame for one season (1 year), field C for two consecutive seasons (2 years) and fields D for three consecutive seasons (3 years). Field A was a freshly established sesame field added at the start of the experiment in 2015. In this study, sesame was cultivated on these four fields A, B, C and D for two consecutive years 2015 and 2016, therein referred to as continuously monocropped sesame fields. The same agronomic practices of sesame was followed throughout the previous years of sesame cultivation before the start of this experiment. The soil chemical properties of the four continuously monocropped fields before start of experiment in 2015 was analysed and shown in **Table 13**.

The meteorological data of the experimental site in 2015 and 2016 included the mean monthly temperature, monthly rainfall, average seasonal temperature and rainfall as shown in **Table 14**. During the two consecutive years, compared with year 2015, year 2016 showed markedly higher temperature and rainfall.

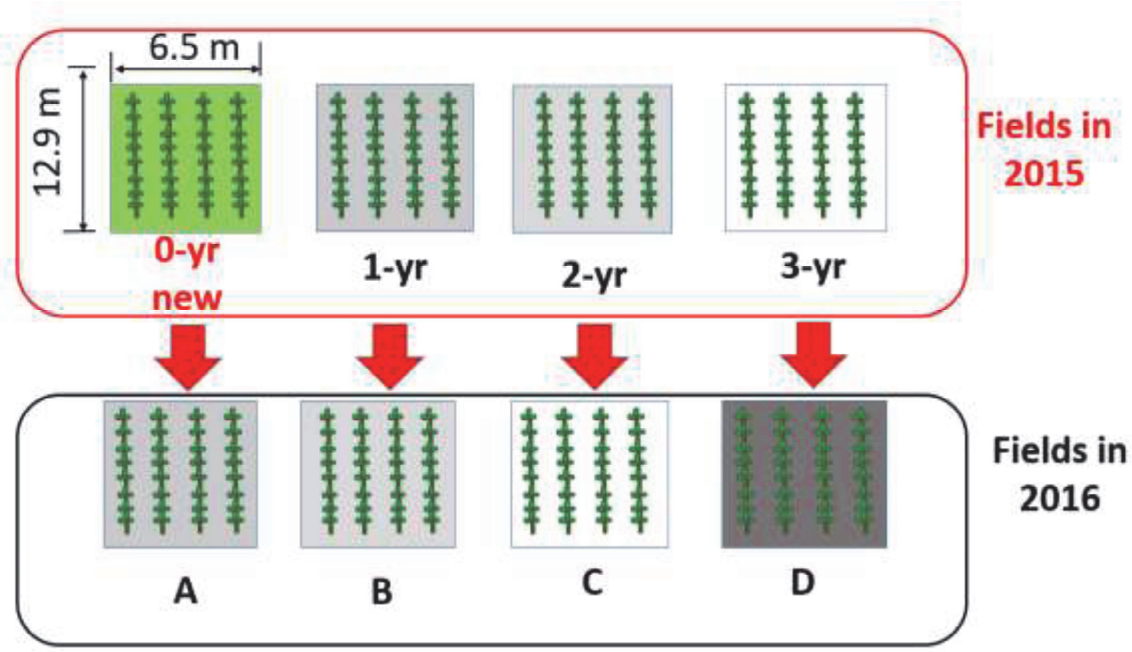


Figure 22. The four continuously monocropped sesame fields in 2015 and 2016.

Table 13. Soil analysis of the four continuously monocropped fields prior to experiment.

Field	pH (H_2O)	EC (dS m^{-1})	TN (g kg^{-1})	C/N ratio	P (mg kg^{-1})	Exchangeable cations K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})
A	5.39	0.05	2.32	9.66	29.0	248	918	108
B	5.44	0.04	2.94	8.11	68.4	112	1220	297
C	5.73	0.04	2.90	7.84	76.2	113	1632	307
D	6.01	0.04	2.86	7.74	46.4	101	1904	353

EC = electrical conductivity; TN = total nitrogen; C/N = total carbon to total nitrogen ratio; P = available phosphorous; K = exchangeable potassium; Ca = exchangeable calcium; Mg = exchangeable magnesium. Data on soil was based on soil samples from depths of 0–15 cm.

Table 14. Mean temperature and rainfall at the experimental site in 2015 and 2016 during the cultivation period from June to September.

Year	Daily/Monthly	June	July	August	September	Average seasonal
2015	Mean daily temperature °C	21	25.0	25.6	21.0	23.2
	Mean monthly rainfall (mm)	132	102.5	123.0	171.5	132.3
2016	Mean daily temperature °C	22	25.7	26.4	22.9	24.3
	Mean monthly rainfall (mm)	135	69.5	126.5	330.0	165.3

4.2.2. Field experimental design

The experimental fields A, B, C and D were ploughed to fine tilth , received the nitrogen-phosphorous-potassium-dolomite basal fertilizer application as 70 kg ha^{-1} N, 105 kg ha^{-1} P_2O_5, 70 kg ha^{-1} K_2O and 1000 kg ha^{-1} dolomite (Total alkali 53%, CaO 39.1% and MgO 10%). The N-P-K fertilizer was supplied in the form of cyclo-diurea (CDU) compound fertilizer (15%-15%-15%) and triple superphosphate for phosphorous (34%). This basal inorganic fertilizer together with dolomite was applied in every sowing season even before start of this experiment. The cultivation was carried out on raised ridges of 75 cm wide separated by 40 cm apart onto which double rows of sesame at spacing of 45 cm x 15 cm were planted (115,942 plants ha^{-1}). Each ridge was covered with black plastic mulch to reduce weeds and maintain soil moisture. Five sesame seeds of cultivar, 'Nishikimaru' were sown per hole and later thinned to one plant after one month (first thinning was done after 2 weeks from sowing). In 2015, sesame was sown on 1 July 2015 and harvested on 28 September 2015 (89 Days after sowing) while in 2016, sowing was done on 7 July 2016 and harvesting on the 27 September 2016 (83 Days after sowing).

4.2.3. Determination of seed yield and 1000-seed weight

At harvest time, 10 randomly selected plants from each plot in three replications were cut and capsules were tied in vinyl bags and allowed to dry in green house after which threshing was done and weight of seeds determined for yield analysis together with weigh of 1000 seeds.

4.2.4. Determination of fatty acids compositions in sesame seeds

Total fatty acids and each composition were determined by using total fat determination unit (Model B-815/B-820 Buchi, Flawil, Switzerland) (Hoffmann et al., 2009). Briefly, 6.0 g of sesame seeds harvested from 10 plants per replicates from each continuous monocropping field was milled in a blender in three replicates (**Figure 23 a-f**). 2.5 g of milled samples was added into the solvent vessel (glass boiling container). Then 45 mL of n-butanol, 7 granules of potassium hydroxide (for saponification) added, 0.26 g of tridecanoic acid C13 were added as internal standard and one spatula of ascorbic acid (about 0.2 g) added to prevent oxidation in the extraction vessel. Extraction and simultaneous saponification of the samples were carried out on extraction unit (Buchi, Flawil, Switzerland) at boiling temperature for 30 minutes.

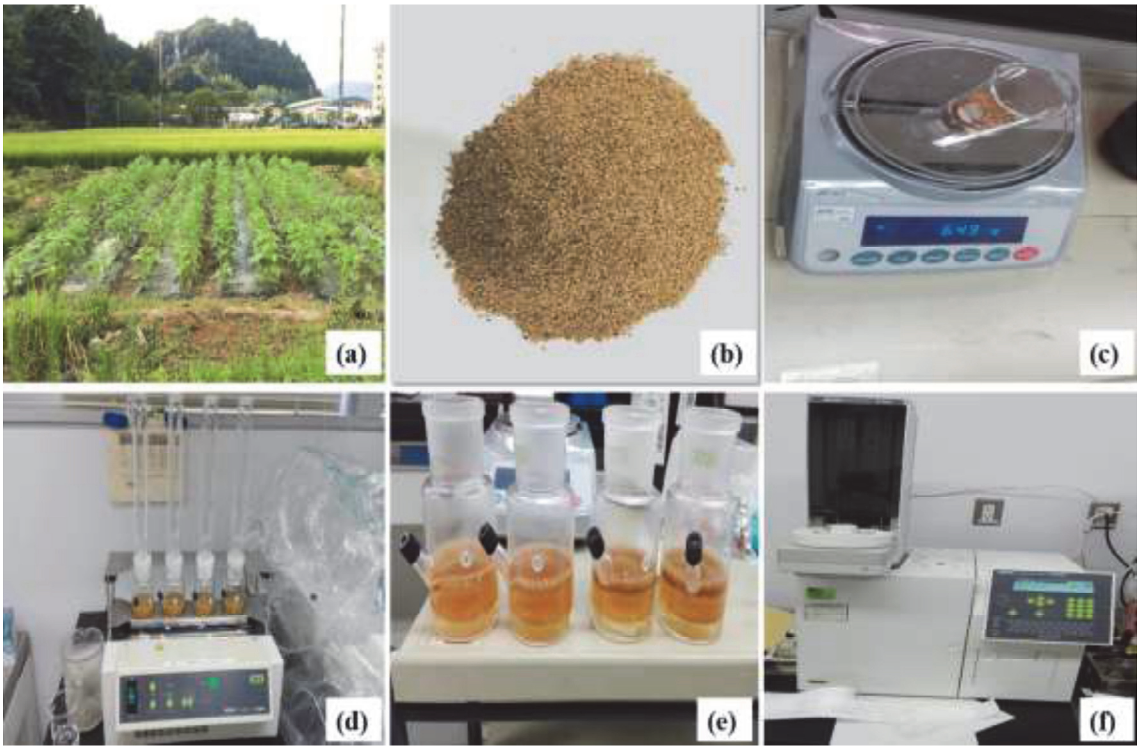


Figure 23. (a) Field cultivation, (b) threshed 'Nishikimaru' seeds, (c) weighing before grinding, (d) saponification of the samples on extraction unit, (e) separated layers and (f) fat determination unit.

Then 40 mL of sodium dihydrogen formic acid mixture was added to convert the potassium salts and fatty acids into free fatty acids and mixture was stirred for 3 minutes. The vessels were later removed from extraction unit and allowed to cool resulting into a two-phase system with organic phase containing fatty acids in the upper phase/layer which was separated. 3 mL of the top layer (upper phase) was transferred using a micropipette into a 3 mL vial. The total fatty acids and compositions were then determined by Fat determination system (Model B-820, Buchi, Flawil, Switzerland) using hydrogen carrier gas at a pressure of 225 kPa and mixture gas pressure of 48 kPa with injection temperature of 220°C and FID detector temperature 260°C. The initial baking temperature was 130°C which was increased at a rate of 6.5°C min^{-1} to the final steady temperature of 260°C, which was held for 4 min before the run was terminated.

4.2.5. Soil sampling and analysis

Immediately after harvesting in each year, soil samples were taken at depth of 0-15 cm, air dried, sieved through 2 mm screen and stored for analysis. Soil samples were analysed for total N using dry combustion by CN-Corder (Macro corder JM 1000CN, J-Science Co., Ltd., Kyoto, Japan) and CN ratio calculated from total N and total C obtained, exchangeable cations K, Ca and Mg by atomic absorption spectrophotometer (Model Z-2300, Hitachi Co., Tokyo, Japan) after extraction of soils using 1 N ammonium acetate. Soil available P was determined using 0.02 N sulphuric acid in mixture of ammonium sulphate as described by Truog (1930). The P concentration was measured by ammonium molybdate-ascorbic acid method on absorption spectrophotometer at 710 nm (Model U-5100, Hitachi Co., Tokyo, Japan).

4.2.7. Data analysis

All experiments were conducted in a completely randomized blocked design replicated three times. Data were analysed using one-way analysis of variance (ANOVA) using SPSS 22.0 (SPSS for windows Inc., Chicago, Illinois, USA) to evaluate the measured parameters affected by the different fields. Multiple comparison was performed using Tukey honestly significant difference at $p < 0.05$. Linear regression analysis was utilized to investigate the relationship between oleic, linolenic, lauric and 1000-seed weight with selected soil mineral nutrients. When considering the differences between the cropping years, a two-way analysis of variance was used with the different years and fields as two fixed factors. In addition, principal component analysis (PCA) was performed to clarify the overall variability with respect to correlations among seed yield, 1000-seed weight, fatty acid contents and soil chemical properties in the different fields which were treated as categorical data, for both 2015 and 2016 cropping.

4.3. Results

4.3.1. Seed yield and 1000-seed weight on the different continuously monocropped fields in 2015 and 2016

Analysis of variance showed that the fields and years had no significant effects on the seed yield, and without significant interactions between fields and years (**Table 15**).

In 2015, the seed yield was in the range of 912–1091 kg ha^{-1} with the lowest seed yield in field B although no significant differences among the four continuously monocropped fields was observed. The 1000-seed weight was in the range of 2.07–2.25 g; however, a significant difference ($p < 0.05$) was found only between fields A and C indicating a decrease in the 1000-seed weight under continuous monocropping.

In 2016, the seed yield and 1000-seed weight showed no significant differences between the four continuously monocropped fields. However, both the seed yield and 1000-seed weight tended to decrease in field C by 25.5% and 5.12% respectively compared with field A. The seed yield was in the range of 953–1279 kg ha^{-1} with the lowest seed yield in field B whereas the 1000-seed weight was in the range of 2.04–2.15 g with the lowest in field C.

Averaged over 2015 and 2016, field C produced the lowest seed yield and 1000-seed weight, with mean values of 1001 kg ha^{-1} and 2.06 g, respectively, although no significant differences was observed between the different years and without significant interactions between fields and years. Overall, field A produced the highest mean seed yield of 1172 kg ha^{-1} accompanied by the highest 1000-seed weight of 2.20 g respectively. In addition, the 1000-seed weight showed significant differences ($p < 0.05$) between fields A and C indicting the decrease of seed weight under long duration of continuous monocropping on field C compared with A, of a short duration of continuous monocropping.

Table 15. Seed yield and 1000-seed weight of sesame seeds from the four continuously monocropped fields A, B, C and D during 2015 and 2016.

Year	Field	Seed yield (kg ha^{-1})	1000-seed weight (g)
2015	A	1066a	2.25a
	B	912a	2.14ab
	C	1050a	2.07b
	D	1091a	2.13ab
ANOVA (*p*-values)		ns	*
2016	A	1279a	2.15a
	B	1279a	2.10a
	C	953a	2.04a
	D	1159a	2.15a
ANOVA (*p*-values)		ns	ns
Source of variation			
Year		ns	ns
Field		ns	*
Year x Field		ns	ns

Different letters within each column are significantly different at $p < 0.05$ (Tukey's test).

* Significant at $p < 0.05$; ns, Non-significant.

4.3.2. Seed fatty acid composition on the different continuously monocropped fields in 2015 and 2016

The total fatty acid (TFA), saturated fatty acids (SFA), mono-unsaturated fatty acids (MUFA), poly-unsaturated fatty acids (PUFA), and individual fatty acids capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids analysed in the sesame seeds from the continuously monocropped fields are shown in **Table 16**. Analysis of variance showed that the year had significant effects on the contents of MUFA, lauric, myristic, palmitic, oleic, and linoleic acids of sesame seeds. The contents of MUFA, oleic, and linoleic acids were significantly higher in 2016 than 2015 whereas the lauric and myristic acids significantly decreased in 2016 compared to 2015. On the other hand, the effect of field was significant on the contents of lauric, myristic, linoleic and linolenic acids. However, there was no year and field interaction on the fatty acid composition of the seeds.

Results also indicated that in the year 2015, SFA significantly decreased from 11.4% in field B to 8.08% in field D indicating a negative influence of continuous monocropping on the saturated fatty acid contents of the sesame seeds. The content of lauric acid was significantly highest in field A (2.15%), with significant difference between fields A and C (1.42%) including field D (1.27%). Results also showed that the content of linolenic acid was significantly increased from 1.13% in field A to 2.26% in field D indicating continuous monocropping increased the content of linolenic acid. In 2016, PUFA showed significant ($p < 0.05$) differences among the fields; the highest PUFA content was in field C (21.3%) that was significantly different from field B (19.2%). In the same year (2016), linoleic also showed significant difference among the fields with highest linoleic acid content detected in field D (39.5%). Furthermore, the linolenic acid content significantly increased from 1.63% in field A to 2.11% in field D without significant differences among the fields A, B and C. Overall, the results showed that continuous monocropping for more than 2 years (fields C and D) tended to increase contents of linoleic, linolenic acids whereas decreasing contents of lauric and myrstic acids.

Averaged over 2015 and 2016, field A produced the highest contents of lauric and myristic acids in sesame seeds compared with field D with mean values of 1.76% and 9.15% respectively. On the other hand, field D produced the highest contents of linoleic and linolenic acids in the sesame seeds compared with fields A and B with mean values of 38.6% and 2.19% respectively.

Table 16. Seed fatty acid composition of the sesame seeds from the four continuously monocropped fields A, B, C and D during 2015 and 2016.

Year	Field	TFA (%)	SFA	MUFA	PUFA	Capric (C10:0)	Lauric (C12:0)	Myristic (C14:0)	Palmitic (C16:0)	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)
			% of Total fatty acid (TFA)									
2015	A	51.4a	10.7ab	16.2a	17.5a	0.29a	2.15a	10.2a	6.84a	30.8a	32.0a	1.13b
	B	54.7a	11.4a	17.6a	19.0a	0.34a	1.82ab	10.3a	7.50a	31.9a	32.8a	1.62ab
	C	52.7a	9.2ab	17.6a	20.6a	0.43a	1.42b	7.68a	6.94a	33.0a	37.0a	2.19a
	D	50.5a	8.08b	17.8a	20.2a	0.45a	1.27b	6.80a	7.24a	34.7a	37.8a	2.26a
ANOVA (*p*-values)		ns	*	ns	ns	ns	*	ns	ns	ns	ns	**
2016	A	53.1a	9.19a	19.5a	19.9ab	0.44a	1.37a	8.15a	6.55a	36.5a	36.0b	1.63b
	B	51.1a	8.82a	18.8a	19.2b	0.41a	1.36a	8.07a	6.58a	36.4a	36.1b	1.70b
	C	51.5a	8.79a	18.9a	19.8ab	0.42a	1.27a	7.36a	6.86a	36.2a	36.4b	2.00b
	D	51.4a	13.2a	19.8a	21.3a	0.42a	0.83a	5.01a	7.10a	37.8a	39.5a	2.11a
ANOVA (*p*-values)		ns	ns	ns	*	ns	ns	ns	ns	ns	**	**
Source of variation												
Year		ns	ns	***	ns	ns	***	*	*	***	*	ns
Field		ns	ns	ns	ns	ns	**	**	ns	ns	**	***
Year x Field		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

TFA = total fatty acid; SFA = saturated fatty acids; MUFA = mono-unsaturated fatty acids; PUFA = poly-unsaturated fatty acids. Different letters within each column are significantly different at $p < 0.05$ (Tukey's test). *** Significant at $p < 0.001$; ** Significant at $p < 0.01$; * Significant at $p < 0.05$; ns, Non-significant.

4.3.3. Soil chemical properties on the different continuously monocropped fields in 2015 and 2016

Analysis of variance showed that the year and field had significant effects on all the measured soil chemical properties, except exchangeable Mg which was not influenced by the field effect (**Table 17**). However, exchangeable Mg, including EC, TN and C/N ratio showed significant interactions between years and fields.

In 2015, soil pH, C/N ratio and exchangeable Ca significantly increased in field D compared to fields A and B. The soil pH increased by 0.88 units, whereas the C/N increased by 1.37 and exchangeable Ca by 519.8 mg kg^{-1} in field D compared with field A. Conversely, the soil EC , TN available P and exchangeable K decreased by 0.08 units, 0.45 g kg^{-1}, 22.9 mg kg^{-1} and 181.5 mg kg^{-1} respectively in field D compared with field A. However, the soil EC, P and K did not show significant difference among the fields A, B and C, whereas significant differences in TN was observed between field B and C. In 2016, soil pH, C/N, exchangeable Ca and Mg significantly increased in field D by 0.64 units, 0.48, 925.8 mg kg^{-1} and 101.6 mg kg^{-1} respectively compared with field A. There was no significant differences in pH, C/N and Mg between fields B, C and D, and between B and C for exchangeable Ca. Conversely soil EC decreased by 0.03 units in field B compared with A; no significant differences in the soil EC between B, C and D, and between A and D. The available P and exchangeable K significantly decreased in field D by 36.1 and 228.9 mg kg^{-1} when compared with field A.

Results also showed that soil EC significantly decreased in 2016 in fields A, B and C compared to 2015 whereas TN, P, K values were lower in 2016 than in 2015 in all fields indicating a significant decrease in the second cropping year. On the other hand, the exchangeable Ca and Mg were significantly increased in field D in 2016 compared with 2015.

Table 17. Soil chemical properties from the four continuously monocropped fields A, B, C and D after harvesting during 2015 and 2016.

							Exchangeable cations		
Year	Field	pH (H_2O)	EC (dS m^{-1})	TN g kg^{-1}	C/N ratio	P (mg kg^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})
2015	A	5.26c	0.15a	2.56a	10.5b	58.4a	389.9a	1191.8b	307.3a
	B	5.39bc	0.10b	2.46b	10.3b	43.5b	177.9b	1182.6b	283.2a
	C	5.66b	0.11ab	2.62a	9.59b	47.6ab	226.4ab	1492.4a	288.0a
	D	6.14a	0.07b	2.11c	11.9a	35.5b	208.4b	1711.7a	255.2a
ANOVA (*p*-values)		***	**	***	***	**	*	***	ns
2016	A	5.46b	0.10a	2.12a	9.81b	53.3a	325.9a	1174.3c	298.9b
	B	5.64ab	0.07b	1.98a	10.3a	24.2b	82.9b	1518.2bc	321.7ab
	C	5.80ab	0.06b	2.13a	10.1ab	29.7b	110.9b	1726.7ab	339.2ab
	D	6.10a	0.07ab	2.00a	10.3a	17.2b	97.0b	2100.1a	400.4a
ANOVA (*p*-values)		*	**	ns	**	***	***	***	*
Source of variation									
Year		*	**	***	**	***	**	***	***
Field		***	**	***	**	***	***	***	ns
Year x Field		ns	*	***	***	ns	ns	ns	**

EC = electrical conductivity; TN = total nitrogen; C/N = total carbon to total nitrogen ratio; P = available phosphorous; K = exchangeable potassium; Ca = exchangeable calcium; Mg = exchangeable magnesium. Data on soil was based on soil samples from depths of 0–15 cm. Different lower case letters within each column are significantly different among fields in each year at $p < 0.05$ (Tukey’s test). *** Significant at $p < 0.001$; ** Significant at $p < 0.01$; * Significant at $p < 0.05$; ns, Non-significant.

4.3.4. Relationship between yield, fatty acid and soil chemical parameters among the continuously monocropped fields

Correlation analysis conducted between selected parameters are shown in **Figure 24**. Correlation analysis indicated a significant positive linear regression (r = 0.458, $p < 0.05$) between the soil exchangeable K and 1000-seed weight (**Figure 24a)**. In addition, there was significant positive correlation between the lauric acid content and soil exchangeable K with a linear regression (r = 0.540, $p < 0.01$) (**Figure 24b)**. Furthermore, a significant positive correlation (r = 0.456, $p < 0.05$) between the oleic content and soil exchangeable Mg in the average data of two years among the different continuously monocropped fields was observed (**Figure 24c)**. On the other hand, a negative correlation (r = -0.404, $p = 0.05$) as shown in **Figure 24d** between linolenic acid and 1000-seed weight was observed.

In addition to the correlation analysis, principal component analysis (PCA) was performed to visualize the relationship between variables and continuous monocropping fields. A summary of the total variation of seed yield, 1000-seed weight, fatty acid composition, and soil chemical properties analysed is presented by the first two factors of the PCA data which explains 53.9% of the total variance (**Figure 25a**). The first principal component (PC 1) attributes to 43.1% of the total variance and shows a high positive correlation mainly with oleic, linoleic, PUFA, linolenic, soil exchangeable Mg content, and a high negative correlation with lauric, myristic, and soil exchangeable K contents. The second factor (PC 2) obtained by the PCA analysis accounts for 10.8% of the total variance among the continuous monocropping fields and is positively correlated with the TFA, SFA, yield whereas negatively correlated with the C/N ratio and palmitic acid content. The score plot of the PCA (**Figure 25b**) shows relatively a good separation of two clusters: group (I) from fields A and B whereas group (II) from fields C and D. Group (I) showed high values for contents of lauric acid, myristic acid, soil exchangeable K and 1000-seed weight (TSW) whereas group (II) showed high values for contents of oleic acid, linoleic acid, linolenic acid, MUFA, PUFA, and soil exchangeable Mg in both years 2015 and 2016.

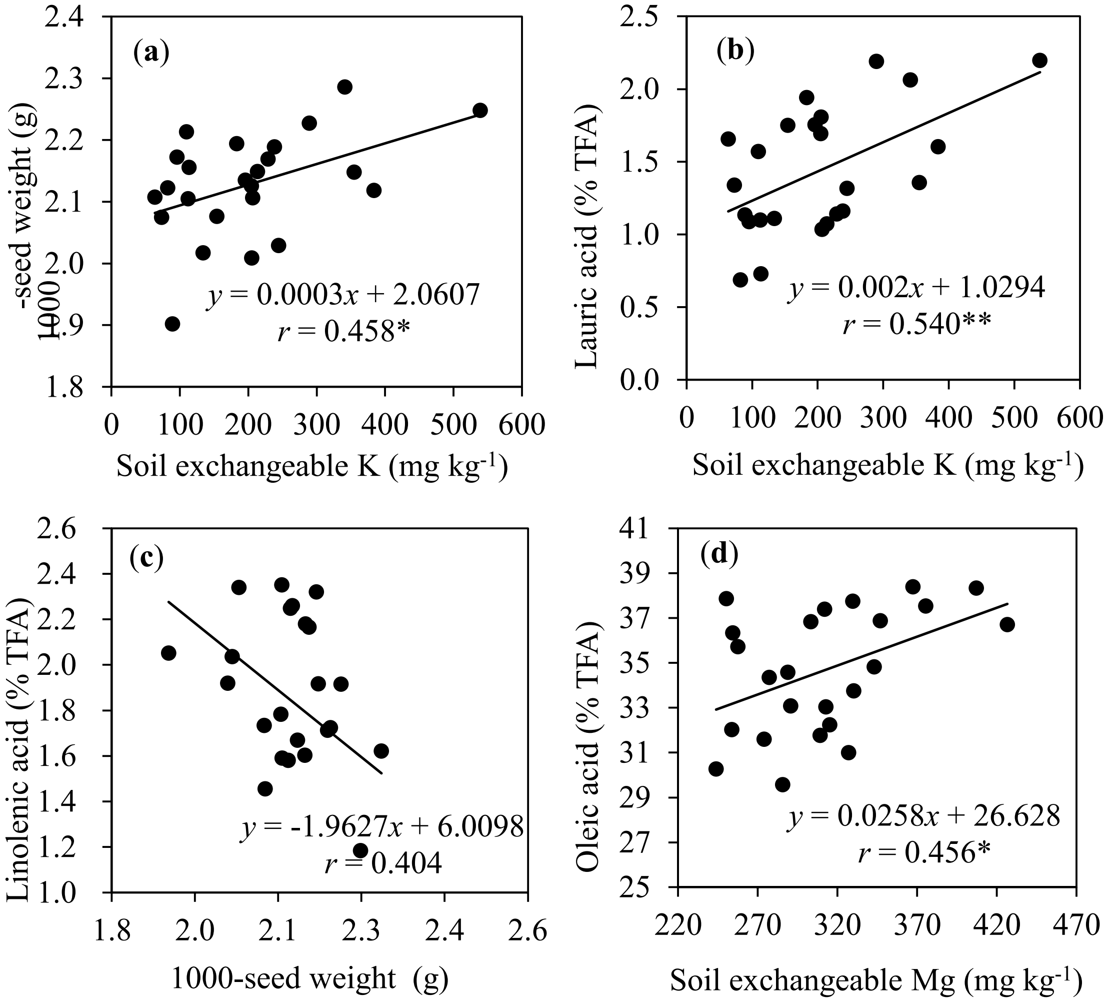


Figure 24. (a) Relationship and model of the 1000-seed weight and soil exchangeable K, (b) lauric acid content and soil exchangeable K, (c) linolenic acid content and 1000-seed weight and (d) oleic acid content and soil exchangeable Mg.

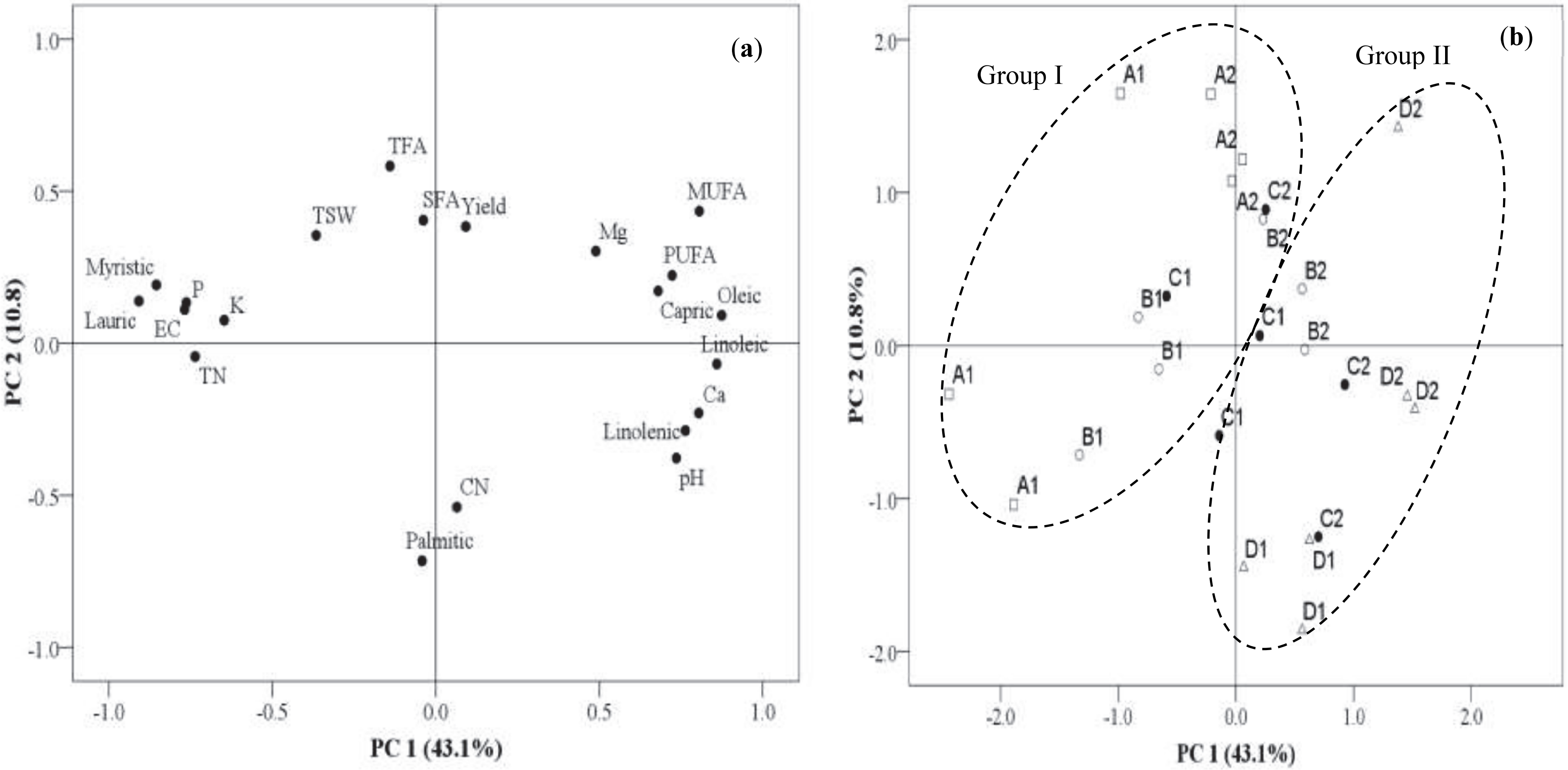


Figure 25. The overall projection of the variables (a) and of the continuous monocropping years on the factor plane (b) obtained by the principal component analysis of fatty acid composition in the sesame seeds, yield, 1000-seed weight and the soil chemical properties from the continuous monocropping fields A, B, C and D. TSW (thousand seed weight) = 1000-seed weight; 1 = year 2015; 2 = year 2016.

4.4. Discussion

The effects of year and continuously monocropped fields were more evident on 1000-sed weight than on the seed yield (**Table 15**). The1000-seed weight gradually decreased from fields A to C suggesting the negative effects of continuous monocropping on sesame. A similar significant decline in seed weights was found in soybean grown in continuous monocropping which accompanied the yield decline of soybean (Kelly et al., 2003). The decreases in the yield of sesame on the different continuous monocropping field could be due to the differences in the soil nutrient status resulting from depletion of nutrients by continuous monocropping. This study showed markedly lower TN and exchangeable K contents in all the continuously monocropped fields in 2016 when compared with those in 2015 (**Table 17**). The decrease in the soil total nitrogen (TN) in continuous monocropping is consistent with early findings that show low total N indicating the loss of soil quality (Reeves, 1997; Wyngaard et al., 2012; Zhong et al., 2014).

On the other hand, soil exchangeable K content also contributed to low yield in fields D when compared with that of field A. However, this study showed that the increase in the exchangeable Ca content from dolomite increased soil pH. This phenomenon could be responsible for the low exchangeable K content. While raising the soil pH of the acidic upland field converted paddy, the increasing levels of exchangeable Ca could upset the soil cation ratios. For instance, the soil Ca/K ratio significantly increased from 3.31 in field A, to 10.4 in field B, to 9.54 in C and 12.5 in field D. This indicated low exchangeable K content in the soils since increase in one cations results into deficiency of one (Hodges, 2010). Furthermore, K deficiency in sesame occurs in soils with high Ca and Mg which have imbalance of Mg/K and Ca/K ratios which often results in yield decline (Kumar and Sharma, 2013). Any deficiency of cation in soils could lead to lower yields. The likely cause of the decline in exchangeable K could be attributed to annual removal of crop because sesame requires high amount of potassium for growth and the seed usually accumulates high amount of K as part of mineral nutrient (Mitchell et al., 1974). Removal of K by crops is usually high resulting into decline in available K which is high in soils with low reserves of K under continuous monocropping (Panda and Patra, 2017). The exchangeable K content is a key factor as one of the most important soil nutrients that could influence sesame seed weight. This is evidenced by the significant positive correlation between soil mineral exchangeable K and 1000-seed weight in the average data of two years between the different continuously monocropped fields.

This study also showed that the yield decline in terms of 1000-seed weight was accompanied by changes in total and individual fatty acid contents, suggesting continuous monocropping could later sesame seed quality (**Table 16**). The contents of lauric and myristic acids decreased on fields with longer duration of continuous monocropping. On the hand, the increase in the oleic, linoleic and linolenic acids were contrarily to as expected in continuous monocropping. The decreases in the SFA including lauric and myristic acids on the different continuous monocropping field could be due to differences in the soil mineral nutrient status resulting from depletion of nutrients by continuous monocropping. Apart from influencing yield, the exchangeable K could have gradually decreased the contents of lauric and myristic acids in sesame seeds as its concentrations in the soil decreased due to continuous monocropping. The decrease in the soil exchangeable K levels could have decreased lauric and myristic acids because these acids mainly exist in form of potassium laurate and potassium myristate which are referred to as potassium fatty soaps with antibacterial and antifungal activities (McBain and Sierichs, 1948; Tanaka et al., 2016; Aid, 2018). For instance, in 2016, lauric acid significantly decreased to 1.37% from 2.15% in 2015 for field A. The decrease in soil exchangeable K also accounted for low saturated fatty acids content in sesame especially in the fields C and D. The decrease in the soil exchangeable K is not only detrimental to yield but also the saturated fatty acids contents of sesame. Since potassium is involved in physiological activities in plant tissues such as increasing disease resistance, protein synthesis and crop quality. It has been reported that increasing potassium (K) fertilization increased saturated fatty acids such as palmitic, stearic and myristic acids in black cumin (Aytac et al., 2017). K in sesame is also associated with the role of carbohydrate, protein and oil synthesis of plants (Jadav et al., 2010). Moreover there was significant positive correlation between the lauric acid content and soil exchangeable K with a positive linear regression showing a good prediction in the lauric acid as soil exchangeable K decreased in the average data of two years among the different continuously monocropped fields (**Table 16**, **Figure 24b**). Furthermore, the high 1000-seed weights corresponds with high lauric acid content in the sesame seeds for shorter duration of continuous monocropping as observed in fields A and B. It has been reported that high 1000-seed weight has a high positive associated with lauric acids in *Cuphea lutea* seeds (Thompson et al., 1990). With increase in continuous monocropping, the 1000-seed weight decreased (**Figure 24a**). This implies that seed yield also decreased leading to fewer seeds harvested but with high fatty acids content such as oleic, linoleic and linolenic acids. To produce high amount of fatty acids, area per hectare would be needed to keep high seed yield. This implies that seed yield should be increased under continuous monocropping.

While on the other hand, the increasing contents of MUFA, PUFA, oleic, linoleic and linolenic acids could be as a result of increase in soil exchangeable Mg in especially in fields C and D which had longer duration of continuous monocropping (**Table 16**). Bellaloui et al., (2010) also reported similar increase in linoleic acid content in continuous monocropping of soybean. The increase in oleic and linolenic including linoleic acids showed that the fatty acid quality of sesame seeds produced on these continuously monocropped fields is high, especially in fields C and D although the yields were low. The results of fatty oleic, linoleic and linolenic acids were on average in the range of 33.7–36.3%, 34.0–38.6% and 1.38–2.19% respectively. The oleic acid and linoleic acid contents were the two most dominant fatty acids in the sesame seeds that is consistent with the contents reported by other researchers (Were et al., 2006; Biglar, 2012; Kurt, 2018). The soil analysis indicated significant increase in the soil exchangeable Mg as result of annual fertilizer application including dolomite that elevated the levels of soil Mg in the continuously monocropped fields. It could be supposed that adequate magnesium supply occurred in the continuously monocropped fields C and D due to the build-up of residual Mg in the soils; which means that sufficient magnesium remained which was readily available for sesame plants. Although not in sesame, but in olive plants, the fatty acids composition especially oleic acid in the oil significantly increased due to foliar application of magnesium and potassium sulphate (Thanaa et al., 2017). Similarly, the application of magnesium sulphate containing 16% MgO and 13% S has also been reported to increase sesame oil content (Mondal, 2016). However, large quantities of soil Mg could result into significant yield reduction not due to Mg toxicity but imbalances in soil cations Ca, K and Mg inducing deficiencies of K (Gerendás and Führs, 2013).

Overall, magnesium is considered as one of the nutrients very important in oil crops when applied in some forms of fertilizers since it is required in oil and fat synthesis. Magnesium (Mg^{2+}) ion regulates fatty acid synthesis in higher plants and adequate Mg^{2+} concentrations are need for maximum rate of fatty acid synthesis in plant tissues (Gupta and Singh, 1996; Singh, 1998). The functions of magnesium are observed in photosynthesis especially through controlling activities of key enzymes involved in photosynthetic carbon metabolism and Mg^{2+} is a structural component of the chlorophyll as well as playing a role in phloem loading of assimilates (Cakmak and Kirkby, 2008). Hence, the increase in the oleic, linoleic and linolenic acids in fields D could be attributed to increasing soil exchangeable Mg. Interestingly, as the linolenic acid contents increased, the 1000-seed weight decreased in the fields C and D which had longer duration of continuous monocropping. Moreover there was significant positive correlation between the oleic content and soil exchangeable Mg in the average data of two years between the different continuously monocropped fields (**Figure 24d**). A negative correlation between linolenic acid and 1000-seed weight was observed indicating inverse relationship between the quantity of seeds and quality of sesame produced in this system of continuous monocropping (**Figure 24c**). Whereas continuous monocropping is not suitable for many crops, the technique adopted for the continuous monocropping may improve the quality of seeds. A similar result was observed in canola seeds, in which the mean linoleic and linolenic acids were increased in in continuous monocropping (Harker et al., 2013).

Unlike continuous monocropping being detrimental to yield, the improvement in fatty acid quality could depended on the management practice. For instance, increasing N inputs including pest control under a high input technology cultivation system significantly improved the oleic, linoleic and linolenic acid contents of rapeseeds under continuous monocropping. This is because of the increased availability of nitrogen N from the soil that increase the fatty acid composition. Fertilizer management influence fatty acid composition since it directly affect soil nutrients (Ali and Ullah, 2012; Shoja et al., 2018; Bellaloui et al., 2018). In this study, soil total N was non-significantly highest in the short duration continuously monocropped fields A and B but had high saturated fatty acid contents suggesting adequate N partly increased synthesis of saturated fatty acids. Zheljazkov et al. (2009) reported N could influence the composition of total saturated fatty acids through increasing individual saturated fatty acids. Several studies showed that oilseed crops respond well to NPK fertilizers in terms of yield and quality (Suzer, 2015; Bahrani and Pourreza, 2016; Kaptan et al., 2017). Therefore, inadequate soil NPK amounts as observed in the fields C and D compared to A could not only limit yield (1000-seed weight) but also oil synthesis and consequently affecting the saturated fatty acid quality (Kaptan et al., 2017).

Furthermore, the increase in unsaturated fatty acids in sesame seeds from long duration of continuous monocropping could be explained by the decrease in soil total N (nutrient depletion) suggesting low uptake of N. N is involved in protein synthesis but occasionally at a high uptake of N promotes protein synthesis (crude protein) while carbohydrates synthesis (oil content) is decreased negatively affecting the content of oil and fats (Rathke et al., 2005). In addition, there was high crude protein with more N uptake at increasing levels of N while reduced oil content of sesame was reported suggesting protein synthesis and storage is favoured over oil synthesis (Mitchell et al., 1974). Therefore, it could be speculated that as the total N decreased in the fields C and D indicating their poor uptake, such phenomenon of high protein synthesis did not occur but rather more carbohydrates was synthesized accompanied by Mg role in fat synthesis. Fatty acid synthesis pathway usually occurs in the chloroplasts of green tissues especially the leaves of plants during photosynthesis from the product acetyl CoA (Podkowinski et al., 2003; Aid, 2019). During photosynthesis, phosphoenolpyruvate is the major substrate involved in the lipid biosynthesis pathway; however, both the protein and lipid synthesis utilize the same substrate (phosphoenolpyruvate) thereby the increase in total oil content or fat content could imply a significant decrease in the total crude protein content of seeds due to the competition for the carbon source (Xu et al., 2016), which could be confirmed by the previous study indicating the seed crude protein content of sesame decreased under continuous monocropping of sesame on upland field converted paddy (See Chapter Two). Therefore, under this system of continuous cropping, decreasing in total N and protein content could have beneficial effect on quality of fatty acids in the presence of adequate Mg.

This study also showed that the fatty acid contents of 2016 were higher than in the 2015 samples. The fatty acid compositions was seemingly affected by the seasonal temperature and rainfall for each year. In year 2016 season, the average temperature and rainfall were 24.25°C and 165.25 mm whereas the average temperature and rainfall in the 2015 season were 23.15°C and 132.25 mm indicating increase in both temperature and rainfall in 2016 (**Table 14**). This showed that there was high night and day temperature during 2016 growing period. It has been reported that high temperatures increased seed palmitic acid and oleic (Izquierdo et al., 2009; Ngure et al., 2015). This could partly occur because the synthesis of fatty acid is regulated by several enzymes such as oleate desaturase whose activity is dependent on temperature (Garcés et al., 1992; Kabbaj et al., 1996). In addition, the variation between 2015 and 2016 all fields could have been due to differences in the water availability that affected the seed fatty acid composition. The growing season of 2016 had nearly twice as much rainfall in September besides more rainfall in August than in 2015 during the development of sesame capsules and this could have contributed to the increased fatty acids compositions. This is in line with other researchers who reported that increased water availability during the development of sesame capsules increase fatty acid compositions especially oleic and linoleic content (Alpaslan et al., 2001).

This study demonstrated that as the yield decreases in longer continuous monocropping years (as shown in field C), the fatty acid quality is improved as indicated by the principal component analysis (PCA). The PCA showed co-relationships between among contents of oleic acid, linoleic, PUFA, MUFA, and linolenic acids, and soil exchangeable Mg which confirmed that continuous monocropping significantly increased these fatty acids due to the increase in the soil Mg in the fields B and C (**Figure 25**). There was also co-relationship among myristic acid, lauric acid with soil exchangeable K, concentration and 1000-seed weight that further confirmed that the contents of lauric and myristic acids were high in the fields A and B of the short duration of continuous monocropping due to the high potassium content in the soil. The high 1000-seed weight observed in the fields A and B is also confirmed by the PCA which showed the seed weight correlated with the potassium content in the soil. With continuous monocropping of sesame, the declining content of potassium has a negative effect on seed yield, lauric acid and myristic acids while the increase in the soil magnesium content increases the mono-uncaptured fatty acids, poly-unsaturated fatty acids, oleic, linoleic and linolenic.

This suggests that Mg content in the soils was the most important factor that influenced oleic, linoleic and linolenic acid content and unsaturated fatty acid whereas the K content influenced the myristic, lauric acid content and the overall saturated fatty acid content. Although there was tendency of yield to decrease, the overall fatty acid quality was enhanced indicating management practices could influence the quality of sesame seeds produced under continuous monocropping systems. Thus, the management practice adopted in this continuous monocropping led to increased soil exchangeable Mg and decreased K content leading to significant increase in the oleic, linoleic and linolenic acids but decreased contents of lauric and myristic acids. Linoleic (omega-6) and alpha-linolenic (omega-3) acids are essential polyunsaturated fatty acids which cannot be synthesized by the human body and therefore required in the diet (Jorgensen et al., 2012). Hence, the sesame seeds produced under this continuous monocropping especially on fields C and D could be highly suitable for human consumption to improve health because the unsaturated fatty acids in sesame seeds which include linoleic and oleic acids are mainly responsible for the oil quality and also increase the suitability of sesame oil for human consumption (Uzun et al., 2008). This study suggests that under continuous monocropping, it is likely that both linoleic and oleic acid will increase when sufficient magnesium is supplied in the continuous monocropping system leading to this significant positive correlations between the two fatty acids.

4.5. Conclusion

In this study, the continuously monocropped fields differed in the yield and fatty acid compositions based on differences in soil mineral nutrient status. This suggested continuous monocropping decrease yield of sesame in the long duration of continuous monocropping as observed in in fields C and D accompanied with high oleic, linoleic and linolenic acids whereas, the individual fatty acids of lauric and myristic acids were high in the short duration of continuous monocropping. This study also found significant positive correlations between the soil exchangeable K content with 1000-seed weigh and lauric acid, also between oleic acid, linoleic, linolenic acids with soil exchangeable Mg whereas a negative correlation between linolenic acid and 1000-seed weight the continuously monocropped fields. This suggests that Mg content in the soils was the most important factor that influenced oleic, linoleic and linolenic acid content and unsaturated fatty acid whereas the K content influenced the myristic acid, lauric acid contents including overall saturated fatty acid content under continuous monocropping. The overall fatty acid composition quality produced on these fields especially of long duration of continuous monocropping could be of high economic value but at the expense of yield. Therefore, whereas continuous monocropping could adversely affect yield in terms of low seed weight, it improved the overall fatty acid composition of sesame seeds especially oleic, linoleic and linolenic acids (unsaturated fatty acids). However, it is still necessary to increase the yield of sesame in continuous monocropping while maintaining the high quality fatty acid composition on the upland field converted paddy.

CHAPTER FIVE

Imbalanced soil chemical properties and mineral nutrition in relation to growth and yield decline of sesame on different continuously monocropped upland fields converted paddy

5.1. Introduction

Sesame (*Sesamum indicum* L.) is an important oilseed crop and widely cultivated in the world for its edible oil and uses in food (Ashri, 1989). However, the production of sesame on upland field converted paddy fields has resulted into yield decline. The mechanisms of growth and yield decline may be associated with changes in nutrient availability, deterioration of soil physicochemical and biological characteristics, phytotoxins accumulation, and soil-borne pathogens (Ventura and Watanabe, 1978; Gentry et al., 2013). This study primarily focused on understanding the changes in soil chemical properties and crop nutrition after long-term continuous monocropping of sesame.

Several studies indicate that continuous monocropping negatively influences soil chemical properties, nutrient absorption and yield of several crops (Wang et al., 2014; Zhong et al., 2014; Ye et al., 2014; Zhou and Wu, 2015). For instance, continuous monocropping leads to decrease in available K due to removal of K by crops (Panda and Patra, 2017). Panda and Patra reported lower dry matter yield accompanied by decrease in K absorption in the second cropping than in the first cropping cycle attributed to decrease in soluble and exchangeable K contents. In maize case, total organic C, total N, and available P were significantly decreased by seven years of continuous monocropping (Wyngaard et al., 2012). Furthermore, soils under continuous monocropping of garlic and onions for 1, 6–10, 11–15 and 16–20 years showed decrease in pH as well as exchangeable Ca and Mg with increase in the duration of continuous monocropping (Kim et al., 2003). Kim et al. reported a decrease in exchangeable cation capacity (CEC) due to decrease in the exchangeable cations and an increase in soil available P caused the imbalance of nutrition, leading to yield and quality decline.

In addition to altering soil nutrient status, the effect of continuous monocropping may influence soil enzyme activities (Wang et al., 2014). Soil enzyme activity is directly or indirectly affected by agricultural practices such as tillage and fertilization, including continuous monocropping (Bowles et al., 2014; Gong et al., 2016). Soil enzymes are important components of soil as they are related to changes in nutrient status. For instance, soil urease is involved in nutrient cycling, releasing available ammonium nitrogen (NH_4^+-N) (Sherene et al., 2017). Soil dehydrogenase plays a significant role in the biological oxidation of soil organic matter by transferring the hydrogen from organic substrates to inorganic acceptors, whereas catalase enzyme is related to soil carbon content and might be a good indicator of soil quality (Salazar et al., 2011; Wang et al., 2013; Sherene et al., 2017). However, little is known on the effect of continuous sesame cropping on soil enzymes that could predict changes in the soil quality.

Although continuous monocropping negatively affects sesame yield, most studies focus on changes in soil microbial diversity leading to soil borne diseases (Nam et al., 2007; Hua et al., 2012). In addition, studies show that upland fields converted from rice paddy for cultivation of crops that require no constant flooding are often associated with changes in biological, chemical and physical properties (Linh et al., 2015; Zhou et al., 2014). Previous studies report that continuous monocropping on upland fields converted paddy causes yield decline in other crops, which is partly attributed to changes in nutrient availability (Matsumoto et al., 2010; Hattori et al., 2013; Nishida et al., 2016). There is scarce information on changes in the soil chemical properties and mineral nutrition under continuous sesame cropping on upland fields converted paddy.

The purpose of this study was to evaluate the effect of continuous sesame cropping on mineral nutrition and changes in the soil chemical properties while comparing leaf tissue nutrient concentrations, soil nutrient contents including pH, EC and soil enzyme activities related to sesame growth and yield on fields of different durations of continuous monocropping on upland field converted paddy.

5.2. Materials and Methods

5.2.1. Location and site description

The experiment was conducted on fields converted from paddy in Tottori, Japan (35° 29′ N, 134° 07′ E) in 2018. The soil chemical properties of the upland field converted from paddy before sesame cultivation was characterized by pH of 5.4 (1:5, soil/water), electrical conductivity (EC) of 0.05 dS m^{-1} (1:5, soil/water), total N (TN) of

2.14 g kg^{-1}, total C (TC) of 22.37 g kg^{-1}, C/N ratio of 10.45, available P of 29 mg kg^{-1}, exchangeable K of 248 mg kg^{-1}, exchangeable of Mg 107 mg kg^{-1}, exchangeable Ca of 918 mg kg^{-1}, and cation exchange capacity (CEC) of 10.4 $cmol_c$ kg^{-1}.

Sesame was cultivated during the warm summer from June to September 2018. The total monthly rainfall received in June, July, August and September was 109.5, 321.0, 19.5 and 609.5 mm, respectively. The average daily maximum temperatures in June, July, August and September were 24.8, 30.7, 32.4 and 25.8 °C, respectively.

5.2.2. Field experimental design

This study compared five different fields with durations of continuous monocropping: where sesame had not previously been cropped (non-continuous monocropping; Year 0) and where sesame had been cropped for two years (Year 2), four years (Year 4), five years (Year 5), and six years (Year 6). To avoid variation due to different cropping years in terms of environmental conditions, we conducted this experiment in 2018 on these five fields with continuous monocropping history. All fields were adjacent to each other and with relatively similar initial soil chemical properties before sesame cultivation.

Briefly, each field measuring 14 m × 6.5 m had previously been divided into micro plots of 3 m × 2.3 m (6.9 m^2) to cultivate four sesame cultivars. However, we selected one cultivar for this study. The same agronomic practices of sesame as recommended by the National Agriculture and Food Research Organization (NARO, Japan) was followed from planting methods to inorganic fertilizer application. Inorganic fertilizer $N–P_2O_5–K_2O$ was used at a rate of 70–105–70 kg ha^{-1} in the form of cyclo-diurea (CDU) compound fertilizer (15%–15%–15%) and triple superphosphate (34%). In addition, CaO and MgO in the form of dolomite (Total alkali, 53%; CaO, 39.1%; and MgO, 10%) were applied at 1000 kg ha^{-1} before sowing every year to raise soil pH of initially acidic paddy field while supplying Mg and Ca for sesame growth. However, in 2017, the Year 2, Year 4 and Year 6 fields received additional K fertilizer after noticing decreasing levels of soil K content. During each harvest, plants were cut down from the base, and all aboveground crop residues were removed from the fields to minimize fungal and bacterial disease, and release of allelopathic compounds exuded from the residues.

Cultivar “Gomazou” was cultivated on raised ridges (75 cm in width; 40 cm between ridges), onto which double rows of plants were sown at spacing of 45 cm × 15 cm. All ridges were covered with black plastic mulch to maintain soil moisture and temperature and to reduce weed growth (**Figure 26**). Sowing was done on 9 July 2018 and seedlings thinned to two plants per hole on 16 July 2018 (one week after sowing), and then finally to one plant per hole on 24 July 2018 (two weeks after sowing).

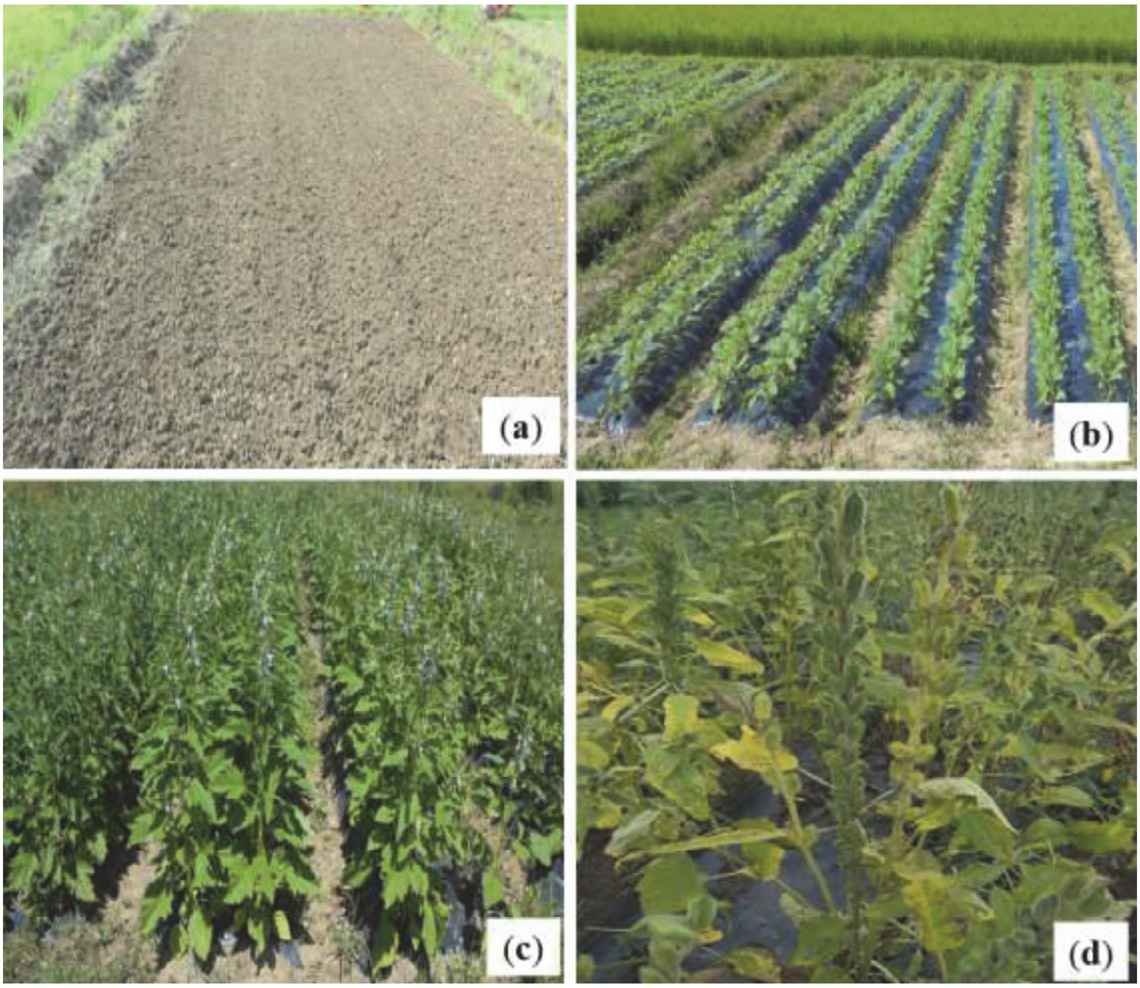


Figure 26. (a) Land preparation, (b) seedling stage, (c) flowering stage and (d) physiological maturity stage.

5.2.3. Growth and yield determination

Sesame growth was determined at full maturity stage from the field. Growth was determined by measuring plant height, height of the first capsule and counting the number of branches per plant of ten representative plants per replication on 24 September 2018. Harvesting from Year 2, Year 4, Year 5 and Year 6 fields was done on 28 September 2018 due to early drying of sesame plants while Year 0 field was harvested a week later (5 October 2018). All plants per micro-plot per replication were cut down and taken to dry in a greenhouse at Tottori University. After drying, the samples were threshed to release seeds. Total seed weight harvested per micro plot was then obtained to calculate yield in kg ha^{-1}, whereas 1000-seed weight was determined by weighing 100 mature seeds in triplicate for each replication to extrapolate the weight of 1000 seeds.

5.2.4. Plant sampling and leaf tissue nutrient concentration analysis

Sesame leaves from ten representative plants per replication were collected at reproductive stage (full bloom) on 23 August 2018 (45 days after sowing). One mature leaf per plant was collected and samples were then oven dried at 72 °C for one week. Prior to analysis, all leaf samples were ground to fine powder and digested with concentrated sulfuric acid (H_2SO_4, 98%) and hydrogen peroxide (H_2O_2, 30%) for analysis of leaf tissue P, K, Ca and Mg concentrations. The leaf tissue P concentration was determined by the vanadomolybdate method (Cavell, 1955) and the yellow vanadomolybdo-phosphoric acid measured colorimetrically by a spectrophotometer at 420 nm (Model U-5100, Hitachi Co., Tokyo, Japan). Leaf tissue K, Ca, and Mg concentrations were determined using an atomic absorption spectrophotometer (Model Z-2300, Hitachi Co., Tokyo, Japan). The leaf tissue N concentration was determined from the ground sample using the dry combustion method on a CN Corder machine (Macro corder JM 1000CN, J-Science Co., Ltd., Kyoto, Japan).

5.2.5. Soil sampling and analysis

5.2.5.1. Soil available nutrients, pH and EC values

At harvest, soil samples were collected with a trowel to a depth of 15 cm, air dried, and passed through a 2-mm sieve. Soil suspension (1:5 w/v, soil: water) was used to measure pH with a pH meter and electrical conductivity with an EC meter (AQUA COND METER F-74, Horiba, Ltd., Japan). Total C and N were analyzed by dry combustion on the CN analyzer (Macro corder JM 1000CN, J-Science Co., Ltd., Kyoto, Japan). For determination of exchangeable ammonium N (NH_4^+-N) and nitrate N (NO_3^--N), fresh soil samples were collected after harvest and stored at −80 °C prior analysis. The exchangeable ammonium N (NH_4^+-N) was extracted with 10% potassium chloride, KCl (1:10, w/v), and extracts determined by indophenol blue method (Maynard and Kalra, 1993) at 693 nm on a spectrophotometer (Model U-5100, Hitachi Co., Japan). The soil NO_3^--N was extracted with 0.01% aluminum chloride, AlCl3.6H2O (1:100, w/v), and determined by rapid ultraviolet (UV) absorption method (Yamaki, 2003), at 210 nm on a spectrophotometer. Soil exchangeable K, Ca, and Mg were extracted in 1 N ammonium acetate (pH 7.1), and analyzed by using an atomic absorption spectrophotometer (Z-2300, Hitachi Co., Tokyo, Japan). Cation exchange capacity (CEC) was measured by the 1 N (pH 7.1) ammonium acetate (NH_4OAc) extraction method, in which the NH_4^+ saturated soil was equilibrated with 10% KCl (Chapman, 1965), and analyzed colorimetrically using Indophenol blue method (Maynard and Kalra, 1993).The CEC was calculated and expressed in $cmol_c\ kg^{-1}$ soil, as shown in Equation (1) (Ross and Ketterings, 2011).

$$CEC\ (cmol_c\ kg^{-1}) = (NH_4^+\text{-N in mg kg}^{-1})/14 \quad (1)$$

Individual cation base saturation (BS%) was calculated from the CEC and the exchangeable cation values. However, Na^+ measurement was not considered in the calculation of the base saturation because of very low concentrations (<1% of the CEC in the soil). Soil available P was determined using 0.002 N H_2SO_4 (pH 3.0) in ammonium sulfate solution according to Truog method (Truog, 1930) The P concentration in soil samples was measured by the phosphomolybdate blue method (

Murphy and Riley,1962) at 710 nm on a spectrophotometer (Model U-5100, Hitachi Co., Tokyo, Japan).

5.2.5.2. Soil enzyme activities

Immediately after harvest, soil samples were collected with a trowel to a depth of 15 cm, passed through a 2-mm sieve and stored at −20 °C prior to enzyme analysis. Soil urease activity was determined by the method of Kandeler and Gerber (Kandeler and Gerber, 1988). Briefly, 5 g of fresh soil were mixed with 2.5 mL of 0.72 M aqueous urea and 20 mL of borate buffer at pH 10.0. The samples were incubated at 37 °C for 2 h and then 30 mL of 1 N KCl to 0.01 N HCl solution were added and shaken for 30 min before filtering. The concentration of ammonium released as a result of urease activity was determined by the indophenol blue method (Maynard and Kalra, 1993) at 693 nm on a spectrophotometer (Model U-5100, Hitachi Co., Tokyo, Japan). The urease activity was expressed as μg (g NH_4^+-N) $^{-1}$ (g dry soil)$^{-1}$ 2 h^{-1}.

Soil dehydrogenase activity was determined by the iodonitrotetrazolium chloride (INT) method of von Mersi and Schinner (von Mersi and Schinner, 1991). Briefly, 1 g of moist soil was mixed with 1.5 mL of TRIS buffer (1 M, pH 7.0), 2 mL of 0.5% (w/v) aqueous solution of INT (10 mg mL−1), and incubated at 40 °C in dark for 2 h. Then, 10 mL of N, N-Dimethylformamide/ethanol in a 1:1 ratio were added to extract the developed iodonitrotetrazolium formazan (INTF), and samples were kept in the dark for 2 h, and shaken vigorously every 20 min. The absorption of the filtrated was measured at 464 nm on a spectrophotometer (Model U-5100, Hitachi Co., Tokyo, Japan) using INTF (iodonitrotetrazolium formazan) as standard. Soil dehydrogenase activity was expressed as nmol INTF (g dry weight soil)$^{-1}$ 2 h^{-1}.

Soil catalase activity was determined by titrating residual hydrogen peroxide (H_2O_2) with potassium permanganate ($KMnO_4$) (Goldblith and Proctor, 1950). Briefly, 2 g of fresh moist soil samples were mixed with 5 mL of 0.3% hydrogen peroxides solution (H_2O_2), shaken on a mechanical shaker at 25 °C for 20 min. Then, 5 mL of 1.5 M H_2SO_4 solution were added and hand shaken for 30 s. Thirty milliliters of the filtrate were then titrated with 0.02 M KMnO4 until the permanganate solution turned pink, persisting for 30 s, and then the titration was stopped. Catalase activity was expressed as mL of 0.02 M KMnO4 g^{-1} soil h^{-1}.

5.2.6. Statistical analysis

In each duration of continuous monocropping, micro-plots were replicated three times. All data were subjected to one-way ANOVA using SPSS version 22.0 (SPSS for windows Inc., Chicago, IL, USA), and means were compared using Tukey's honestly significant difference (HSD) test at $p < 0.05$. In addition, principal component analysis (PCA) was performed with SPSS version 22.0 to clarify total data variability with respect to correlations among growth, yield, soil chemical properties parameters and mineral nutrient concentrations in sesame leaf tissue under the different continuous monocropping years.

5.3. Results

5.3.1. Effect of continuous monocropping on sesame growth and yield under the different duration of cropping years

Continuous monocropping significantly decreased sesame plant height, height of the first capsule and the number of branches per plant (**Figure 27**, **Table 18**). Compared to Year 0 (control), plant height significantly ($p < 0.001$) decreased by 18.76%, 15.22%, and 13.64% in the Year 4, Year 5 and Year 6 fields, respectively. The height of first capsule significantly increased by 5.11% and 1.52% in the Year 2 and Year 6 fields, respectively, whereas a decrease of 16.81% and 7.89% occurred in the Year 4 and Year 5 fields, respectively, compared to Year 0. The number of branches per plant was significantly higher in the Year 2 field, whereas it decreased in Year 4 and Year 5, indicating fewer branches in the long duration of continuous monocropping than the short duration.

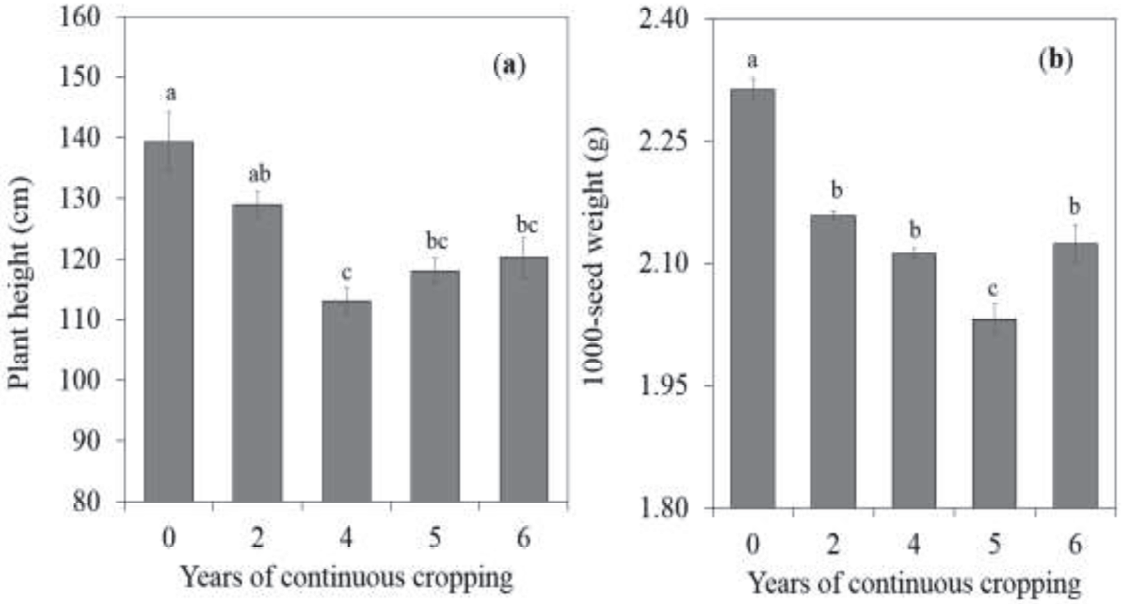


Figure 27. (a) The seed yield and (b) 1000-seed weight of sesame. Bars show mean values with standard errors (*n* = 3). Different letters above the bars indicate significant differences by Tukey HSD test ($p < 0.05$).

Continuous monocropping significantly decreased sesame seed yield (**Table 18**) and the 1000-seed weight (**Figure 27a**). Seed yield (1952 kg ha^{-1}) was highest in Year 0 (non-continuous monocropping) and significantly ($p < 0.001$) decreased in the continuously monocropped fields (**Table 18**). Compared to the Year 0 field, seed yield decreased by 52.86% in Year 2, and no significant differences in the seed yield among the Year 2, Year 4, Year 5 and Year 6 fields was observed. The 1000-seed weight significantly ($p < 0.001$) decreased with increase in continuous monocropping years (**Figure 27b**). The 1000-seed weight was significantly higher in Year 0 (2.31 g). Compared to the Year 0 field, 1000-seed weight decreased by 6.68% and 12.20% in the Year 2 and Year 5 fields, respectively. However, there were no significant differences among Year 2, Year 4 and Year 6 fields. In addition, the 1000-seed weight tended to recover in the Year 6 field after decreasing in the Year 5 field.

Table 18. The effect of continuous monocropping on the sesame growth and yield parameters under the different duration of cropping years.

Year ^{z}	Height of First Capsule (cm)	Number of Branches/Plant	Seed Yield (kg ha^{-1})
0	61.27a	2.37ab	1952.16a
2	64.40a	2.80a	920.29b
4	51.00c	1.77c	723.17b
5	56.43b	1.70c	617.32b
6	62.20a	1.90bc	641.45b
ANOVA (*p*-values)	***	***	***

^{z} Years of continuous monocropping. Different letters indicate significant differences among years by Tukey HSD test ($p < 0.05$). Significance levels are indicated as * ($p < 0.05$), ** ($p < 0.01$) and *** ($p < 0.001$).

5.3.2. Effect of continuous monocropping on sesame leaf tissue nutrient concentrations under the different duration of cropping years

Continuous monocropping did not significantly affect leaf tissue P and Mg concentration, whereas leaf tissue N, K and Ca concentrations were significantly affected (**Table 19**). The leaf tissue N and K concentrations significantly decreased with increase in the duration of continuous monocropping. Compared to the Year 0 field (non-continuous monocropping), the leaf tissue N concentration significantly ($p < 0.01$) decreased by 22.10% and 16.27% in the Year 5 and Year 6 fields. However, the Year 0 and Year 2 fields significantly differed from the Year 5 and Year 6 fields in leaf tissue N concentrations, indicating decrease in N concentrations in long duration of continuous monocropping.

Similarly, continuous monocropping significantly ($p < 0.01$) decreased leaf tissue K concentration. The leaf tissue K concentrations decreased in the Year 6 field by 46.44% compared to Year 0 (non-continuous monocropping). Furthermore, the leaf tissue K concentration in Year 2 was significantly higher than in both Year 5 and Year 6 fields, indicating decrease in K nutrition in the long duration of continuous monocropping. Although there were no significant differences in the leaf tissue K concentrations among Year 0, Year 2, Year 4 and Year 5 fields, leaf tissue K concentration tended to decrease gradually with increase in the duration of continuous monocropping.

The leaf tissue Ca concentration tended to significantly ($p < 0.05$) increase in the Year 6 field compared to the Year 5 field. In addition, the leaf tissue Ca concentration of Year 5 field was significantly lower than in the Year 2 field. However, there were no significant differences in the leaf tissue Ca concentration among Year 0, Year 2, Year 4 and Year 5 fields.

Table 19. The effect of continuous monocropping on the sesame leaf tissue nutrient concentrations under the different duration of cropping years.

Year ^{z}	N%	P (%)	K (%)	Ca (%)	Mg (%)
0	2.69a	0.80a	2.91ab	0.44ab	0.47a
2	2.68a	0.79a	3.13a	0.48a	0.48a
4	2.38ab	0.55a	2.21abc	0.40ab	0.43a
5	2.09b	0.83a	1.86bc	0.27b	0.37a
6	2.25b	1.21a	1.56c	0.56a	0.45a
ANOVA (*p*-values)	**	ns	**	*	ns

^{z} Years of continuous monocropping. Different letters indicate significant differences among years by Tukey HSD test ($p < 0.05$). Significance levels are indicated as * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) and NS (Non-significant).

5.3.3. Effect of continuous monocropping on soil chemical properties under the different duration of cropping years

The effect of continuous sesame cropping on the soil chemical properties are shown in **Tables 20** and **21**. With the exception of soil EC, C/N ratio and NO_3^--N, all soil macronutrients including base saturations were significantly affected by continuous monocropping. Continuous monocropping significantly ($p < 0.001$) increased soil pH from 5.29 in the Year 0 field to 6.30 in the Year 6 field (**Table 20**). Soil pH gradually increased from Year 2 to Year 5, indicating increase in pH with increase in continuous monocropping years. Conversely, soil total C ($p < 0.05$) and N contents ($p < 0.001$) contents significantly decreased in the long duration of continuous sesame cropping. Although there were no significant differences among the Year 0, Year 2, Year 4 and Year 5 fields in the soil total C and N contents, Year 6 had significantly lower soil total C and N contents.

Continuous monocropping also significantly ($p < 0.01$) decreased soil exchangeable NH_4^+-N content. Soil exchangeable NH_4^+-N was significantly lower in Year 5 (5.84 mg kg^{-1}), followed by Year 6 (5.94 mg kg^{-1}) field compared to the Year 2 field. However, no significant differences between Year 0 and Year 2 or Year 0 and Year 4 in the soil exchangeable NH_4^+-N content were observed. Although the effect of continuous monocropping was not significant on the NO_3^--N content, there was an overall decrease in the available N content indicated by the decreasing exchangeable NH_4^+-N content in the Year 6 field compared with Year 0 field. Furthermore, the soil available P content was also significantly ($p < 0.001$) higher in Year 0 and lower in the continuously monocropped fields, indicating tendency of continuous sesame cropping to decrease available P content, although no significant differences among the Year 2, Year 4, Year 5 and Year 6 fields were observed.

Table 20. The effect of continuous monocropping on the soil chemical properties under the different duration of cropping years.

	1:5 (H_2O)					Available N		
Year^z	pH	EC (dS m^{-1})	Total C (g kg^{-1})	Total N (g kg^{-1})	C/N ratio	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	Available P (mg kg^{-1})
0	5.29c	0.053a	26.36ab	2.57ab	10.23a	9.71ab	13.10a	71.35a
2	5.64bc	0.052a	28.56a	2.71a	10.54a	10.91a	9.79a	49.88b
4	5.91ab	0.052a	24.83ab	2.40bc	10.36a	6.53bc	8.06a	45.17b
5	6.02ab	0.052a	25.86ab	2.41bc	10.71a	5.84c	9.58a	43.31b
6	6.30a	0.046a	24.50b	2.30c	10.67a	5.94c	11.28a	40.84b
ANOVA (*p*-values)	***	ns	*	***	ns	**	ns	***

^{z} Years of continuous monocropping. Different letters indicate significant differences among years by Tukey HSD test ($p < 0.05$).

Significance levels are indicated as * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) and NS (Non-significant).

Continuous sesame cropping significantly altered the soil exchangeable cations K, Ca and Mg and their base saturations including soil cation exchange capacity and CEC values (**Table 21**). Although soil exchangeable K content was significantly ($p < 0.01$) affected, there were no significant differences among the Year 0, Year 5 and Year 6 fields. However, soil exchangeable K content was higher in the Year 2 (250.47 mg kg^{-1}) and Year 4 (229.9 mg kg^{-1}) fields compared to the Year 0 (157.4 mg kg^{-1}) field with no decreasing trend in continuous monocropping observed. Both the soil exchangeable Ca and Mg contents significantly ($p < 0.001$) increased in the Year 5 and Year 6 fields compared to the Year 0 field. Soil exchangeable Ca content increased from 799.12 mg kg^{-1} in Year 0 to 1478.36 mg kg^{-1} in Year 2 and 1252.35 mg kg^{-1} in Year 4. No significant differences in soil exchange Ca content were observed among Year 2, Year 5 and Year 6. Similarly, exchangeable Mg content tended to gradually increase from Year 0 to Year 6 without significant difference among Year 4, Year 5 and Year 6.

Continuous sesame cropping also significantly ($p < 0.001$) increased the soil CEC values. Soil CEC significantly increased from 10.62 cmol$_c$ kg^{-1} in Year 0 to 11.67 and 11.90 cmolc kg^{-1} in Year 5 and Year 6 respectively. Continuous sesame cropping also significantly affected the soil cation base saturations. The soil K saturation in the Year 0 field did not significantly differ from Year 4, Year 5 and Year 6. The Year 2 (5.73%) field had significantly higher K saturation compared with the Year 0 (3.80%) field, although no significant difference between Year 2 and Year 4 was observed. The soil Ca and Mg saturations significantly increased in continuous monocropping compared to the Year 0 field. The Ca saturation increased from 37.6% in Year 0 to 66.0% and 56.0% in Year 2 and Year 4, respectively, with no significant differences among Year 2, Year 5 and Year 6. Similarly, Mg saturation increased from 15.2% in Year 0 to 20.9% and 26.1% in Year 2 and Year 4, respectively, with no significant differences among Year 4, Year 5 and Year 6.

Table 21. The effect of continuous monocropping on soil exchangeable cation, CEC and base saturations under the different duration of cropping years.

	Exchangeable Cations				Base Saturation (%)		
Year [z]	K ($mg\ kg^{-1}$)	Ca ($mg\ kg^{-1}$)	Mg ($mg\ kg^{-1}$)	CEC ($cmol_c\ kg^{-1}$)	K	Ca	Mg
0	157.42b	799.12c	193.47c	10.62b	3.80bc	37.63c	15.18c
2	250.47a	1478.36a	281.15b	11.20ab	5.73a	65.97a	20.91b
4	229.90a	1252.35b	349.60a	11.18ab	5.28ab	56.00b	26.06a
5	161.98b	1478.44a	356.68a	11.67a	3.56c	63.37ab	25.48a
6	217.69ab	1658.71a	381.09a	11.90a	4.70abc	69.74a	26.72a
ANOVA (*p*-values)	**	***	***	**	**	***	***

[z] Years of continuous monocropping. Different letters indicate significant differences among years by Tukey HSD test ($p < 0.05$). Significance levels are indicated as * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) and NS (Non-significant).

5.3.4. Effect of continuous sesame cropping on soil enzyme activities under the different duration of cropping years

Continuous sesame cropping significantly altered soil enzyme activities (**Table 22**). Soil urease activity was significantly ($p < 0.05$) higher in the Year 0 (97.17 µg of NH_4^+-N g^{-1} soil 2 h^{-1}) and Year 2 (106.18 µg of NH_4^+-N g^{-1} soil 2 h^{-1}) fields than the Year 5 (80.38 µg of NH_4^+-N g^{-1} soil 2 h^{-1}) field, indicating a decrease in urease activity due to continuous monocropping. However, there were no significant differences in the urease activities among the Year 0, Year 2, Year 4 and Year 6 fields, suggesting a slight recovery in the urease activity. The soil catalase activity was significantly ($p < 0.05$) decreased in the long duration continuous monocropping than in the short duration.
The catalase activity was significantly higher in the Year 4 (6.89 mL of 0.02M KMnO4 g^{-1} soil h^{-1}) field compared to the Year 6 (4.13 mL of 0.02M KMnO4 g^{-1} soil h^{-1}) field, indicating a gradual decrease from Year 5 in continuous sesame cropping.

Continuous sesame cropping significantly ($p < 0.001$) decreased the soil dehydrogenase activity. Soil dehydrogenase activity was significantly higher in the Year 0 field (0.70 INTF µ moles g^{-1} soil 2 h^{-1}), decreasing with increase in duration of continuous monocropping to the lowest value in the Year 5 field (0.39 INTF µ moles g^{-1} soil 2 h^{-1}). However, the soil dehydrates activity slightly increased in the Year 6 field, although still significantly lower than the Year 0 field.

Table 22. The effect of continuous monocropping on the soil enzyme activities under the different duration of cropping years.

Year ^{z}	Urease (μg NH_4^+-N g^{-1} soil 2 h^{-1})	Catalase (mL of 0.02M $KMnO_4$ g^{-1} soil h^{-1})	Dehydrogenase (INTF μ moles g^{-1} soil 2 h^{-1})
0	97.17ab	6.311ab	0.70a
2	106.18a	6.29ab	0.55b
4	88.13ab	6.89a	0.47bc
5	80.38b	6.30ab	0.39c
6	87.63ab	4.13b	0.50bc
ANOVA (*p*-values)	*	*	***

^{z} Years of continuous monocropping. Different letters indicate significant differences among years by Tukey HSD test ($p < 0.05$). Significance levels are indicated as * ($p < 0.05$), ** ($p < 0.01$), *** ($p < 0.001$) and NS (Non-significant).

5.3.5. Relationship among soil chemical properties, leaf tissue nutrient concentrations, growth and yield decline parameters under the different duration of cropping years

Principal component analysis (PCA) was performed to visualize the relationships between the variables and duration of continuous monocropping years. The PCA explained 62.1% of the total variance and clustered continuous monocropping fields into three distinct groups (I, II and III) (**Figure 28a, b**). PC 1 accounted for 45.6% of total variance and showed a high positive correlation with soil TN content, total C content, exchangeable NH_4^+-N content, leaf tissue K concentrations, plant height, number of branches per plant, and soil urease activity that were high in Year 0 (non-continuous monocropping field) represented by Group I. Groups I and II, representing variables from Year 0 and Year 2, respectively, were correlated and accounted for the highest variability explained by the PCA in the continuous monocropping. PC 2 accounted for 16.5% of total variance among the parameters, and was positively correlated with the soil exchangeable Ca content, CEC and Ca saturation, while negatively correlated with the soil catalase activity and EC. PC 2 was a measure of variability in Group III (mainly Year 6 field) due to the high exchangeable cations including soil pH. Group I (Year 0) differed from continuous monocropping Groups II (Year 2) and III (Year 4, Year 5 and Year 6) but Groups I and II, and Groups II and III were strongly correlated. Group III was clustered based on high soil exchangeable Ca, pH, Mg, base saturation, and CEC, whereas Group I due to the high seed yield, 1000-seed weight, number of branches per plant, soil total N and C contents, exchangeable NH_4^+-N content, leaf tissue K concentration, soil dehydrogenase and urease activity, and Group II due to high height of the first capsule, leaf tissue Ca concentration, and exchangeable K and K saturation.

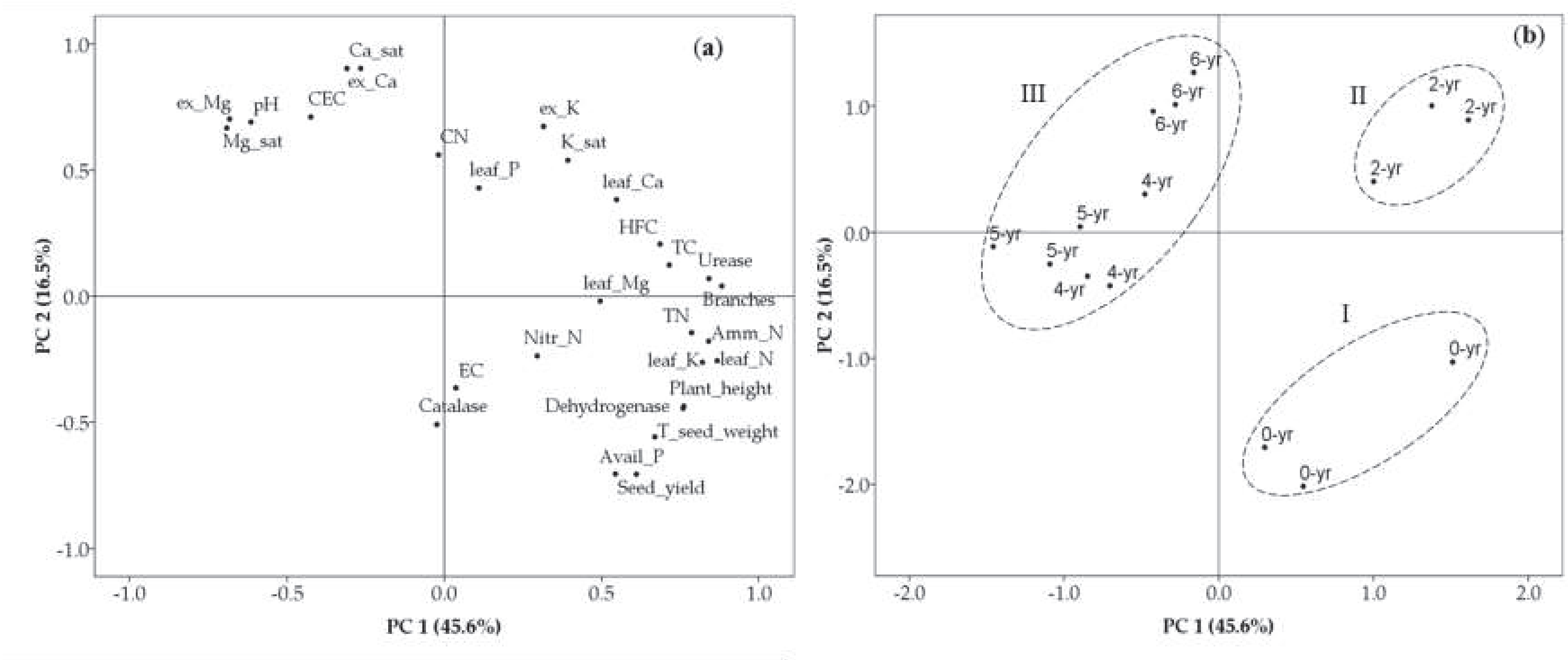


Figure 28. Principal component analysis (PCA) showing variation in growth, seed yield, nutrient concentration, and soil chemical properties. (a) Loading plot of the first two components showing the relationship between measured variables. HFC, height of first capsule; T_seed_weight, thousand seed weight (1000-seed weight); TN, total soil N; TC, total soil C; Avail P, available P; Amm_N, NH_4^+-N; Nitr_N, NO_3–-N; Ca_sat, Ca saturation (%); Mg_sat, Mg saturation (%); and K_sat, K saturation (%). (b) The score plot of the PCA with clusters corresponding to continuous monocropping years (Year 0, non-continuous monocropping; 0-yr, 2-yr, 4-yr, 5-yr and 6-yr indicate Year 0, Year 2, Year 4, Year 5 and Year 6 fields, respectively).

5.4. Discussion

In this study, soil chemical properties, especially pH, total N, and C contents, exchangeable NH_4^+-N content, available P, exchangeable Ca and Mg contents, CEC values, Ca and Mg saturations, and soil enzyme activities, were significantly altered by continuous sesame cropping, indicating the negative influence of long-term continuous sesame cropping. Similar studies show that continuous monocropping can alter soil mineral nutrients and other chemical properties, negatively affecting crop growth and yield (Sun et al., 2018; Zhao et al., 2018). The results show that soil pH gradually increased with the increase in the duration of continuous monocropping, suggesting the acidic nature of the upland field converted paddy could be alleviated with sesame cultivation (**Table 20**). Kusumawardani et al. (2017) reported that conversion of rice paddy fields to upland crop cultivation could increase soil pH attributed to the change in land use and cropping system. However, in this study, the rise in the soil pH was mainly attributed to the continuous addition of dolomite lime containing Ca that greatly increased soil pH. It is reported that dolomite addition to acidic soils results in an increase in soil pH (Anderson et al., 2013). The results is contrary to several studies that show continuous monocropping of other crops significantly decreases soil pH, attributed to the annual fertilizer application and increase in allelochemicals exuded from roots and decomposing residues that have acidic nature (Xiong et al., 2015; Li et al., 2016). In this study, sesame residues were removed from the fields to prevent occurrence of allelopathy from decomposing residues limiting decrease in pH. Hence, soil pH in different continuous monocropping systems depends on the residue management practice and fertilizer use.

The results also show that continuous sesame cropping significantly decreased the soil total N and C contents in Year 6 compared with Year 2, suggesting long-term continuous sesame cropping could reduce the organic C and N stocks, resulting from nutrient depletion due to the annual crop harvest. In this study, sesame residues after each harvest were removed from each field, thereby decreasing the quantity of organic materials from the continuously monocropped fields. Liu et al. (2006) reported that decreases in soil organic carbon under continuous monocropping could be accelerated by little crop residue return to the soil. Reducing quantities of crop residues decreases organic matter content from soil leading to low total N and C contents decreasing soil quality. Similar decrease in the soil total N and C in continuous monocropping have been reported, indicating loss of soil quality (Reeves et al., 1997; Wyngaard et al., 2012; Zhong et al., 2014). Hence, with continuous sesame cropping, decreasing levels of total N and C could indicate loss in the soil nutrient status since organic matter is responsible for increasing soil fertility. Moreover, Nishimura et al. (2008) reported increase in soil organic matter content in paddy fields under rice cultivation but significantly decreases when a paddy is converted into upland crop cultivation. Furthermore, the decrease in the total N could have caused the decrease in the soil exchangeable NH_4^+-N content, indicating decrease in available N under long-term continuous sesame cropping. The findings agree with several studies that show decrease in available N with continuous monocropping (Horst and Hardter, 1994; Nie et al., 2008; Wang et al., 2014). In this study, the decrease in the available N could also be enhanced by leaching of soil mineral nutrients in the upland field converted paddy. Usually, paddy-rice fields are characterized by hard plow pans that prevent leaching and increase the water for plants in the puddled layer when under continuous rice cropping (Janssen et al., 2007). However, the hard plow pans are easily disintegrated as a result of desiccation of cracks during the dry periods, which leads to increasing percolation of water when converted to upland crop cultivation (Zhou et al., 2014). Therefore, as the water leaching occurs under percolation, soil mineral nutrients could easily be lost to the subsoil layers (Katoh et al., 2004). This could be aggravated by increasing duration of continuous sesame cropping whereby the hard pans initially created under rice cultivation are broken by repeated tillage as in continuous monocropping.

Although continuous sesame cropping significantly had lower soil available P content than the Year 0 field, the differences could be attributed to the initial soil P status of the freshly opened field rather than continuous monocropping. On the other hand, the significantly higher soil exchangeable K content in the Year 2 and Year 4 fields compared to the Year 0 field could be due to the additional K applied into the soil in the previous cropping year. Therefore, both available P and exchangeable K were adequate for sesame growth. The results also show that soil exchangeable Ca and Mg contents significantly increased in the Year 5 and Year 6 fields compared to the Year 0 field, suggesting long-term duration of continuous sesame cropping under this management practice could lead to build up of base cations on upland fields converted paddy (**Table 21**). The increase in Ca and Mg cations including their base saturations could be due to the high quantity of dolomite added annually (Anderson et al., 2013). In addition, the observed increase in the soil CEC content in the Year 5 and Year 6 fields compared to the Year 0 field could be explained by the increase in Ca and Mg tightly attached on soil colloidal particles. Although soil CEC is a measure of nutrient retention and supply to crops (Jones and Jacobsen, 2001), the increase in the soil CEC in this study may not indicate an increase in nutrient availability since soil total N and C contents tended to decrease in continuous sesame cropping. Instead, the increase in the soil CEC values could be attributed to increase in the Ca and Mg ions from dolomitic lime on the soil colloidal particles. It is reported that liming soils can increase CEC since at high soil pH, Ca, Mg and other base cations can be tightly held to the soil (Jones and Jacobsen, 2001; Bolan et al., 2003).

In this study also showed that the impact of continuous sesame cropping was significant on enzyme activity, indicating the negative influence of continuous monocropping on both the chemical and biological properties of soil. Soil enzymes activities are useful indicators of soil fertility status (Schloter et al., 2003). In this study, urease activity was significantly lower in Year 5 compared to Year 0, indicating a decrease in N nutrient cycling and release. The decrease in the soil total N could have enhanced the decrease in urease activity that consequently led to the decrease in the available N in the long duration of continuous monocropping. Furthermore, soil catalase activity was significantly decreased in Year 6 compared to Year 4, suggesting low levels of substrates such as organic matter in the long duration of continuous monocropping. The results are consistent with previous studies that show continuous monocropping of other crops led to decrease in soil enzymes activities (Xiong et al., 2015; Sun et al., 2018). Xiong et al. (2015) also reported decrease in the urease and catalase activities in continuous black pepper cropping that corresponded with the low organic matter content, limiting the growth. In general, urease and catalase enzymes have a strong co-relationship with soil carbon content and are good indicators of soil quality (Wang et al., 2013). Therefore, the decreasing soil total N and C contents in the long duration continuous monocropping suggests low organic matter could explain the decrease in the enzyme activities (**Tables 20**, **22**). Moreover, soil dehydrogenase activity was decreased in continuous monocropping due to decreasing soil carbon content (Liu et al., 2006). In this study, dehydrogenase was significantly higher in the Year 0 field compared to the Year 5 and Year 6 fields, indicating tendency to decrease soil fertility. The results are consistent with the findings reported that continuous cotton cropping led to decrease in soil enzyme activities indicating a negative impact of continuous monocropping on soil function and sustainability (Acosta-Martinez et al., 2002). Since increase in the soil enzyme activities is directly related to soil nutrient cycling and availability (Wang et al., 2014) the low enzyme activities in the long duration of continuous sesame cropping suggests low nutrient availability, especially N that could influence sesame growth and yield.

Continuous sesame cropping significantly affected N and K nutrition in sesame plant that could be directly linked to the growth and yield decline. The leaf tissue N concentration significantly decreased in the Year 2, Year 4 and Year 6 fields compared to the Year 0 field, indicating low uptake of N (**Table 19**). Several studies also reported a decrease in N uptake as a result of either limited absorption or depletion in soil N due to continuous monocropping (Nie et al., 2008; Riedell et al., 2009). The decrease in the leaf tissue N concentration could be attributed to the decrease in the soil available N that could arise from its depletion in continuous monocropping.

Furthermore, there was a decrease in the leaf tissue K concentration in Year 6 compared to Year 0, indicating a gradual decrease in K uptake in long duration of continuous sesame cropping that could negatively influence sesame growth and yield. In this study, the decrease in the leaf tissue K concentration could be caused by competitive ion effect in which absorption of K by sesame plants was hindered by high levels of soil exchangeable Ca and Mg in the long duration of continuous monocropping. It is known that high levels of Ca and Mg could affect the uptake of K (Weil and Brady, 2016).

The leaf tissue Ca concentration tended to increase in Year 6 compared to Year 5, possibly due to the increase in soil exchangeable Ca indicating its adequate absorption. The leaf tissue P and Mg was unaffected by continuous sesame cropping, signifying these nutrients were adequate in the soil for sesame growth. The significantly lower soil available P in the continuously monocropped fields, however, did not affect P nutrition.

Increasing duration of continuous monocropping years significantly decreased sesame plant height and number of branches per plant, indicating growth decline under continuous monocropping. The non-significant difference in the seed yield between the continuously monocropped fields but significant differences in the 1000-seed weight could suggest continuous sesame cropping is more pronounced on 1000-seed weight than overall seed yield. Hence, the decreasing 1000-seed weight confirmed that continuous sesame cropping could lead to yield decline in terms of low seed weight. A similar decrease in seed weights is reported for continuous soybean cropping that led to the yield decline (Kelley et al., 2003). Usually, the overall sesame yield is composed of number of capsules per plant, number of seeds per capsule and the seed weight (Dossa et al., 2017). Furthermore, plant height and height of the first capsule are all associated with sesame seed yield (Biabani and Pakniyat, 2008). With the decrease in 1000-seed weight and plant height under continuous sesame cropping, these two parameters could be the most influenced in continuous monocropping other than total seed weight that may be affected by factors such as shattering and loss of seeds during harvesting.

The overall growth and yield decline could be attributed to low uptake of N and K, indicated by the decreasing leaf tissue nutrient concentrations in long duration of continuous sesame cropping. The findings agree with other studies that show decrease in nutrient absorption can lead to growth and yield decline in continuous monocropping (Ye et al., 2014;

Wang et al., 2014). Moreover, sesame requires adequate N and K for both dry matter and yield (Shehu et al., 2009; Jadav et al., 2010; Shehu, 2014). These macronutrients are important for growth and any imbalance or inadequate absorption could result in poor growth and consequently yield decline. In this study, sesame growth and yield could have been limited by decrease in N and K nutrition caused by decreasing available N and either decreasing K or poor absorption of K due to competitive ionic effect. Thus, the high growth indicated by increase in plant height and number of branches per plant and yield indicated by increase in 1000-seed weight in Year 0 could indicate sufficient uptake of N and K. It is reported that K deficiency in sesame occurs at levels of K leaf tissue concentration of ≤1.5% and values of 1.5–2.4% show moderate deficiency levels of K. Under low K, sesame plants are smaller primarily because of short internodes (Mitchell et al., 1974). The increase in the leaf tissue K in Year 0 could indicate long internodes in sesame leading to high plant heights. Conversely, the short plants observed in the long duration continuous monocropping could have had shorter internodes and fewer branches per plant leading to low growth. The decrease in the 1000-seed weight possibly due to low K uptake could result from low accumulation of photosynthate in sesame plants. K plays a role in photosynthesis because it stimulates activity of ribulose bisphosphate carboxylase (Demmig and Gimmler, 1983).

The cause of competitive ion effect could be attributed to large quantities of dolomite added annually. The increase in soil exchangeable Ca and Mg added from dolomite lime and the decrease in the K led to higher Ca/K, Mg/K and Ca/Mg ratios consequently affecting availability and uptake of K by sesame plants. Loide (2004), reported that incorrect use of lime fertilizers alters the Ca/Mg and Mg/K ratios, which is detrimental to plants and decreases yields. In addition, K deficiency in sesame occurs in soils with high Ca and Mg, which have wide Mg/K and Ca/K ratios, and often results in yield decline (Kumar and Sharma, 2013). In the study, the cation ratios Ca/K and Mg/K increased with the long duration continuous monocropping. For instance, the Ca/K ratio increased from 5.1 in Year 0 to 9.3 in Year 5 and Mg/K from 1.2 in Year 0 to 2.3 in Year 5, suggesting imbalances in soil exchangeable cations. This could be sufficient to hinder absorption of K leading to K deficiency in plants including sesame (Hodges, 2010). A study reported that continuous eggplant leads to yield decline due to imbalance in cation ratios (Yoshimura et al., 1976). Yoshimura et al. observed slight chlorosis between the veins of leaves in the middle parts of the plants with chlorotic eggplant leaves containing 2–4% of K and above 2.2% of Mg (K/Mg ratios of 1.3–2.7) being attributed to Mg excess and K deficiency. In this study, the leaf tissue Ca/K increased in Year 6 (0.37) compared to Year 0 (0.15), whereas Mg/K significantly increased from 0.15 in Year 0 to 0.20

in Year 6, indicating excess of Ca and Mg in sesame plant tissue and decrease in K could occur in a long-term continuous sesame under this management practice. Hence, the increase in Mg and Ca in soil led to the excess Mg in sesame plant tissues and near deficiency in K negatively affected sesame yield and growth in the long duration of continuous monocropping.

The growth and yield decline in continuous sesame cropping due to low available N and decreased absorption of K as a result of competitive ion effect was further confirmed by the principal component analysis (PCA) that accounted for 62.1% of variability in the data. The short duration of continuous monocropping (Year 0) was clearly distinguished from the long durations based on high soil available N, total N, soil enzyme activities, and leaf tissue N and K concentrations that significantly increased sesame growth and 1000-seed weight (**Figure 28**). The decrease in soil enzyme activities was confirmed by the PCA that showed correlation among soil urease, dehydrogenase, soil total N and C contents, and exchangeable NH_4^+-N content, suggesting decrease in the soil enzyme activities resulted from decrease in the total N and C contents. Urease enzyme plays a role in hydrolyzing urea to CO_2 and NH_3, thereby releasing ammoniacal N for plants after urea fertilization (Bolata and Chaves, 2010). In this study, urease activity was significantly lower in the long duration continuous monocropping, suggesting decrease in the available N as a result of decrease in hydrolysis of urea fertilizer. Furthermore, the high dehydrogenase activity in Year 0 and significantly lower activity in Year 5 indicated loss in soil organic matter that directly influenced soil fertility. Moreover, significant positive correlation existed among soil total organic carbon, organic matter and dehydrogenase activity (Wolinska and Stepniewska, 2012). Hence, under continuous monocropping of sesame, the loss in the total C and N reflecting low organic matter content could suggest low mineralization of nutrients leading to decreasing growth and yield.

In addition, the PCA confirmed that, with increase in continuous sesame cropping under this management, a gradual build-up of soil exchangeable Ca and Mg from dolomite could occur, leading to competitive ion effect. This was evidenced by the negative correlation between exchangeable Ca and Mg, and 1000-seed weight and plant height in the long duration of cropping, as indicated by the PCA. Although dolomite lime was intended to increase soil pH to alleviate acidity, the plant height and 1000-seed of sesame were significantly higher in the Year 0 with low pH compared to Year 5 or Year 6 with high pH, indicating that growth and yield of sesame was not influenced by the change in the soil pH. It is reported that sesame growth can be favorable at pH range of 5.0–8.0 (Langham, 2008). This further suggests that a one-time application of dolomite in the first cropping season could be sufficient for sesame growth. Hence, it may not be necessary to increase soil pH with dolomite in subsequent cropping since the soil pH of the non-continuous monocropping field was in the range preferred by sesame. However, it is important to identify alternative sources of Mg and Ca fertilizer for sesame cropping on upland field converted paddy to avoid competitive ionic effect in continuous monocropping. The increase in the soil Ca and Mg limiting absorption of K by sesame plants could suggest that erroneous fertilization practice is one of the possible causes of growth and yield in continuous monocropping of several crops including sesame. This could also suggest that fertilization programs in continuous monocropping should consider balancing nutrients to overcome imbalances while managing N availability. Therefore, adequate N and increasing K availability could be the most important factors in maintaining high sesame growth and yield on upland field converted paddy under continuous monocropping.

5.5. Conclusions

In this study showed that continuous sesame cropping could lead to decreasing N availability and decreased absorption of K. Results show a decrease in soil total N and C contents, soil enzyme activities, and leaf tissue N and K concentrations that led to the decrease in plant height and 1000-seed weight of sesame in the long duration of continuous monocropping. The gradual decrease in soil urease and dehydrogenase including catalase activities showed a decrease in the available nutrients in the long duration continuous sesame cropping. For instance, urease activity was significantly lower in the Year 5 field compared to the Year 0 field, suggesting decrease in N cycling and its release for sesame growth. The results also show that the large quantities of soil exchangeable Ca and Mg from dolomite application led to competitive ion effect causing decrease in absorption of K, as indicated by the low leaf tissue K concentration in Year 6 compared to Year 0. The decrease in the leaf tissue N was caused by decreasing soil available N that could rapidly be depleted through annual cropping of sesame without returning residue back to the soil. The low leaf tissue K concentration was attributed to increased levels of Ca and Mg as a result of large dolomite addition. The results also suggest that possible N and K deficiency leading to decrease sesame productivity could occur if continuous sesame cropping is prolonged on upland fields converted paddy. Therefore, further research should focus on increasing N availability and establishing appropriate use of dolomite lime in continuous sesame cropping on upland field converted paddy for sustainable sesame production.

CHAPTER SIX

Growth and cation uptake of sesame seedlings as affected by balancing soil cations of continuously monocropped soil from upland fields converted paddy

6.1. Introduction

Sesame (*Sesamum indicum* L.) is an important oilseed crop cultivated worldwide (Ashri, 1989). However, the productivity of sesame depends on the availability of mineral nutrients such N, P, and K applied as chemical fertilizer in the soil (Shehu et al., 2009). For instance, seed yield of sesame was increased by supplying adequate K in soil, which was initially low in K (Jadav et al., 2010). Potassium is one of the nutrients that limit sesame productivity. Potassium is a monovalent cation essential for growth of higher plants and protein synthesis, and also it is the most abundant cation in plant tissues and plays an important role in various physiological and biochemical processes, including photosynthesis (Munson, 1985; Leigh and Wyn Jones, 1986). The availability of K is affected by several factors. Usually, potassium nutrition in plants is affected by balance of both calcium and magnesium. A deficiency of one element would imply an excess of the other in plants. According to Bergmann (1992), Mg/K ratio in the soil is important for uptake of mineral nutrients by plants because excess concentrations of one can negatively influence plant growth. Loide (2004), reported that incorrect use of lime fertilizers alter the Ca/Mg and Mg/K ratios detrimental to plants through decreasing yields. Therefore, it is important to maintain the balance of soil cations for crops that show poor growth due to the imbalances.

Sesame requires high concentration of K in the tissue, between 1.5 to 2.4%, and low K in the soil caused poor growth (Mitchell et al., 1974). However, the imbalance in exchangeable cations resulted into growth and yield decline of sesame due to decrease in the leaf tissue K content of continuous monocropping (see Chapter Five). The decrease in K uptake was attributed to increase in soil exchangeable Ca and Mg in the long duration continuous monocropping for 4 to 6 years that caused competitive ionic effect in the soil. Moreover, the cation ratio of Ca/K and Mg/K significantly increased leading to relatively higher ratio of both Mg/K and Ca/K in the sesame leaf tissues suggesting low content of plant K. Furthermore, Hannan (2011) reported that magnesium ions could cause K deficiency in grapevine plant at the ratio of K/Mg of less than 0.30 in the soil. Hence, care should be taken while supplying high amount of Ca and Mg in soil in order to avoid cation imbalances.

The imbalances in base cation ratios could be corrected through increasing concentration of the limiting nutrient to raise the base saturation percentages to optimal ranges. The ideal range was stated as 65% Ca, 10% Mg and 5% K, resulting into ratios of Ca/K as 13, Ca/Mg as 6.5 and Ca/Mg as 2 and any deviations in one could cause a deficiency of another (Hodges, 2010). Balancing the ratios through adding more nutrients is important in correcting a deficiency of a particular cation. For instance, increasing the concentration of K in the soil in which it was limited by high exchangeable Mg content, led to improved cotton growth (Pettiet, 1988). Furthermore, high yields of annual grass was achieved with soil exchangeable sites occupied by 50 to 60% Ca, 8-12%, by Mg and 4-5% by K, and these base saturations allowed better uptake of K (Zalewska et al., 2018). Although the cation ratios in continuous monocropped sesame soils are reported to change, the effect of balancing these ratios on growth of sesame is still unknown.

Several potassium fertilizers including muriate of potash could supply adequate soil exchangeable K in continuously monocropped soil. However, other organic materials such as biochar could be used to balance soil nutrients together with K inorganic fertilizers. Biochar is a soil amendment produced from thermal degradation of these organic materials through pyrolysis and can supply high amount of potassium (Lehmann and Joseph, 2009; Biederman and Harpole, 2013). Therefore, biochar addition could be used to supply the extra K required to improve sesame growth on continuously monocropped upland field converted paddy soil.

The objective of this study was to determine the effect of balancing the base saturations of continuously monocropped sesame soils from upland field converted paddy on the growth and nutrient uptake of sesame seedlings under different cropping years.

6.2. Materials and Methods

6.2.1. Collection, preparation and balancing cations of continuously monocropped soils from the field

6.2.1.1 Soil collection and preparation

Continuously monocropped sesame soils were collected from the fields of Tottori (35°29′14.85″N, 134°07′47.01″E), Japan on 6 May 2017. The collected samples were dug at a depth of 15 cm in the rhizosphere soils of continuous monocropping of sesame for 1 year (1-yr, soil used to crop sesame for the last one year), 2 years (2-yr, soil used to crop sesame for the last two years) and 4 years (4-yr, soil used to crop sesame for the last four years). The soil samples were air dried, after removing all impurities, crushed and sieved through 2 mm mesh before use in the experiment.

6.2.1.2 Balancing soil cations

The collected continuously monocropped sesame soils were analyzed for exchangeable cation prior to cation balancing (**Table 23**).

Table 23. Soil chemical properties of the different continuously monocropped soils (1, 2 and 4-yrs) before experiment.

Year	K mg kg^{-1}	Ca (mg kg^{-1})	Mg (mg kg^{-1})
1	171.4	1143	244.0
2	142.7	1432	320.3
4	114.1	1645	323.0

Analysis of soil indicated decrease in exchangeable K whereas exchangeable Ca and Mg were high in the 2 and 4-yr cropping soils compared with 1-yr soil which necessitated soil cation balancing. According to the results of the soil chemical properties in **Table 23**, the cation ratios in the 1-yr soils were as Ca/K 6.7, Mg/K, 1.4 and Ca/Mg 4.7, in 2-yr soils; Ca/K 10.0, Mg/K 2.3 and Ca/Mg 4.5; and in the 4-yr soils as Ca/K 14.6, Mg/K 2.9 and Ca/Mg 5.1. This showed that the Ca/K and Mg/K ratios were higher than the acceptable range (Hodges, 2010). Therefore, balancing cations was aimed at achieving optimal base saturations (CaO, 75%; MgO, 25% and K_2O, 10%) to decrease the wide Ca/K and Mg/K ratios. In order to balance the soil cation ratios, the soil cation exchange capacity (CEC) of each continuously monocropped soil was determined and the formula below was used to calculate the deficit or surplus of each cation. However, the individual cation was converted into the oxide to ease calculations, i.e. Ca to CaO, Mg to MgO and K to K_2O and used kg $10a^{-1}$ instead of kg ha^{-1}.

Formulae;

CaO in kg $10a^{-1}$ =

$$\left(\left(\left(\frac{CEC\ x\ optimal}{100}\right) x\ 28\right) - CaO\ in\ \frac{mg}{100g}\right) x\ prov.specific\ gravity\ x\ \frac{soil\ depth}{10} cm \ \ldots.. \text{(1)}$$

K_2O in kg $10a^{-1}$ =

$$\left(\left(\left(\frac{CEC\ x\ optimal}{100}\right) x\ 47\right) - K2O\ in\ \frac{mg}{100g}\right) x\ prov.specific\ gravity\ x\ \frac{soil\ depth}{10} cm \ \ldots.. \text{(2)}$$

MgO in kg $10a^{-1}$ =

$$\left(\left(\left(\frac{CEC\ x\ optimal}{100}\right) x\ 20\right) - MgO\ in\ \frac{mg}{100g}\right) x\ prov.specific\ gravity\ x\ \frac{soil\ depth}{10} cm \ \ldots.. \text{(3)}$$

Where; optimal = optimal base saturations, prov. specific gravity = provisional specific gravity of soil. Note: optimal base saturations were CaO = 75%, MgO = 25% and K_2O = 10%. CaO meq = 28, MgO meq = 20 and K_2O meq = 47. Provisional specific gravity = 1.2 and soil depth = 15 cm. Any values in negative was considered a surplus.

The calculated deficient or surplus in **Table 24** were then converted to mg/100g of soil considering 1 ha = 1800,000,000 g of soil. According to the results, CaO was deficient by 28.9, and 8.22 mg/100g in 1-yr and 4-yr soils respectively, whereas MgO was deficient by 4.35, and 2.96 mg/100g in 1-yr and 4-yr soils respectively and K_2O was deficient by 21.66 25.29, and 39.6 mg/100g in 1-yr , 2-yr and 4-yr soils respectively.

Table 24. Soil CEC, cation oxides and calculated deficient or surplus amount of each soil exchangeable cation for different continuously monocropped soils of 1, 2 and 4-yrs.

		mg/100 g soil			Deficit or surplus		
Year	CEC ($\mathrm{cmol_c\ kg^{-1}}$)	CaO	MgO	K_2O	CaO ($\mathrm{kg\ 10a^{-1}}$)	MgO ($\mathrm{kg\ 10a^{-1}}$)	K_2O ($\mathrm{kg\ 10a^{-1}}$)
1	9.00	160.1	40.7	20.6	52.1	7.82	39.0
2	9.04	200.5	53.4	17.2	-19.2	-14.7	45.5
4	11.4	230.3	53.8	13.7	14.8	5.33	71.3

Negative = surplus and therefore no additional cation

Since the pot size was 15 cm diameter and carrying 740 g of dry soil, in the 1-yr soil, 305.8 mg (74 kg $10a^{-1}$) of CaO from quick lime was added and in the 4-yr soil, 86.9 mg (21 kg $10a^{-1}$) of CaO was added. In the 1-yr soil, 53.6 mg (13 kg $10a^{-1}$) of MgO from inorganic fertilizer of magnesium (60%, MgO) was added and 36.5 mg (21 kg $10a^{-1}$) of MgO was added into the 4-yr soil. 2-yr soil did not require additional CaO since it showed surplus (**Table 24**). For balancing of K, in the 267.2, 311.9, and 488.9 mg of K_2O were added from muriate of potash (60% KCl) into the 1, 2 and 4-yr soils respectively to balance the soil exchangeable K ratio. In kg $10a^{-1}$, these rates of K_2O are 65, 76, and 119 kg of K_2O inorganic fertilizer for 1-yr, 2-yr and 4-yr soil balancing respectively.

For balancing exchangeable K with biochar, rice husk biochar was used. The rice husk biochar had a pH (1:5 water) of 10.47; EC (1:5 water), 1.66 dS m^{-1}; C, N and C/N ratio of 39.8%, 0.51% and 78.3, respectively; available P (Truog-P), 647.9 mg kg^{-1}; exchangeable K, 3640.7 mg kg^{-1}; exchangeable Ca, 1207. 8 mg kg^{-1}; exchangeable Mg, and 369.3 mg kg^{-1}. For the biochar addition, to balance the soil exchangeable K (supplemental K), 36.5 g (88.9 kg $10a^{-1}$), 42.7 g (103.8 kg $10a^{-1}$) and 66.9 g (162.6 kg $10a^{-1}$) respectively of rice husk biochar. There was no additional K from KCl added into biochar treatment.

The balancing treatments included the control (normal soil), adjusted with inorganic fertilizer (CaO and K_2O), and biochar addition.

6.2.2. Experiment design, sesame cultivation and growth determination

The experiment was set up in a 3 x 3 consisting of three levels of balancing treatments and the three continuously monocropped soil of 1-yr, 2-yr and 4-yr collected from sesame field, laid out in a randomized block design with three replicates under greenhouse condition of the Tottori University, Japan. To all pots, were added basal inorganic fertilizer $N–P_2O_5–K_2O$ of 70–105–70 kg ha^{-1} in the combination of both cyclo-diurea (CDU) compound fertilizer (15%-15%-15%) and triple superphosphate (34%).

Sesame cultivar 'Maruhime' was sown on the 8th September 2017 in pots in the greenhouse. This cultivar was selected because it is sensitive to continuously monocropped soil. All pots were watered twice a day maintaining relative moisture content of 60%. At 40 days after sowing, the sesame plants were harvested (17 October 2017). Prior to harvesting, the plant height and SPAD value were measured on 16 October 2017. Fresh weight was also determined after harvest and plant samples were oven dried at 72°C for one week until the plants attained a constant dry weight and dry weight was determined.

6.2.3. Plant and soil analysis

6.2.3.1. Plant cation nutrient uptake in sesame plants

All sampled sesame were oven dried at 72°C until a constant weight was attained (after one week). Samples were then ground to fine powder and digested in a mixture of concentrated H_2SO_4 (98%) and H_2O_2 (30%). Briefly, 50 mg of plant sample was added into digestion test tubes and 1 mL of concentrated H_2SO_4 (98%) was added. The test tubes were allowed to stay overnight and digested by heating on the digestion block to 180°C. During the digestion, 0.6 mL of H_2O_2 (30%) was periodically added after 2-3 h from the start of heating. The H_2O_2 (30%) was to ensure complete digestion until the solution became clear. The solution was cooled and transferred to 50 mL volumetric flasks and brought to mark with RO water. The plant K, Ca, and Mg concentration in the solution was determined by using an atomic absorption spectrophotometer with known standards (Model Z-2300; Hitachi Co.). The cation uptake was calculated from the concentration and the dry weight of each sesame plant in the treatment.

6.2.3.2. Soil exchangeable cations and cation exchange capacity

After removal of plants from each pot, soil samples were air-dried, crushed and all residues removed and sieved through 2 mm sieve and stored for analysis. Soil exchangeable cation potassium K, calcium Ca and magnesium Mg were extracted using 1 N ammonium acetate solution (NH_4OAC) pH adjusted to 7.1 and extracted cations measured using atomic absorption spectrophotometer. Briefly, 2 g of each soil samples were weighed and saturated with 30 mL of neutral normal NH_4OAC (1N, pH 7.1), shaken on a mechanical shaker for 15 minutes and then centrifuged at 3,000 rpm for 3 minutes. The supernatant was collected into a 100 mL flat bottomed flask over a filter paper (Advantec 110 nm). To the remaining residue, was added 30 mL of neutral normal NH_4OAC (1N, pH 7.1) and centrifuged at 3,000 rpm for 3 minutes and supernatant filtered again into the flask. The procedure was repeated with 30 mL neutral normal NH_4OAC (1N, pH 7.1) and again filtered. The total filtrate collected, about 90 mL in the flask was brought to mark with excess neutral normal NH_4OAC (1N, pH 7.1). The filtrated was again filtered using a 0.45 μ pore filter paper suing a syringe before analysis of cation on an atomic absorption spectrophotometer with known standards (Model Z-2300; Hitachi Co.).

The cation exchange capacity (CEC) was measured by the 1N (pH 7.1) ammonium acetate (NH_4OAc) extraction methods (Chapman, 1965) and expressed to $cmol_c\ kg^{-1}$ soil. 2 g of the soil were saturated with neutral normal NH_4OAC (1N, pH 7.1), shaken for 15 minutes and filtrate was discarded and excess NH_4OAC was removed by washing thrice with 80 % methanol. The NH_4^+- saturated soil was equilibrated with 10% KCl and shaken for 15 minutes and filtered. The filtrate was used for the determination of NH_4^+- by micro-kjeldahl distillation and the NH_3 liberated was collected in 4% boric acid (with mixed indicator-red ethanol solution) and titrated with standard 0.1 N H_2SO_4. The titre was used for the determination of the CEC of the soil samples.

The soil base saturations were calculated as the sum of Ca^{2+}, + Mg^{2+} and K^+. The Na^+ measurements were not considered in the calculation of base saturation because of very low concentrations (<1% of the CEC). The exchangeable base saturations were calculated as in equation 2.

$$\text{Exchangeable base saturation percentage} = \frac{\text{Exchangeable cation}}{\text{CEC}} \times 100 \text{ .... (2)}$$

6.2.4. Statistical analysis

All experimental data presented are the means of three replicates. All data were analysed using one-way analysis of variance (ANOVA) using SPSS version 22.0 (SPSS for windows Inc., Chicago, Illinois, USA). Tukey's multiple comparison test at $p < 0.05$ was used to compare means. When considering the differences between the continuous cropped soil years, a two-way analysis of variance was used with the different amelioration treatments and cropping considered as two fixed factors.

6.3. Results

6.3.1. Effect of soil cation balancing on the fresh weight, SPAD value, plant height and dry weight of sesame seedling in the greenhouse experiment

The effect of balancing soil cations and continuous monocropping soil was significant in overall fresh weight (**Table 25**). Although fresh, dry weight and plant significantly decreased in the 4-yr soils compared to those in 1-yr, adding more K fertilizer and rice husk biochar to supply K while balancing cation ratios significantly increased fresh and dry weight with more pronounced effect in 4-yr than in 1 and 2 years soils (**Figure 39, 30)**.

For instance, the plant height, fresh weight and dry weight increased by 24.1, 35.6 and 40.4% respectively for the biochar addition, whereas the plant height, fresh weight and dry weight increased by 18.1, 25.9 and 17.8% respectively in the adjusted soil with inorganic fertilizer suggesting balancing with biochar was more beneficial than with inorganic fertilizers in the 4-yr soils. There was no significant differences between balancing with biochar and K fertilizer. In addition, the SPAD value was not affected by the balancing treatments. On the other hand, plant height was only significantly affected by balancing treatments with more growth observed in the K adjusted and biochar treatments compared with the control. Plant height response to mineral balancing was more effective in the 4-yr soils than 1 and 2 years. There was no significant effect of continuously monocropped soils on the plant height although the growth was non significantly decreased with increase in the duration of continuous monocropping years.

Table 25. The SPAD value and fresh weight of sesame plants under the different balancing treatments in the different continuously monocropped soils of 1, 2 and 4-yrs.

Year	Treatment	SPAD value	FW (g/plant)
1	Control	47.8a	15.4a
	Fertilizer	40.8a	16.0a
	Biochar	45.4a	17.5a
	ANOVA (*p*-values)	ns	ns
2	Control	43.1a	14.3a
	Fertilizer	42.5a	15.9a
	Biochar	41.8a	16.2a
	ANOVA (*p*-values)	ns	ns
4	Control	41.6a	10.0b
	Fertilizer	42.3a	13.5ab
	Biochar	44.0a	15.5a
	ANOVA (*p*-values)	ns	*
Source of variation			
Treatment (Y)		ns	*
Year (T)		ns	*
YxT		ns	ns

Means followed by different within a column in the same cropping are significantly different at $p < 0.05$ according to the Tukey test. *** Significant at $p < 0.001$; ** Significant at $p < 0.01$; * Significant at $p < 0.01$; ns, Non-significant.

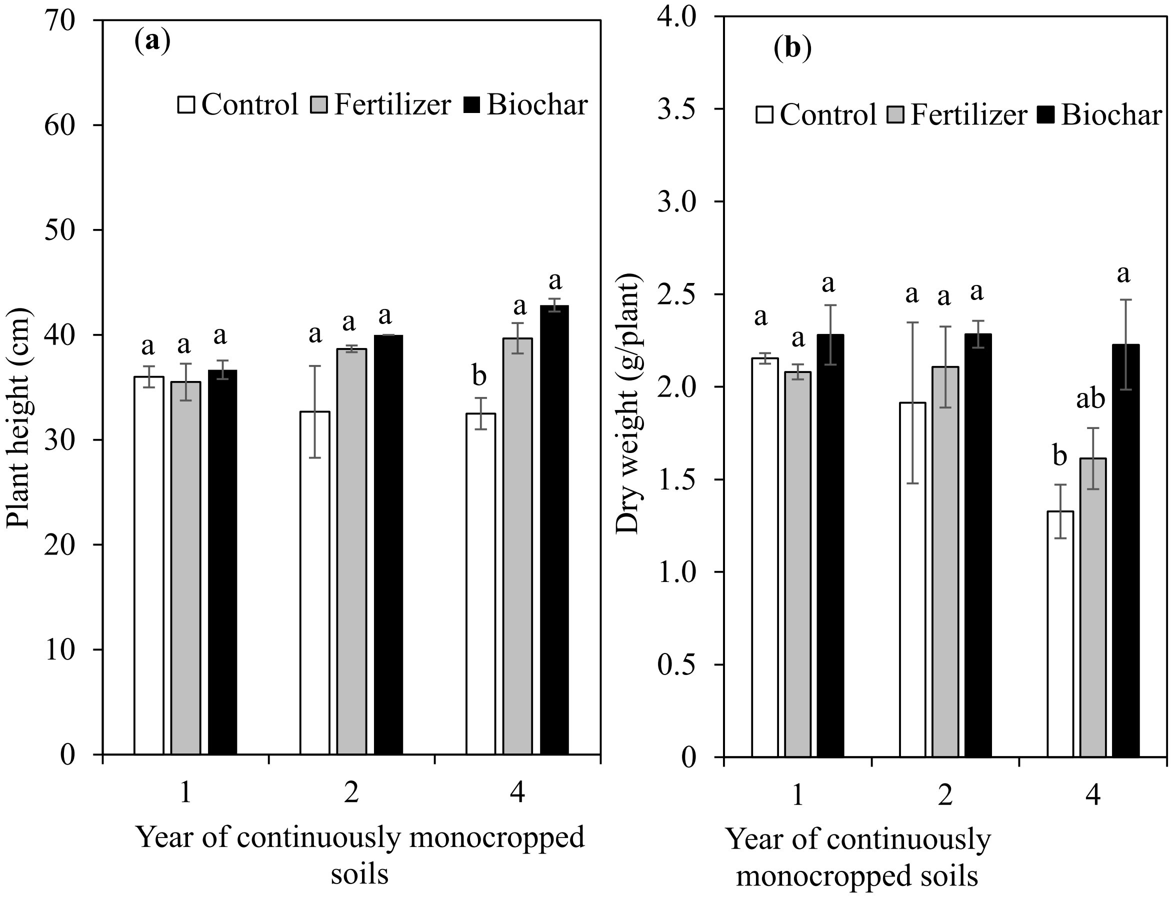


Figure 29. (a) Plant height and (b) dry weight per plant of sesame plants as affected by balancing of soil cation imbalance with inorganic adjustment and biochar addition. The error bars represent standard errors (*n* = 3). Different letters on each bar are significantly different at ($p < 0.05$).

Addition of inorganic fertilizers to balance for cations and rice husk biochar showed a non-significant increase in the plant height of sesame seedlings in the 1 and 2-years continuously monocropped soils whereas a significant increase in the plant height occurred with balancing the 4-year continuously monocropped soil. However, there was no significant differences between balancing with inorganic fertilizer and biochar in the 4-year continuously monocropped soil.

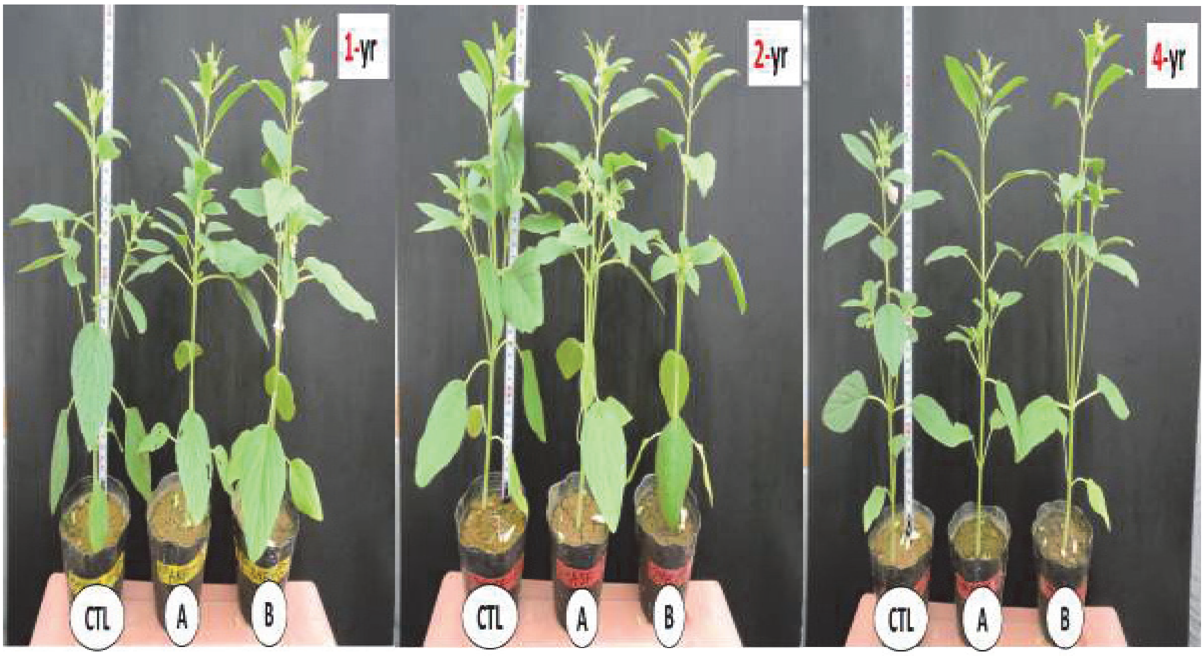


Figure 30. Plant heights of sesame plants in 1, 2 and 4-yr soils after harvest as affected by amelioration of soil cation imbalance with inorganic fertilizer and biochar addition. CTL, control; A, balanced with additional fertilizer; B, balanced with rice husk biochar.

6.3.2. Effect of soil cation balancing on the cation concentration and uptake in the shoot of sesame seedling grown in continuously monocropped soils in green house experiment

The effects of balancing soils on the plant nutrient concentration of K, Ca, Mg and their uptake are shown in **Table 26**. In the 1-yr soil, the balancing of soil showed a significant difference between the control, adjusted and the biochar addition treatments in the concentration of K and the uptake of K in the shoot tissue. The concentration of K was significantly increased by 29.8% in the adjusted with the inorganic fertilizer and 22.2% with biochar addition. Similarly the uptake of K was significantly increased by 27.3% in the adjusted with inorganic fertilizer and by 26.7% in the rice husk biochar addition suggesting balancing significantly increased concentration and uptake of K by the sesame plant in the 1-yr soil. There was no significant effect of balancing on the plant Ca and Mg concentrations and their uptake. In the 2-yr soil, the concentration of K was significantly increased by 29.7% in the adjusted with inorganic fertilizer and 19.0% with the biochar addition. Similarly the uptake of K was significantly increased by 38.5% in the adjusted with inorganic fertilizer and by 35.0% in the rice husk biochar addition suggesting balancing significantly increased concentration and uptake of K by the sesame plant in the 2-yr soil. Conversely, the concentration of Ca was significantly decreased by 1.2% in the adjusted with inorganic fertilizer and 52.8% with biochar addition but Ca uptake showed no significant differences among treatments. There was no significant effect of balancing on plant Mg concentration and uptake.

In the 4-yr soil, concentration of K was significantly increased by 19.6% in the adjusted with inorganic fertilizer and 5.4% with biochar addition. Similarly, the uptake of K was significantly increased by 34.2% in the adjusted with inorganic fertilizer and by 43.4% in the rice husk biochar addition, suggesting balancing significantly increased concentration and uptake of K by the sesame plant in the 2-yr soil. The concentration of Ca was significantly increased by 3.9% in the adjusted with inorganic fertilizer but decreased by 62.0% with biochar addition but Ca uptake showed no significant differences among treatments. In addition, there was no significant effect of balancing on plant Mg concentration and uptake.

The analysis of variance showed that balancing soil cations significantly improved the plant K concentration and uptake that was initially decreased by continuous monocropping as observed in the unbalanced soils. In addition, balancing soil cations with additional K and rice husk biochar could decrease the concentration and uptake of Ca.

Table 26. The concentrations and uptake of K, Ca and Mg of sesame plants under the different amelioration treatments in the different continuously monocropped soils of 1, 2 and 4-yrs.

		Concentration (%)			Uptake (mg/plant)		
Cropping	Treatment	K	Ca	Mg	K	Ca	Mg
1	Control	2.88c	1.95a	0.37a	62.0b	41.9a	8.01a
	Fertilizer	4.11a	1.90a	0.37a	85.3a	39.6a	7.78a
	Biochar	3.71b	1.66a	0.43a	84.6a	38.3a	10.20a
	ANOVA (*p*-values)	***	ns	ns	*	ns	ns
2	Control	2.43b	1.95a	0.49a	44.6b	36.1a	9.87a
	Fertilizer	3.45a	1.92ab	0.35a	72.5a	40.2a	7.43a
	Biochar	3.0ab	1.27b	0.43a	68.6ab	29.4a	9.73a
	ANOVA (*p*-values)	*	*	ns	*	ns	ns
4	Control	2.92b	2.31a	0.56a	38.4b	30.3a	7.64a
	Fertilizer	3.63a	2.41a	0.40a	58.3a	38.8a	6.57a
	Biochar	3.08ab	1.43b	0.36a	67.8a	31.2a	8.16a
	ANOVA (*p*-values)	*	*	ns	**	ns	ns
Source of variation							
Year (Y)		***	***	ns	***	ns	ns
Treatment (T)		***	*	ns	***	ns	ns
TxY		ns	ns	ns	ns	ns	ns

Means followed by different within a column in the same cropping are significantly different at $p < 0.05$ according to the Tukey test. *** Significant at $p < 0.001$; ** Significant at $p < 0.01$; * Significant at $p < 0.01$; ns, Non-significant.

6.3.3. Effect of soil cation balancing on the soil exchangeable cations, cation ratios and bases saturations of continuously monocropped soils in greenhouse experiment

The cation balancing treatments significantly increased soil exchangeable K content by 243.9 mg kg^{-1} in the fertilizer balancing and by 517.8 mg kg^{-1} in the biochar addition compared with the control (**Table 27**). In addition, the exchangeable Ca content was higher in the adjusted and biochar addition than the control. On other hand, balancing cations did not affect soil exchangeable Mg content in 1-yr soil. Similarly, exchangeable K increased by 252.6 mg kg^{-1} in the adjusted soil and by 665.0 mg kg^{-1} in the biochar addition balancing compared to the control in the 2-yr soils. Conversely, exchangeable Ca significantly decreased in the balanced soils compared to control with lowest value (3302 mg kg^{-1}) in the rice husk biochar treatment. Soil exchangeable Mg was not significantly affected by the balancing treatments. Furthermore, the exchangeable K in the 4-yr soil was significantly higher in the biochar addition (1046.3 mg kg^{-1}) than in the adjusted with inorganic fertilizer (542.5 mg kg^{-1}) suggesting increasing K levels with biochar addition whereas the exchangeable Ca was significantly highest in the adjusted with inorganic fertilizer (6386.1 mg kg^{-1}). The results showed that the treatments and cropping soils affected the soil exchangeable K and Ca levels and the effect showed significant interactions indicating increase in exchangeable K and decrease in Ca with balancing. In addition, continuous monocropping soils significantly showed decrease in exchangeable K and increase in Ca.

Balancing soil cations with additional K fertilizer and rice husk biochar significantly decreased the soil Ca/K, Mg/K and Ca/Mg ratios except in the 4-yr soil where Ca/Mg ratio was unaffected (**Table 28**). However, there were no significant differences between balancing with adjusted inorganic fertilizers and biochar addition for the Ca/K and Mg/K ratios. The Ca/K ratio was significantly decreased from 22.0 to 9.1 in the yr-1 soil, and from 42.3 to 10.4 in the 2-yr soil and then from 50.2 to 11.8 in the 4-yr soil with treatment and cropping showing significant effect and interactions. Similarly, the Mg/K ratio decreased from 2.3 to 0.8 in the 1-yr soil, from 3.8 to 1.0 in the 2-yr soil and from 3.3 to 0.7 in the 4-yr soil. The Ca/Mg ratio was increased by the adjustment with inorganic fertilizer in the 1-yr soil whereas it was decreased in the 2-yr soil.

Table 27. The soil chemical properties under the different amelioration treatments in the different continuously monocropped soils of 1, 2 and 4-yrs.

Year	Treatment	K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})
1	Control	129.6c	2753b	281.7a
	Fertilizer	373.5b	3347a	296.5a
	Biochar	647.5a	3329a	299.4a
	ANOVA (*p*-values)	***	**	ns
2	Control	98.1c	3921a	352.0a
	Fertilizer	350.7b	3644a	343.4a
	Biochar	763.1a	3302b	331.5a
	ANOVA (*p*-values)	***	**	ns
4	Control	117.4c	5645a	373.5a
	Fertilizer	542.5b	6386a	386.8a
	Biochar	1046.3a	4623b	333.9a
	ANOVA (*p*-values)	***	**	ns
Source of variation				
Year (Y)		***	***	*
Treatment (T)		***	***	ns
T xY		***	***	ns

Means followed by different within a column in the same cropping are significantly different at $p < 0.05$ according to the Tukey test. *** Significant at $p < 0.001$; ** Significant at $p < 0.01$; * Significant at $p < 0.01$; ns, Non-significant.

On the other hand, soil CEC was unaffected by balancing treatments whereas the soil base saturations of K was significantly increased in the 1, 2 and 4-yer soils with higher increment in the 4-yr soil than the other soils, mostly in the biochar balance treatment. The Ca and Mg saturations increased in the adjusted with inorganic fertilizer the 4-yr soil significantly by 35.8% and 1.9% respectively compared to the control whereas the biochar addition had negative effect on the these base saturations. The analysis of variance showed that the effect of continuous monocropping years had significant increase in the Ca/K, Mg/K and Ca/Mg ratios but balancing soil cations by additional K fertilizers and rice husk biochar significantly decreased these ratios. In addition, balancing cations with biochar increased K saturation while decreasing Ca saturation more than with additional K fertilizer.

Table 28. The soil exchangeable cation ratios, CEC and base saturations under the different balancing treatments in the different continuously monocropped soils of 1, 2 and 4-yrs.

Year	Treatment	Ca/K	Mg/K	Ca/Mg	CEC (cmol$_c$ kg^{-1})	K sat (%)	Ca sat (%)	Mg sat (%)
1	Control	21.96a	2.26a	9.76b	13.41a	2.55c	103.45a	17.63a
	Fertilizer	9.10b	0.80b	11.33a	13.28a	7.26b	126.14a	18.62a
	Biochar	5.18b	0.47b	11.12ab	13.81a	12.04a	121.47a	18.21a
	ANOVA (*p*-values)	**	***	*	ns	***	ns	ns
2	Control	42.29a	3.79a	11.15a	14.70a	1.74c	133.93a	20.03a
	Fertilizer	10.41b	0.98b	10.61a	12.84a	7.06b	142.58a	22.42a
	Biochar	4.35b	0.44b	9.96b	13.21a	14.83a	125.30a	20.95a
	ANOVA (*p*-values)	**	**	**	ns	***	ns	ns
4	Control	50.26a	3.33a	15.11a	14.89a	2.06c	191.46ab	21.09a
	Fertilizer	11.83b	0.72b	16.63a	14.05a	9.90b	227.31a	22.96a
	Biochar	4.42b	0.32b	13.84a	14.94a	17.98a	154.62b	18.63a
	ANOVA (*p*-values)	***	***	ns	ns	***	**	ns
Source of variation								
Year (Y)		**	ns	***	ns	***	***	**
Treatment (T)		***	***	*	ns	***	***	ns
Y*T		**	ns	*	ns	***	**	ns

Means followed by different within a column in the same cropping are significantly different at $p < 0.05$ according to the Tukey test. *** Significant at $p < 0.001$; ** Significant at $p < 0.01$; * Significant at $p < 0.01$; ns, Non-significant.

6.4. Discussion

In this study, results indicated that balancing the soil with additional K fertilizer and biochar had no significant effect on the sesame plant height, fresh and dry weight in 1-yr and 2-yr soils (**Table 25** and **Figure 29**), suggesting short duration of continuous monocropping of sesame does not alter the cation ratios capable of causing nutrient imbalance. However, significant decrease in the plant highest, fresh and dry weight of sesame seedlings occurred in the 4-yr soils control when compared to balancing suggesting soil cation ratios of Ca/K, Mg/K and Ca/Mg were out of range capable of causing competitive ionic effect. Hence, balancing of cations with more K fertilizers and rice husk biochar alleviated competitive ion effect and supplied more potassium allowing increase in sesame growth. This result was consistent with other researches that showed balancing cation ratios, increase K availability significantly improved crop growth and yield (Pettiet, 1988; Zalewska et al., 2018). Moreover, the results also showed that balancing soil cations was more effective in rice husk biochar than inorganic fertilizers suggesting biochar could offer other benefits apart from increasing K. Biochar addition is known to increase crop growth (Carter et al., 2013; Alburquerque et al., 2013). Therefore, rice husk biochar addition could be recommended for improving sesame growth.

In this study, balancing the cation ratios using inorganic fertilizer and biochar addition significantly increased the exchangeable K in the soil, its concentration and uptake in the sesame plants (**Table 26**). The balancing treatments increased soil exchangeable K because of the additional K from the fertilizer and high K content of the biochar used. It is reported that rice husk biochar contain high amount of K that is readily available for plants indicated by its high ash content (Major et al., 2010; Abrishamkesh et al., 2015). This increase in availability of K was indicated by the increase in the soil K saturations that were more than 5% in both K balancing with fertilizer and rice husk biochar. Because K is also required for protein synthesis, which directly determines tissue growth (Leigh and Wyn Jones, 1986), the increased growth in the balanced soil indicates sufficient uptake of K suggesting that addition of more K in continuously monocropped sesame soils could improve yield under field conditions. Furthermore, results also showed that inorganic fertilizer and biochar balancing significantly decreased the soil Ca/K, Mg/K and Ca/Mg ratios indicating increase in availability of exchangeable K for sesame plant (**Tables 27, 28**). The Ca/K and Mg/K ratios decreased to acceptable levels of less than 13 with balancing treatments that is consistent with the report of Hodges (2010). The additional K could readily be supplied either in the form of K fertilizers or rice husk biochar to balance the soil cations in continuous monocropping sesame. On the other hand, the increasingly high cation ratios (Ca/K, Mg/K) in the control especially in the 4-yr continuously monocropped soil compared with the inorganic fertilizer and biochar adjustments balancing could be attributed to the increase in exchangeable Ca and Mg supplied through dolomite in the continuous monocropping fields (Anderson et al., 2013). The Ca/Mg ratio was increased by the adjustment with inorganic fertilizer in the 1-yr soil whereas it was decreased in the 2-yr soil possibly due to the addition of extra Ca in the 1-yr soil while there was no more addition in the 2-yr soil. Hence, the decrease in the growth of the control could be mainly attributed to lack of absorption of K since the uptake of K was lower than in biochar and K inorganic fertilizer additions due to high Ca and Mg. This study agrees with the findings that high exchangeable Ca content in the soils could lead to competitive ionic effect between Ca, Mg and K (Garcia et al., 1999). The results also consistent with the report of Huu Nguyen et al. (2017) that high contents of Mg in the soil could inhibit the uptake of K in tomato plants. It was also demonstrated that the content of plant Ca ions depends on the lime content in the soil and excess of this could hinder physiological roles of potassium in plants (Gluhic et al., 2009). The high content of exchangeable Ca and Mg in the unbalanced soil significantly affected the uptake of K suggesting that decreasing dolomite application could alleviate ionic competition effect. Since poor nutrition of K occur in sesame plants when Ca/Kg and Mg/K ratios are high (Kumar and Sharma, 2013), in the balanced soil, increased uptake of K occurred indicating of continuous monocropping obstacles of sesame could be mitigated through adding more K and reducing Ca and Mg to a balanced ratio. On the other hand, there was no significant effect of balancing on the uptake of Ca and Mg by sesame seedlings, and it could be speculated that changes in the Ca/Mg ratio does not affect sesame yield in the field. This was also consistent with the report that incising the Ca/Mg ratio does not necessarily affect yield (Rosanoff et al., 2015). In addition, it could suggest that balancing of soil cations should focus more on K than other Ca and Mg on continuously monocropped soils.

Although it is reported that if the soil contains adequate absolute quantities of Ca, Mg and K, the ratios of these cations will not usually affect crop yields in the ratios found in most agricultural soils (Kopittke and Menzies, 2007; Vieweger et al., 2017). This study indicated that the soil cation ratios under continuous monocropping of sesame could influence the growth and consequently yield. This finding agreed with Lombin (1981) who reported that soil Ca/K ratio influenced maize yield and there was a significant negative correlation between yield and exchangeable K while a significant positive correlation between yield and (Ca+Mg)/K ratio. The imbalance in the ratio caused Ca with low base saturation on soil to become a critical limiting nutrient for maize yield when higher rates of K and Mg were applied (Lombin, 1981). Hence, imbalances in mineral nutrition might limit the growth and yield of the crop with deviations in cation ratios. In this study, the high contents of Ca and Mg could easily displace K from the soil exchange complex inducing low uptake of K in a phenomenon referred to as 'competitive ion effect'. It is reported that a high magnesium and calcium content could induce to competitive ion effect in which K is less absorbed by plants (Hannan, 2011; Marschner, 2012). However, this imbalance in soil cations could be successfully mitigated with additional K from fertilizers and biochar which contain high amount of K, as observed in this study.

6.5. Conclusions

In this study, balancing the soil cation ratios with additional fertilizer and biochar significantly decreased the Ca/K and Mg/K ratios to acceptable levels suggesting more availability of K in the soil to enhance sesame growth. It was observed that balancing cations is more effective in the long duration of continuous monocropping (4-yr soil) than in short durations (1 and 2-yrs) suggesting cation imbalances occur in long term continuous monocropping of sesame on upland field converted paddy. The non-significant effect on the Ca/Mg ratio suggested that sufficient Ca and Mg existed in the soils and thus balancing of soil should focus more on K than other exchangeable cations in continuously monocropped sesame soils. In addition, the existence of exchangeable K might not necessarily mean its availability to sesame plant due to increase in Ca and Mg causing competitive ion effect. A balanced nutrition and fertilization should take into consideration the ratios among Ca, Mg and K for sesame production. This study indicated that increase in sesame growth could be recovered through balancing cations of continuously monocropped soil through either additional K fertilizers or rice husk biochar that could increase K saturations to above 5% to increase its availability.

CHAPTER SEVEN

Growth, seed yield, mineral nutrients and soil properties of sesame (*Sesamum indicum* L.) as influenced by biochar addition on upland fields converted paddy

7.1. Introduction

Sesame *(Sesamum indicum* L.) is an important oilseed crop cultivated worldwide for its edible oil and food (Ashri, 1989). The demand for sesame seeds is increasing due to the increasing knowledge on their dietary and health benefits (Dossa et al., 2017). However, sesame production is being hampered by continuous monocropping on upland fields converted paddy (see previous chapters). A study indicates that crop yields on upland fields converted from paddy may decrease due to declining soil fertility status of the paddy soils that could require soil amendment with organic materials (Nishida, 2016). Hence, addition of biochar soil amendment that has become popular in most farming systems across the world could restore the productivity of sesame on upland fields converted paddy.

Biochar is a soil amendment produced from thermal decomposition of organic materials through pyrolysis and it has the potential to increase crop yields (Lehmann and Joseph, 2009; Jones et al., 2012). Earlier research has shown that biochar addition can improve plant growth and soil quality (Lehmann et al., 2006; Chan et al., 2007; Major et al., 2010). The positive responses in crop yield on biochar addition were attributed to improved soil properties, such as a decrease in soil bulk density, and subsequent increase in porosity and water holding capacity (Lu et al., 2014; Nelissen et al., 2015), increase in the cation exchange capacity (CEC) which enhances the retention of basic nutrients (Lehmann et al., 2003), increased uptake of N and its availability in soil (Xu et al., 2015), adsorption of soil phytotoxins (Elmer and Pignatello, 2011; Rogovska, et al., 2014), liming effects (van Zwieten et al., 2010), and increased plant nutrient concentration (Uzoma et al., 2011; Woldetsadik et al., 2018). For instance, cultivation on sandy soils using biochar increased maize yield by 150% and 98% over the control at rates of 15 t ha^{-1} and 20 t ha^{-1} respectively (Uzoma et al., 2011). It has also been reported that rice husk biochar addition at 41.3 t ha^{-1} significantly increased rice grain yield by 16–35% attributed to increased water holding capacity, available N and cation exchange capacity

(CEC) (Haefele et al., 2011). Zhang et al. (2010) also reported an increase in rice yields of 14% over the control in paddy soils with wheat straw biochar rate of 40 t ha^{-1}. Furthermore, it has been shown that rice husk biochar addition rates of up to 50 t ha^{-1} significantly increased maize seed protein by 27% compared to without biochar while increasing plant height by 23% compared with control (Ali et al., 2017). The authors attributed these increases to the increase in soil fertility status improving nutrition required for maize grain quality improvement. There are also several reports on an increase in plant height, growth and grain quality with biochar application in crops (Alburquerque et al., 2013;Varela Milla et al., 2013; Baronti et al., 2010) , which indicate a positive effect of biochar on crops.

Biochar addition to soils is expected to promote sustainable crop production through a positive effect on yield, but these may depend on the cropping seasons. For instance, Cornelissen et al. (2018) studied the effect of rice husk biochar and cacao shell biochar applied to Indonesia Utisol soil at rates of up to 15 t ha^{-1} and found that the maize yield with rice husk biochar become lower and faded in the second cropping while with cacao shell biochar was highest in the second cropping through third and fourth, but faded in the fifth cropping seasons. In addition, biochar applied to soil and tested over four cropping seasons on acidic soil in Brazil showed positive effects in the first cropping, but these faded in the following cropping (Steiner et al., 2014). Carter et al. (2013) also reported a high yield of lettuce and cabbage in the first cropping, but yield decreased significantly in the third cropping with rice husk biochar rates of up to 167 t ha^{-1} field equivalent. Furthermore, rapeseed yield with wheat straw biochar faded after third cropping that suggested biochar needed to be applied after every three years to maintain positive effects on crop yield (Jin et al., 2019). These lack of positive responses of crop yield could be attributed to the changes in biochar chemistry over time as it ages in the soil environment (Mukherjee et al., 2014). Hence, the properties of biochar responsible for crop improvement may be altered consequently leading to no effect on growth and yield.

Several pieces of research have focused on biochar effects on grain crops, such as rice, maize, wheat and vegetable of which plant growth responses to biochar addition varied (Rogovska et al., 2014; van Zwieten et al., 2010; Haefele et al., 2011; Varela Milla et al., 2013; Alburquerque et al., 2013; Carter et al., 2013) . However, there is still a scarcity of information of biochar addition on sesame (Furtado et al., 2016; Ndor et al., 2015), indicating a need to generate understanding of how biochar addition can effectively be used to increase sesame production. An earlier research has shown that rice and saw dust biochar addition at 10 t ha^{-1} significantly increased sesame seed yield by 55.5% compared to without biochar attributed to improved soil physico-chemical properties, such as bulk density and porosity, increased pH, total N, K, Mg and CEC after biochar addition on a highly leached ultisols with low base saturation and strongly acidic soils (Ndor et al., 2015). Although in a pot experiment, biochar addition to sesame has been shown to significantly increase plant height at increasing rates where the optimal rate of 11.21 g kg^{-1} (equivalent to 22.42 t ha^{-1}) was obtained beyond which biochar decreased pant height becoming harmful to sesame growth (Furtado et al., 2016). The authors attributed this negative effect on sesame growth to increase in pH due to biochar application on already neutral soil that had pH 6.4 before the experiment. Furthermore, coconut shell biochar addition at 10 t ha^{-1} on sandy coastal has been shown to increase sesame yield when grown on sandy coastal soil (Nurhayati, 2017). Therefore, biochar addition shows positive results on sesame. However, crop responses to biochar application depend on biochar type, application rates, soil properties and climatic conditions (Hussain et al., 2017). It important to explore the utilization of biochar in sesame to understand how seed yield and growth are influenced on upland fields converted paddy in Japan under different field climatic conditions, paddy soils with low pH and higher biochar rates in order to close existing gaps on biochar use for sesame.

In this study, we hypothesized that biochar addition would increase sesame growth, yield and nutrient availability with increasing rates of biochar in first and second cropping. To investigate the effect of biochar addition on sesame performance, we cultivated sesame on two fields of first and second cropping on upland field converted from paddy. The specific objectives this study were to determine the effect of biochar addition on the (a) growth and yield of sesame in the first and second cropping; (b) leaf tissue nutrient concentration and seed mineral nutrient contents; and (c) soil physicochemical properties of the upland field converted paddy under continuous monocropping.

7.2. Materials and Methods

7.2.1. Location and site description

This field experiment was conducted in 2017 at the Tottori Prefecture, Japan (35° 29′ 14.85′ ′ N, 134° 07′ 47.01′ ′ E). Most precipitation occurred between June to September during the cultivation period. The total monthly rainfall received in the region in 2017 were 66.5, 158.5, 161 and 224.5 mm in June, July, August and September respectively. The average daily maximum temperatures were 24.2 °C in June, 30.6 °C in July, 30.5 °C in August and 26.0 °C in September favorable for sesame growing. The region has primarily one sesame crop harvested per year. The dominant soil at this site was classified as Cambisols (FAO/IIASA/ISRIC/ISS-CAS/JRC, 2012)

7.2.2. Soil and Biochar Properties

Analysis of the basic physicochemical properties of the soil samples (0–15 cm) taken from the experiment field before sowing in 2016 indicted that the topsoil had a pH (1:5 H_2O) of 5.39; electrical conductivity (EC) of 0.05 dS m^{-1}; bulk density, 1.27 g cm^{-3}; porosity, 49.09%; total C, 26.25 g kg^{-1}; total N, 2.29 g kg^{-1}; C/N ratio, 11.46; available P (Truog-P), 6.99 mg kg^{-1}; exchangeable K, 109.62 mg kg^{-1}; exchangeable Ca 1931.22 mg kg−1; and exchangeable Mg 383.29 mg kg^{-1}. The commercial rice husk biochar added to the study soils was manufactured and bought from a local store in Tottori, Japan. The rice husk biochar was surface applied by hand in June 2016 in the old field and then in June 2017 in the newly opened field before sesame sowing and immediately incorporated into the soil to a depth of 0–15 cm with base fertilizer utilizing a rotatory power tiller (**Figure 31**). The rice husk biochar had a pH of 10.47 and EC of 1.66 dS m^{-1} determined from biochar suspension (1:5 w/v, biochar: water) mechanically shaken for 1 h and measured with a pH and EC meter (Horiba Aqua Cond Meter F-74). Total C and N were analyzed by dry combustion on the CN-corder (Macro corder JM 1000CN, J-Science Co., Ltd., Kyoto, Japan) and reported as C, N and C/N ratio of 39.76%, 0.51% and 78.26, respectively. The rice husk biochar had available P of 647.94 mg kg^{-1} determined according to Truog method (Truog, 1930). Exchangeable K, Ca, and Mg in the rice husk biochar were K, 3640.73 mg kg^{-1}, 1207.78 mg kg^{-1}; and 369.26 mg kg^{-1} respectively, determined upon extraction of biochar with 1 N ammonium acetate (pH 7.1) and analysed by atomic absorption spectrophotometer (Model Z-2300, Hitachi Co., Tokyo, Japan). The biochar cation exchange capacity (CEC) was 7.53 $cmol_{c}$ kg^{-1}, measured by the 1N (pH 7.1) ammonium acetate (NH_4OAc) as described by Chapman (1965). The bulk density of the biochar was determined by measuring the weight of compacted biochar in 100 cm3 steel cylinders and was found to be 0.29 g cm^{-3} whereas the ash content was measured by igniting the biochar sample at 550 °C for 5 h in a muffle furnace and found to be 38.04%.

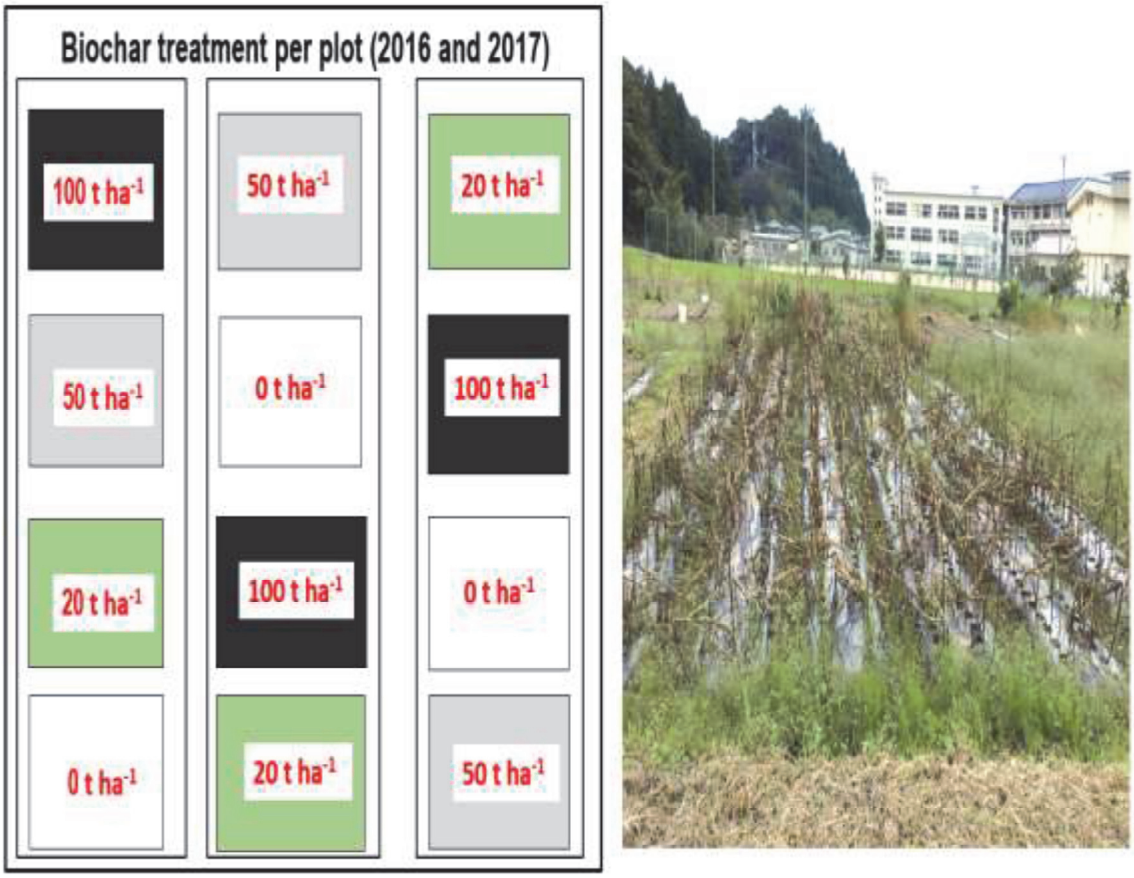
7.2.3. Field experimental design

In this study, the experiment was conducted on two different plots: One in which sesame was previously cultivated with rice husk biochar for one year (season) and a new field where sesame had not been cultivated before. Each of these fields measuring 10.5 m by 6.5 m were divided into micro plots measuring 2.4 m by 1.9 m (4.56 m^2) onto which biochar was incorporated. Each micro plot was separated by 0.4 m as buffer space. The one-year old sesame field had rice husk biochar rates of 0, 20, 50 and 100 t ha^{-1} applied already in the previous year's cultivation (**Figure 32a**). This field was under cropping in 2016 with biochar and sesame, but due to typhoon winds that destroyed the sesame plants, and data could not be collected (**Figure 32b**).

Therefore, in 2017, the new field opened adjacent to the old field received a similar amount of rice husk biochar treatments at the start of the experiment. The new field and old field are considered as first and second cropping respectively.

Prior to sowing, all fields were ploughed by a power tiller, harrowed to a fine tilth and basal inorganic fertilizer applied at a rate of N—P_2O_5—K_2O, 70:105:70 kg ha^{-1}, including dolomite ($CaMg(CO_3)_2$) at a rate of 1,000 kg ha^{-1}. Sesame cultivar 'Gomazou' was planted on ridges of 75 cm wide separated by 40 cm and plant spacing of 45 cm between rows and 15 cm between plants. Sowing date was 11 July 2017 in which five sesame seeds were sown per hole, then thinned to two plants at 14 days after sowing and then one plant per hole at 21 days after sowing. The fields were kept without weeds by hand weeding whenever necessary until harvesting at the on 22 September 2017. Growth was determined by measuring plant height, height of lowest capsule and number of branches while the seed yield and 1000-seed weight were determined after drying sesame seeds in a greenhouse.

Figure 31. (a) Rice husk biochar addition, (b) treatment plots, (c and d) cultivation ridge showing sesame seedlings, (e) sesame plants at juvenile stage and (f) sesame plants at flowering.



(a)

(b)

Figure 32. (a) Biochar treatment and (b) sesame plants damage by typhoon winds in 2016 prior to harvest.

The growth and seed yield were determined by randomly selecting ten plants per replicates in each treatment whereas the total number of seeds per plant was calculated after obtaining a weight of 1000 seeds and total seed weights from 10 plants.

7.2.4. Sampling and Analyses

7.2.4.1. Plant Sampling

For leaf tissue nutrient concentration analysis, three representative plants were selected at the reproductive stage (50 Days After Sowing) whereas remaining plants were used for growth and yield determination at harvest. Mature leaves from the representative samples were separated from stem and roots and oven dried at 72 °C until a constant weight was attained (after a week). Leaf samples were then ground to fine powder and digested in a mixture of concentrated H_2SO_4 (98%) and H_2O_2 (30%) for P, K, Ca and Mg concentration. Plant P concentration was determined colorimetrically with a spectrophotometer at 420 nm (Model U-5100, Hitachi Co., Tokyo, Japan). Plant K, Ca, and Mg concentration was determined by using an atomic absorption spectrophotometer (Model Z-2300, Hitachi Co., Tokyo, Japan). The plant N was determined from the ground sample with the dry combustion method on a CN Corder machine (Macro corder JM 1000CN, J-Science Co., Ltd., Kyoto, Japan).

For the seed mineral nutrient, the analysis was conducted on sesame seeds after harvesting and drying. Seed mineral nutrient concentrations were determined by means of dry ashing as described by Estefan et al. (2013). Ground sesame seed samples (1.0 g) was placed in a crucible, ignited, and burnt to ash in a muffle furnace at 550 °C for 5 h. The ash was cooled and dissolved into 5 mL of 2 N HCl, diluted to 50 mL in a volumetric flask with reverse-osmosis water, and filtered through Advantec 110-mm filter paper. Phosphorous (P) concentration was determined from the filtrates by means of the ammonium vanadate-ammonium-molybdate yellow colorimetric method using a spectrophotometer (Model U-5100, Hitachi Co., Tokyo, Japan), with absorbance measured at 420 nm. The seed K, Ca and Mg contents were determined from the filtrates by means of atomic absorption spectrophotometry (Model Z-2300, Hitachi Co., Tokyo, Japan). The seed N concentration was measured by means of the dry combustion method using a CN-corder (Macro corder JM 1000CN, J-Science Co., Ltd., Kyoto, Japan) and the values of total N values (%) were multiplied by the N factor 6.25 to obtain crude protein values (N × 6.25%) (Mitchell et al., 1976).

7.2.4.2. Soil Sampling

To evaluate the effect of the rice husk biochar addition on soil physical properties, soil samples from fields were collected at 0–15 cm from the sesame fields after harvest. Three soil phases in each biochar treatment of the first and second cropping were calculated from two samples collected per replication at the top 10 cm layer by applying a soil three-phase meter (Model DIK-1130, Daiki Rika Kogyo Co., Ltd., Saitama, Japan) to a 100 cm^3 undisturbed soil core. The surface soil bulk density and porosity were determined on the undisturbed soil cores collected using sampling cores of 100 cm^3 after oven drying at 105 °C for 24 h.

For chemical analysis, at harvest, soil samples were collected with a trowel to a depth of 15 cm, air dried, and passed through a 2-mm sieve. Soil suspension (1:5 w/v soil: water) was used to measure pH and electrical conductivity with a pH meter and EC meter (Horiba Aqua Cond Meter F-74). Total C and N were analyzed by dry combustion on the CN-corder (Macro corder JM 1000CN, J-Science Co., Ltd., Kyoto, Japan). Soil exchangeable K, Ca, and Mg were extracted in 1 N ammonium acetate (pH 7.1), and analyzed by using an atomic absorption spectrophotometer (Model Z-2300, Hitachi Co., Tokyo, Japan). Cation exchange capacity (CEC) was measured by the 1N (pH 7.1) ammonium acetate (NH_4OAc) extraction methods in which the NH_4^+ saturated soil was equilibrated with 10% KCl and steam distilled by micro–kjeldhal distillation before titration with 0.1 N H_2SO_4 (Chapman, 1965), and expressed to $cmol_c$ kg^{-1} soil. Soil available P was determined using 0.002 N H_2SO_4 (pH 3.0) in ammonium sulphate solution according to Troug method (Troug, 1930). The P concentration in soil samples was measured by the ammonium molybdate–ascorbic acid method at an absorption wavelength of 710 nm on a spectrophotometer (Model U-5100, Hitachi Co., Tokyo, Japan).

7.2.5. Data Analyses

All results were the means of the three replicates. Data were analyzed using one-way analysis of variance (ANOVA) using SPSS version 22.0 (SPSS for windows Inc., Chicago, Illinois, USA) to evaluate the measured parameters as affected by the different rates of biochar addition. The pairs of means were also compared on significant ANOVA tests using Tukey's honestly significance difference (HSD) test ($p < 0.05$). A nonlinear regression analysis was utilized to investigate the relationship between sesame seed yield, plant height and the biochar rates. When considering the differences between the cropping fields, a two-way analysis of variance was used with the different biochar treatments and cropping as two fixed factors.

7.3. Results

7.3.1. Effect of rice husk biochar on the growth and yield components of sesame

The plant height, height of the lowest capsule, number of branches per plant and 1000-seed weight as affected by varying rates of biochar addition in first and second cropping fields are shown in **Table 29**.

The biochar rate showed a significant influence on the plant height with a significant interaction between biochar and cropping. However, there were no significant differences in the height of lowest capsule and 1000-seed weight and number of seeds per plant although a number of branches per plant and 1000-seed weight were higher compared to the control in the first and second cropping respectively. In comparison with the control (F), the plant height of the F+50B was non-significantly higher in the first cropping by 10.8% whereas the F+100B was significantly higher in the second cropping by 18.1%. There were no significant differences between control with F+20B and F+50B. The number of branches per plant in the first cropping were significantly increased by 42.7% in the F+50B treatment compared to the control that indicated biochar increased vegetative growth of the sesame. However, in the second cropping, this significant effect was not observed although the F+50B and F+100B treatments tended to have a greater number of branches per plant compared to the control and no significant interaction between biochar addition and cropping was observed.

The seed yield and a total number of seeds per plant of sesame affected by varying rates of biochar in the first and second cropping are shown in **Figure 33**.

Table 29. The plant height, height of the lowest capsule, number of branches per plant and 1000-seed weight of sesame under the different biochar treatments in different cropping.

Cropping	Biochar Treatment	Plant Height (cm)	Height of First Capsule (cm)	Number of Branches/Plant	1000-Seeds Weight (g)
First cropping	F	140.60ab	68.12a	1.72b	2.16a
	F+20B	137.85b	64.26a	2.03b	2.08a
	F+50B	157.63a	67.48a	3.01a	2.23a
	F+100B	152.47ab	70.37a	2.07ab	1.97a
Second cropping	F	114.42b	55.93a	2.34a	1.93a
	F+20B	134.71ab	57.50a	2.29a	2.13a
	F+50B	124.30ab	58.14a	2.48a	2.03a
	F+100B	139.63a	59.02a	2.45a	2.03a
Source of variation					
Biochar (B)		**	ns	ns	ns
Cropping (C)		***	***	ns	ns
B x C		*	ns	ns	ns

Means followed by different lowercase letters within a column in the same cropping are significantly different at $p < 0.05$ according to the Tukey test. *** Significant at $p < 0.001$; ** Significant at $p < 0.01$; * Significant at $p < 0.05$; ns, Non-significant.

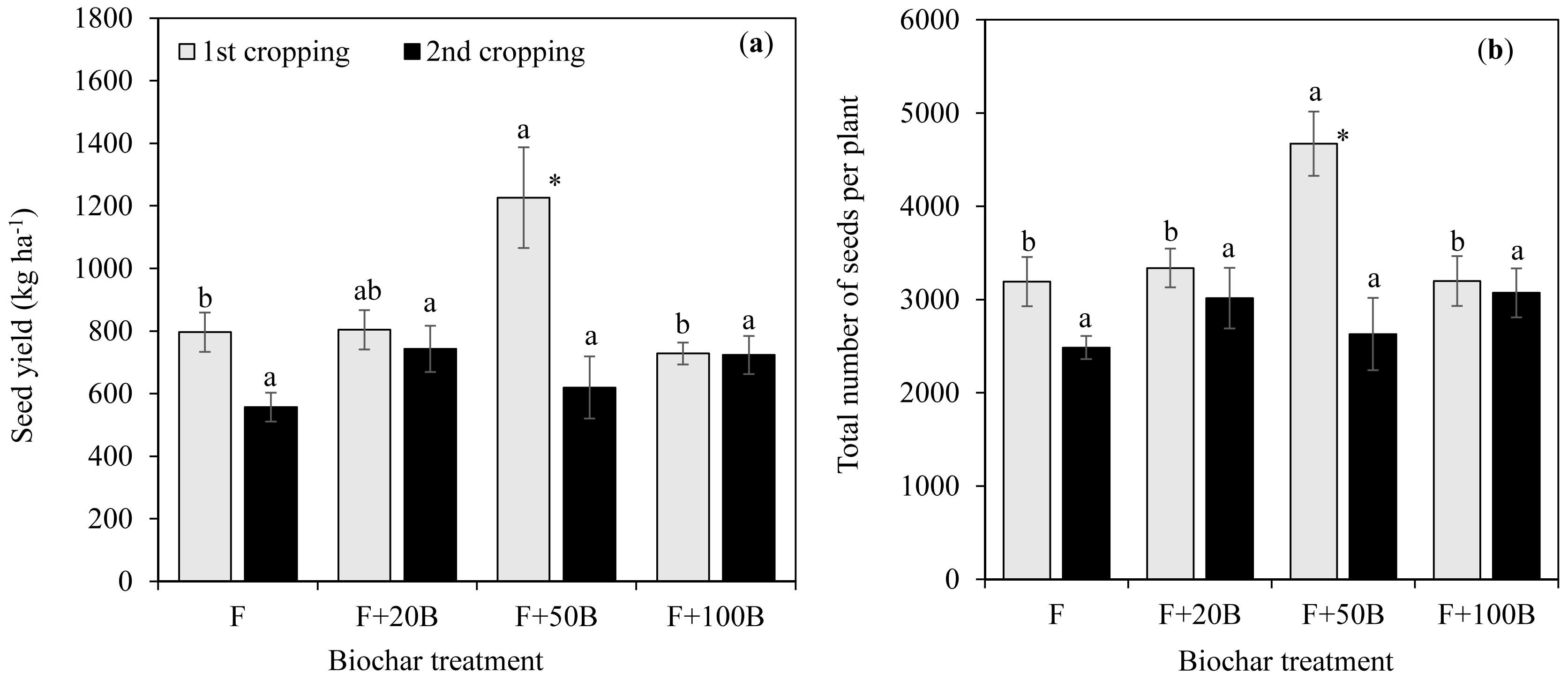


Figure 33. (a) The seed yield and (b) total number of seeds per plant as affected by rice husk biochar treatments. The bars represent the standard errors ($n = 3$). Different lower case letters indicate significant difference ($p < 0.05$) among treatment means. * Significant difference ($p < 0.05$) between first and second cropping of the F+50B treatment.

The higher rate of biochar addition significantly improved the seed yield of sesame in the first cropping. In comparison with the control (797 kg ha^{-1}), the F+20B and F+50B treatments significantly increased seed yield by 0.9% and 35.0% (1226 kg ha^{-1}) whereas the F+100B significantly decreased seed yield by 9.4% (Figure 1a). However, there were no significant differences between control, F+20B and F+100B treatments. The increase in the seed yield was accompanied by an increase in the total number of seeds per plant (**Figure 33b**). The number of seeds per plant significantly increased by 31.7% in the F+50B treatments whereas there were no significant differences between the number of seeds per plant in the control, F+20B and F+100B although F+20B and F+100B increased by 2.4% and 0.2% respectively over the control. In the second cropping field, biochar addition did not significantly influence seed yield and the number of seeds per plant. However, the positive effects of biochar addition were observed. In comparison with the control, the seed yield of sesame in the second cropping increased in the F+20B, F+50B, F+100B treatments by 25.1%, 10.1% and 23.0% respectively. Similarly, the total number of seeds per plant non-significantly increased in the F+20B, F+50B, F+100B treatments by 17.6%, 5.5% and 19.1% respectively. In both the first and second cropping, the addition of biochar improved sesame yield with F+50B and F+20B showing higher seed yield compared to the control.

The analysis of variance indicated that the biochar rate did not exhibit statistically significant influences on both the seed yield and total number of seeds per plant while cropping exhibited a statistically significant ($p < 0.01$) influence and the interaction between the biochar rate and cropping was significant ($p < 0.01$). As the biochar addition increased, the sesame seed yield and number of seeds per plant increased and then decreased. A positive nonlinear relationship between the two cropping seed yields, plant height and biochar rates were observed (**Figure 34**). The determination coefficient (R^2) and the significance for the overall plant height were higher than that of seed yield indicating growth of sesame was significantly influenced more than seed yield.

The plant height showed a tendency to increase with the increasing rate of biochar without decreasing at high addition rates indicated by the nonlinear relationship. However, this increase in the plant height at the rate of biochar, above 100 t ha^{-1}, did not result into a significant increase in seed yield.

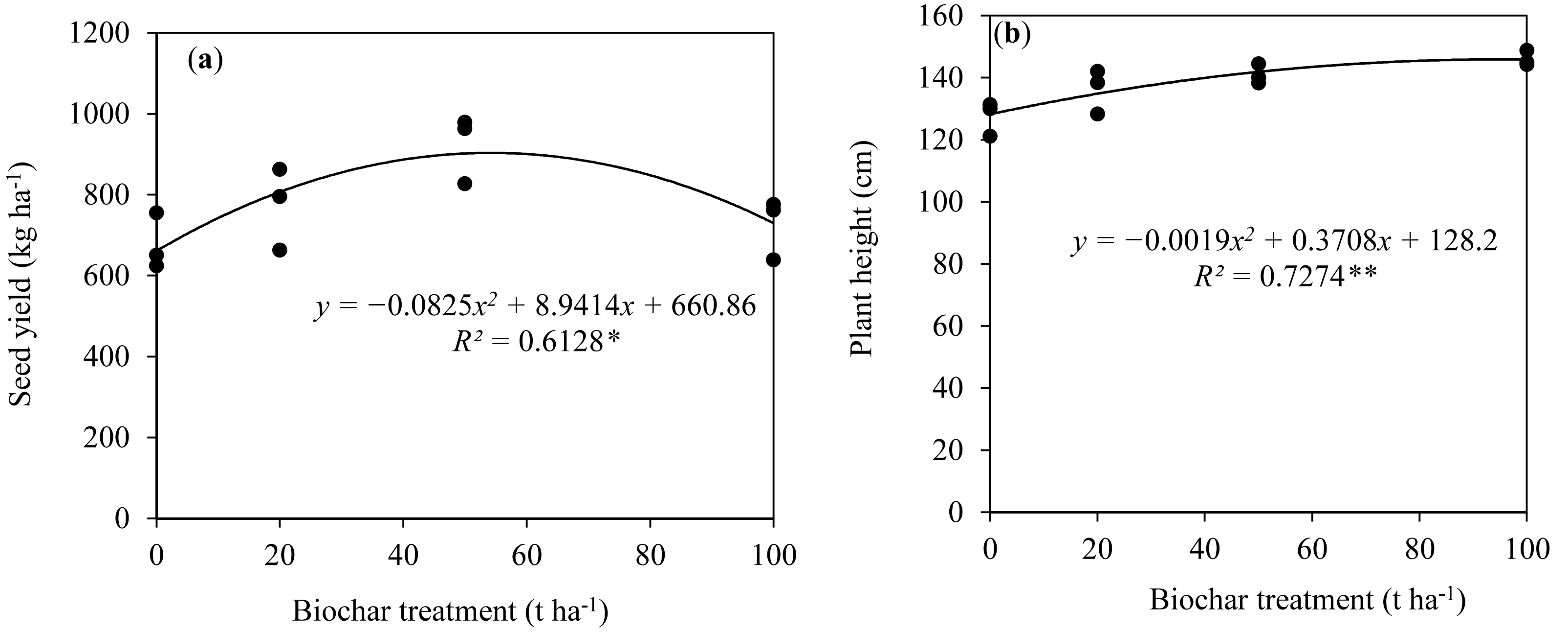


Figure 34. (a) The seed yield and (b) plant height response to rice husk biochar treatments. Relationships were fitted to first and second cropping data. * Significant at $p < 0.05$; ** Significant at $p < 0.01$.

7.3.2. Effect of rice husk biochar on the leaf tissue and sesame seed nutrient concentration

The leaf nutrient concentrations of sesame plants as affected by varying rates of biochar addition in the first and second cropping fields are shown in **Table 30**. The F+50B treatment significantly increased the leaf K concentration by 41.4% compared with the control in the first cropping and although there were no significant differences in leaf Mg concentration between control with biochar rates, the F+50B had a significantly higher leaf Mg than F+20B and F+100B in the first cropping. Biochar addition did not have a statistically significant effect on the leaf N, P and Ca concentrations in the first cropping. In the second cropping field, biochar addition did not have a statistically significant effect on any of the leaf nutrient concentrations. However, a non-significant increase in the N and K were observed compared to the control whereas leaf P increased in the F+20B, F+50B, but decreased in the F+100B. The leaf Ca and Mg concentrations were all non-significantly lower compared to the control.

The analysis of variance indicated that the biochar rate ($p < 0.01$) and cropping ($p < 0.001$) exhibited statistically significant influences on the concentration of leaf K, but no significant interaction whereas leaf Mg indicated significant ($p < 0.05$) interaction between biochar rates and cropping. The concentration of leaf P, Mg and K were significantly decreased by in the second cropping compared with first cropping irrespective of biochar addition.

The crude protein and mineral nutrient contents of sesame seed affected by varying rates of biochar addition in the first and second cropping fields are shown in **Table 31**.

Table 30. The leaf nutrient N, P, K, Ca and Mg of sesame under the different biochar treatments in different cropping.

Cropping	Biochar rate	N (%)	K (%)	P (%)	Ca (%)	Mg (%)
First cropping	F	2.79a	2.89b	0.70a	1.72a	0.36 ab
	F+20B	2.99a	3.10ab	0.68a	1.66a	0.33 b
	F+50B	3.26a	4.94a	0.71a	1.98a	0.49 a
	F+100B	3.31a	3.46ab	0.67a	1.58a	0.32 b
Second cropping	F	2.91a	2.41a	0.44a	1.53a	0.35a
	F+20B	3.04a	2.69a	0.49a	1.47a	0.34a
	F+50B	3.15a	2.75a	0.51a	1.46a	0.32a
	F+100B	3.11a	2.49a	0.44a	1.33a	0.30a
Source of variation						
Biochar (B)		ns	**	ns	ns	*
Cropping (C)		ns	***	*	ns	*
B x C		ns	ns	ns	ns	*

Means followed by different lowercase letters within a column in the same cropping are significantly different at $p < 0.05$ according to the Tukey test. *** Significant at $p < 0.001$; ** Significant at $p < 0.01$; * Significant at $p < 0.05$; ns, Non-significant.

Table 31. The seed macronutrient mineral nutrient contents of sesame seeds under the different biochar treatments in different cropping after harvesting.

Cropping	Biochar rate	Crude protein (%)	mg/100 g seed			
			P	K	Ca	Mg
First cropping	F	20.0a	612.6a	744.7b	1222.2a	419.0a
	F+20B	19.8a	640.7a	794.9ab	1275.6a	416.2a
	F+50B	20.6a	636.6a	846.7a	1321.5a	409.6a
	F+100B	19.1a	617.6a	833.8a	1280.8a	403.2a
Second cropping	F	18.9b	560.6a	820.9a	1252.2a	396.9a
	F+20B	20.4ab	640.7a	794.6a	1419.7a	398.7a
	F+50B	21.2a	617.3a	857.6a	1315.5a	389.8a
	F+100B	20.9a	608.9a	859.0a	1382.3a	422.4a
Source of variation						
Biochar (B)		ns	ns	**	ns	ns
Cropping (C)		ns	ns	ns	ns	ns
BxC		ns	ns	ns	ns	ns

Means followed by different lowercase letters within a column in the same cropping are significantly different at $p < 0.05$ according to the Tukey test. *** Significant at $p < 0.001$; ** Significant at $p < 0.01$; * Significant at $p < 0.05$; ns, Non-significant.

The higher rates of biochar addition except in the F+100B, improved the crude protein, P, K, and Ca in the first cropping. However, there were no significant differences between biochar rates and control in the crude protein, P, Ca and Mg in first cropping. In comparison with control, the F+50B and F+100B treatments significantly increased the seed K content by 12.1% and 10.7% respectively whereas there was no significant difference between F+20B and control in the first cropping. However, no significant effect of biochar addition on seed K was observed in the second cropping. Biochar addition in the second cropping field significantly improved the crude protein content of sesame seeds. The crude protein of the F+50B and F+100B treatments were significantly higher than that of the control by 10.9% and 9.6%, respectively, whereas there were no significant differences between the F+20B treatment and control although an increase by 7.4% occurred in the F+20B compared with control. In the second cropping, biochar addition did not have a statistically significant effect on the seed P, K, Ca and Mg. The analysis of variance indicated that the biochar rate ($p < 0.01$) exhibited statistically significant influences on the seed K content, but no significant interaction between biochar rates and cropping. The content of seed crude protein, P, Ca and Mg were not significantly influenced by either biochar rates or cropping.

7.3.3. Effect of biochar on soil physico-chemical properties in first and second cropping fields

The soil physical properties of bulk density and porosity as affected by varying rates of biochar addition in the first and second cropping fields are shown in **Figure 35**. Biochar addition in the first and second cropping fields significantly decreased soil bulk density with increasing rates of biochar. The F+100B treatments significantly decreased bulk density to 0.76 g cm^{-3} compared to the control (1.09 g cm^{-3}) in the first cropping and to 1.01 g cm^{-3} from 1.15 g cm^{-3} in the control of the second cropping field (**Figure 35a**).

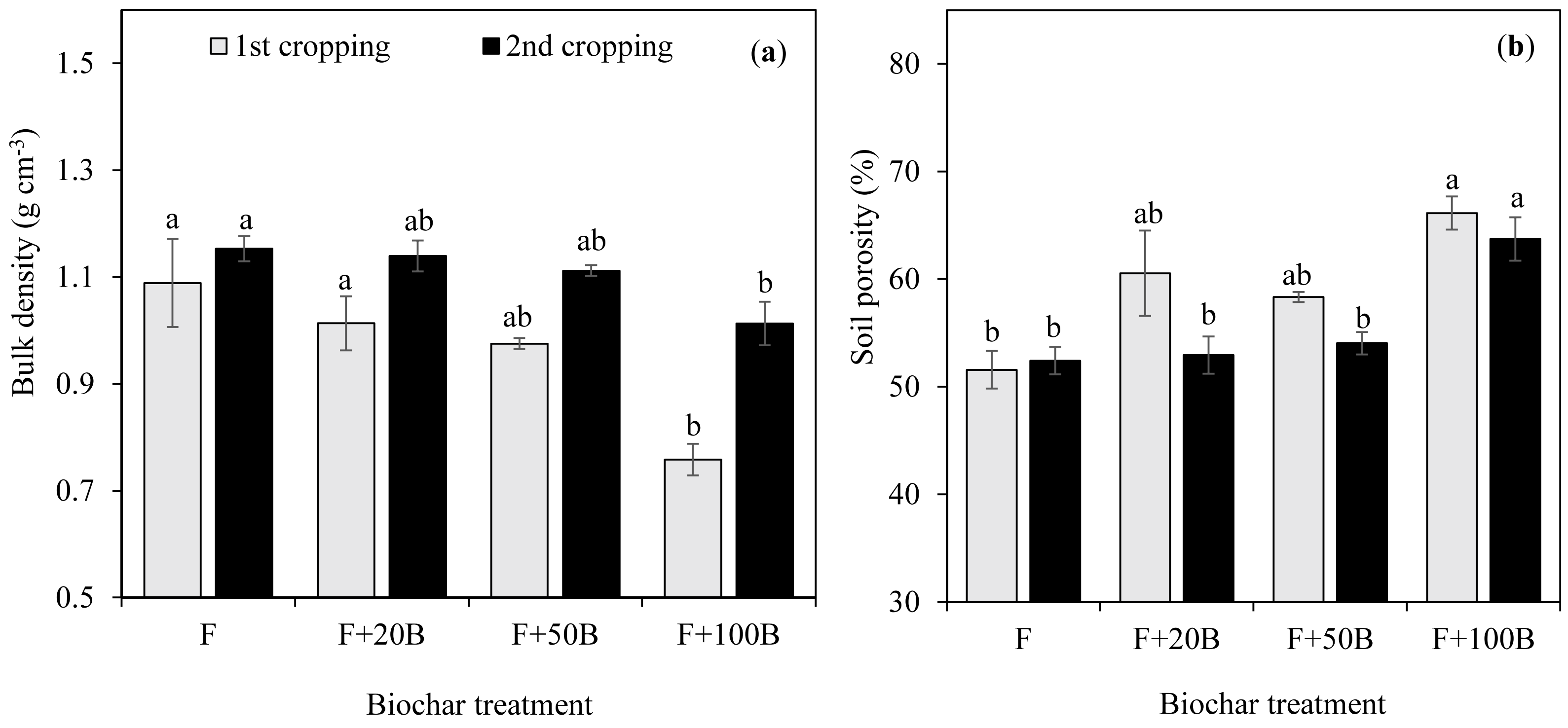


Figure 35. (a) The soil bulk density and (b) soil porosity as affected by rice husk biochar treatments. The bars represent the standard errors ($n = 3$). Different letters indicate significant difference ($p < 0.05$) among treatment means.

Similarly, the F+100B treatments significantly increased soil porosity to 66.1% compared to the control (51.6%) in the first cropping and to 63.7% from 52.4% in the control of the second cropping field (**Figure 35b**). In both the first and second cropping, the addition of biochar improved the physical property of the soil with higher rates of biochar addition. The analysis of variance indicated that the biochar rate exhibited statistically significant influences on both the soil bulk density ($p < 0.001$) and porosity ($p < 0.001$). Although the cropping had a significant influence on soil bulk density ($p < 0.001$) and porosity ($p < 0.05$), there were no significant interactions between the biochar rate and cropping.

The soil chemical properties affected by varying rates of biochar addition in the first and second cropping fields are shown in **Table 32**.

Table 32. The soil chemical properties of sesame under the different biochar treatments in different cropping after harvesting.

Cropping	Biochar rate	pH	EC (dS m^{-1})	TN (g kg^{-1})	C/N ratio	P (mg kg^{-1})	CEC ($cmol_c$ kg^{-1})	Exchangeable cations		
								K (mg kg^{-1})	Ca (mg kg^{-1})	Mg (mg kg^{-1})
First cropping	F	5.65b	0.10ab	2.40a	10.1c	13.3a	9.9b	190.7c	3272.2a	281.6a
	F+20B	5.52b	0.18a	2.40a	14.4bc	46.4a	12.3ab	292.6bc	3303.7a	250.0ab
	F+50B	5.97ab	0.09b	2.60a	25.1b	20.9a	12.5ab	408.0b	2217.1a	224.6ab
	F+100B	6.38a	0.12ab	2.89a	50.6a	31.2a	14.5a	686.1a	1683.5a	198.1b
Second cropping	F	5.54a	0.08b	2.09b	10.3c	40.2a	11.5b	179.5b	3213.1a	248.7a
	F+20B	5.84a	0.08b	2.18ab	15.6bc	19.1a	13.2ab	158.8b	1326.6a	215.8a
	F+50B	5.49a	0.20a	2.29ab	19.1b	36.9a	12.9ab	447.4a	2575.9a	244.7a
	F+100B	5.68a	0.09b	2.53a	31.4a	33.5a	14.3a	386.6a	1767.3a	215.8a
Source of variation										
Biochar (B)		ns	*	*	***	ns	***	***	ns	*
Cropping (C)		ns	ns	**	**	ns	ns	**	ns	ns
B x C		ns	***	ns	**	ns	ns	**	ns	ns

Means followed by different lowercase letters within a column in the same cropping are significantly different at $p < 0.05$ according to the Tukey test. *** Significant at $p < 0.001$; ** Significant at $p < 0.01$; * Significant at $p < 0.05$; ns, Non-significant.

Biochar addition in the first cropping significantly increased soil pH, EC, C/N ratio, exchangeable K and soil cation exchange capacity (CEC) compared to the control while the soil exchangeable Mg was significantly decreased. The F+100B treatment significantly increased soil pH by 0.73 units compared to the control (5.65) whereas the soil EC of the F+20B and F+50B were significantly different while of the control was not statically different from F+100B treatment. The C/N ratio significantly increased with increasing biochar rates by 4.3, 15.0 and 40.5 in the F+20B, F+50B and F+100B treatments respectively which indicated high carbon content in the soil with biochar treatments. The biochar addition significantly increased the soil exchangeable K with increasing rates of biochar treatments. The exchangeable K significantly increased by 101.9, 217.3 and 495.4 mg kg^{-1} in the F+20B, F+50B and F+100B respectively. Conversely, the exchangeable Mg significantly decreased by 31.55, 57.0 and 83.5 mg kg^{-1} in the F+20B, F+50B and F+100B respectively. However, there were no significant differences between the F+20B and F+50B treatments. The higher rates of biochar addition improved the soil CEC. The F+100B treatment significantly increased the CEC by 4.7 $cmol_c$ kg^{-1} compared to the control. In the first cropping, there were no significant effect of biochar addition on the available P and exchangeable Ca. However, these parameters showed higher values in the biochar addition treatments compared with the control, except exchangeable Ca which tended to decrease in the F+50B and F+100B treatments.

In the second cropping field, the addition of biochar significantly influenced soil EC, total N, C/N ratio, exchangeable K, and CEC whereas biochar did not show a significant effect on the soil pH, available P, exchangeable Ca or Mg. The F+50B treatment significantly increased soil EC by 0.12 dS m^{-1} compared to the control and no obvious significant differences were observed between the control, F+20B and F+100B treatments. The total N and C/N ratio significantly increased with increasing biochar rates and the in the F+100B treatments were significantly higher by 0.4 g kg^{-1} and 21.0 for total N and C/N ratio respectively when compared with control. The F+50B and F+100B treatments significantly increased exchangeable K by 267.9 and 207.1 mg kg^{-1} compared with the control. However, there was no significant difference between control and F+20B treatment. Similar to the first cropping, the higher rates of biochar addition improved the soil CEC. The F+100B treatment significantly increased the CEC by 0.23 cmolc kg^{-1} compared to the control.

In the second cropping, there was no obvious significant effect of biochar addition on the soil pH, available P and exchangeable Ca and Mg. However available P, exchangeable Ca and Mg were non-significantly decreased in the biochar addition treatments compared to the control. The analysis of variance indicated that the biochar rate exhibited statistically significant influences on EC, total N, C/N ratio, exchangeable K and Mg, and CEC whereas cropping significantly influenced total N, C/N ratio and exchangeable K (**Table 4**). There were also significant interactions between biochar rates and cropping for EC, C/N ratio, and exchangeable K that indicated an improvement of continuous monocropping soil with biochar.

7.4. Discussion

In this study, biochar additions significantly improved the soil physical properties of bulk density and porosity in both first and second cropping fields (**Figure 35**). A similar finding was reported in sesame that rice husk and saw dust biochar application at 10 t ha^{-1} significantly increased soil porosity and decreased the bulk density of a highly leached acidic Ultisol soil (Ndor et al., 2015). The increase in soil porosity and decrease in bulk density after biochar addition allow easy root penetration for water and nutrient absorption as a result of increased water holding capacity and reduced tensile strength of the soil (Chan et al., 2007; Lu et al., 2014; Nelissen et al., 2015). This increase in soil porosity and decrease in bulk density is attributed to the low particle density and high porosity of the rice husk biochar compared with soil which enables the soil to hold more water and air (Downie et al., 2009). In this study, the rice husk biochar used had a low bulk density of 0.29 g cm^{-3} which therefore greatly increased the soil porosity and decreased the soil bulk density after biochar addition on the upland field converted paddy soil. In addition, decreasing the bulk density and increasing soil porosity by biochar addition is important in the second cropping since continuous cropping results into a deterioration in the soil physical quality (Reeves, 1997). This suggests the integration of rice husk biochar in continuous monocropping systems would improve soil conditions and overall crop performance.

Furthermore, biochar addition in the first cropping significantly increased soil pH with increasing rates of the rice husk biochar. The result agrees with other findings that biochar addition increased soil pH (Alburquerque et al., 2014; Wang et al., 2014). Therefore, with rice husk biochar addition to slightly acidic upland field converted from paddy, the acidity would gradually be decreased due to the liming effect of the biochar. Rice husk biochar has been reported to increase soil pH of tea garden soil (acidic soil) from 3.33 to 3.63 (Wang et al., 2014). In this study, increase in soil pH could be due to the alkaline nature of the biochar used since the rice husk biochar had a pH of 10.47 besides its high ash content (38.04%). The ash content of biochar plays important role in increasing soil pH and consequently determines the soil CEC (Sollins et al., 1988). This positive effect on soil pH was only observed in the first cropping. This study showed that biochar in the second cropping did not have a significant effect on the soil pH (**Table 32**). The pH of the control did not significantly differ from the biochar treatments in the second cropping. The addition of dolomite to increase soil pH and alleviate acidity in the second cropping possibly affected biochar liming effect. Whereas the biochar had a liming effect in the first cropping, this effect could have been offset by the addition of dolomite in the second cropping. This suggested that addition of both dolomite and biochar may not be good and could lead to loss of functional properties of biochar in the subsequent cropping although this hypothesis remains untested. However, Cornelissen et al. (2018) reported that rice husk biochar addition on acidic Ultisol soil of Indonesia showed a less pronounced effect on the soil pH as the cropping season increased due to decreasing liming potential, which also agrees with these result. In addition to pH, biochar addition showed tendency to increase soil EC especially in the second cropping that could be attributed to the highly soluble minerals contained in the rice husk biochar that was readily added into the soil.

Although biochar addition significantly increased the soil total N and C/N ratio, possibly N immobilization occurred with increasing biochar rates (**Table 32**). Similar findings have been reported that with biochar addition indicating an increase in the soil total C from 2.27% to 2.78% and total N from 0.24% to 0.25% (Jones et al., 2012). In addition, research indicates that microbial immobilization of N with a C/N ratio of biochar above 16 occurs (Alburquerque et al., 2014; Bargmann et al., 2014). Furthermore, an increase in the soil total N, added from the rice husk biochar may not indicate N availability to plants and microbes (Biederman and Harpole, 2013). Hence, the increase in the C/N ratio with increasing rates of biochar in both the first and second cropping could possibly be high enough to immobilize N thereby limiting N availability to sesame plant. The non-significant effect on soil available P and exchangeable Ca suggested the rice husk biochar had little ability to supply these nutrients. Possibly, the high Ca and P in the biochar was insoluble and remained in its micro-porous fabric of the biochar (Limwikran et al., 2018) Limwikran et al. reported that rice husk biochar could not readily release Ca into soil thus acted as a sink rather than a source for exchangeable Ca.

On the other hand, the biochar addition significantly increased the soil exchangeable K with increasing rates of biochar treatments whereas exchangeable Ca and Mg tended to decrease although not significantly different between biochar rates. This is contrary to Wang et al. (2014) and Carter et al. (2013) who reported the increase in the exchangeable cations Ca, Mg and K with rice husk biochar addition. In this study, only exchangeable K was significantly increased with increasing biochar rates. Wang et al. (2014) reported that with biochar application, K levels in soil increased from 42 to 324 mg kg^{-1}. The rice husk biochar used had an ash content of 38.04% that was very high to increase soil K content in the soil. A similar increase in the exchangeable K was observed and attributed to high ash content in rice husk biochar and being a source of K itself (3640.73 mg kg^{-1} K in the rice husk biochar used) (Major et al., 2010; Abrishamkesh et al., 2015) This increased exchangeable K with biochar addition could also be due to the increased soil pH which enhances release K into the soil (Atkinson et al., 2010). Due to the increase in soil K, rice husk biochar could enhance crop growth and yield, including sesame on soils where K is a limiting factor.

This study also showed that the higher rates of biochar addition improved the soil CEC which agrees with the findings of Ndor et al. (2015) who reported increased CEC with rice husk and saw dust biochar in sesame cultivation. The biochar addition on a strongly acidic soil decreased in soil acidity through an increase in pH and the increasing the soil CEC thereby retaining nutrients into the soil for sesame (Ndor et al., 2015) In this study, the observed increase in the soil pH due to biochar addition and the rice husk biochar CEC (7.53 cmolc kg−1) itself could also have increased the soil CEC. The increase in soil CEC levels is in agreements with findings of Laird et al. (2010) who reported that biochar treatment increased soil CEC from by 4 to 30% more than the control while Jien and Wang (2013) reported the CEC of a highly weathered soil was raised from 7.41 to 10.8 cmol$_c$ kg^{-1} after biochar application. In this study, the increase in the soil CEC is the indicator of nutrient retention suggesting that rice husk biochar could improve soil nutrient status in continuous monocropping as observed in the second cropping. Similar results have shown that rice husk biochar addition to acid sulfate soils in Indonesia has been reported to increase CEC, in addition to improving soil porosity and exchangeable K (Masulili et al., 2010). Therefore, this result suggests that cultivation of sesame with rice husk biochar could improve not only soil physical properties promoting proper plant growth, but also hold sufficient nutrients through an increase in CEC upland fields converted paddy soils.

Furthermore, biochar addition did not have a significant effect on leaf tissue N, P and Ca concentration and their contents in the sesame seeds (**Tables 30**, **31**). Overall, biochar addition had a significant influence on the leaf tissue K and Mg concentration of, and content of the seeds. This result agrees with other findings, including a meta-analysis from different studies that biochar addition had no significant effect on plant tissue N and P concentrations whereas it increased the K tissue concentration (Biederman and Harpole, 2013; Si et al., 2018). Although the overall effect of biochar addition was not significant on crude protein, a significant increase in the crude protein content was observed in the second cropping suggesting an increase in protein synthesis in sesame. This could be attributed to the adequate supply of soil K from biochar allowing increased plant tissue K concentration. Potassium, K is an important nutrient that plays a significant role in protein synthesis in plants (Hasanuzzaman et al., 2018). Hence, adequate supply of K from the rice husk biochar could enhance protein quality of sesame seeds. In addition, the increase in the crude protein content could be attributed to the slight increase in soil total N with biochar compared to the control in the second cropping. A similar finding shows that biochar addition (from acacia) of up to 50 t ha^{-1} in maize cropping system increased maize grain protein by 13% compared without biochar (Ali et al., 2017). Therefore, it could be speculated that the protein content of sesame is increased with rice husk biochar addition although leaf tissue N concentration did not show statistical significance.

Biochar addition significantly increased leaf tissue K concentration and K content in the sesame seeds especially in the first cropping attributed to the high K content in the rice husk biochar reflected in the higher soil exchangeable K compared to the control. The soil K concentration in the 50 t ha^{-1} was twice the value in the control whereas it tripled in the 100 t ha^{-1} compared to control suggesting the rice husk biochar could possibly replace the 70 kg K_2O ha^{-1} inorganic fertilizer rate supplied to sesame in this study as a K source. A meta-analysis of several pieces of research shows that biochar addition treatments performed better than fertilizer at increasing plant tissue K concentration (Biederman and Harpole, 2013). Hence, rice husk biochar with a high K content could be an alternative source of K fertilizers for sesame especially for resource poor farmers. Conversely, the leaf tissue Mg concentration tended to decrease compared to the control in the first cropping indicating that increasing biochar rates and cropping, the leaf tissue Mg concentration of sesame is significantly decreased that could influence seed yield at high rates of biochar addition. The content of Mg in the sesame seeds with biochar addition was also non-significantly lower than the control indicating the negative effect of high increasing rates of biochar addition on the sesame seed quality. Koyama and Hayashi (2017) also found a similar decrease in the Mg concentrations in rice straw tissue with an increase in rice husk biochar addition rate up to 2 kg m^{-2} (equivalent to 20 t ha^{-1}). The result also agrees Syuhada et al. (2016) who found out that biochar addition at 10 and 15 g kg^{-1} from oil palm feedstock applied on to podsol soils significantly increased concentration and uptake of K while decreasing the Mg concentration in maize tissue. They attributed the low uptake of Mg to competitive ion effect between the uptakes of Mg and K. Although in this study dry matter yield was not measure the, the increased nutrient concentrations of K also implies high uptake of K since nutrient uptake is governed by the concentration of nutrients in plant tissues and the dry matter yield. Usually competitive ion effect occurs when there is a high concentration and uptake of K in plant tissue which decreases the concentration and uptake of Mg (Butnan et al., 2015; Weil and Brady, 2016; Zemanová et al., 2017). Major et al. (2010) also found a similar decrease in the concentration of Mg in maize seeds in high wood biochar addition treatments (8 and 20 t ha^{-1}) attributed to declining stock of Mg in the soil due to this ion competition effect. In this study, the F+50B treatment significantly increased the leaf tissue K concentration by 41.4% compared with the control and increased seed K content by 12.1% in the first cropping, whereas Mg content in the seeds non-significantly decreased by 0.7%, 2.3% and 3.9% in the biochar addition of F+20B, F+50B and F+100B respectively. Therefore, the competitive ion effect is likely to occur when higher rates of the rice husk biochar with a high content of K is applied in sesame due to luxury consumption and decreased uptake of Mg. This could negatively affect yield and mineral quality of sesame.

Furthermore, biochar addition increased the overall sesame yield compared with the control that was consistent with Ndor et al. (2015) who reported significant increase in the seed yield of sesame to 925 kg ha^{-1} in the 10 t ha^{-1} rice husk and sawdust biochar compared with the 595 kg ha^{-1} in the control in a field experiment. In particular, the yield increase with biochar addition, in the first cropping, suggested that sesame positively responds to biochar (**Figure 33**). In addition, 10 t ha^{-1} of coconut shell biochar added together with chicken manure to sesame resulted into higher seed weight per plant than the control on a sandy coastal soil (Nurhayati, 2017). In this study, the 50 t ha^{-1} (35.0% increase over control) in the first cropping significantly increased the sesame seed yield that could be attributed to increased number of seeds per plant rather than increase in the seed weight since there was no significant increase in the 1000-seed weight with biochar addition. The significant increase in the number of branches per plant with the biochar treatments in the first cropping could explain the increase in the total number of seeds per plant consequently increasing sesame yield. A similar increase in the number of branches per plant was reported that increased the seed yield of rapeseed (*Brassica napus* L.) with biochar addition (Khan, 2015). In the second cropping, the F+20B (20 t ha^{-1}) non-significantly increased seed yield by 25.1% compared with control. The non-significant differences between the biochar treatments in the second cropping could be attributed to loss in the functional properties of the biochar although the seed yield increased in the 20 t ha^{-1}. This finding agrees with the recent report that rice husk biochar on acidic Ultisol soil of Indonesia at 15 t ha^{-1} significantly increased maize yield only in the first season, but the effect faded from the second season onwards (Cornelissen et al., 2018).

The biochar addition also significantly increased sesame plant height in both the first and second cropping; and that could possibly explain the increase in the seed yield. It has been reported that poultry litter biochar at an optimal rate of 11.21 g kg^{-1} (equivalent to 22.4 t ha^{-1})

significantly increased sesame plant height (Furtado et al., 2016) which is consistent with these results. The increase in the seed yield and plant height at 50 t ha^{-1} of rice husk biochar for sesame cropping on upland field converted paddy is consistent with other researchers who applied high biochar rates and obtained good crop performance (Haefele et al., 2011; Schulz et al., 2013). Haefele et al. (2011) reported that rice husk biochar applied at 4.13 kg m^{-2} (equivalent to 41.3 t ha^{-1}) in on Humic nitisols (pH 4.3) increased grain yield of rice by 16–35% at Sinilioan Phillipines whereas Schulz et al. (2013) observed increased growth of oat plants with addition of 100 t ha^{-1} of composted biochar to sandy and loamy soil. Several pieces of field research indicated that biochar addition increased crop growth and yield (Lehmann et al., 2006; Major et al., 2010). For instance, cultivation on sandy soils using biochar increased maize yield by 150% and 98% over the control at rates of 15 t ha^{-1} and 20 t ha^{-1} respectively (Uzoma et al., 2011). Zhang et al. (2010) also reported an increase in rice yields of 14% over control in paddy soils with wheat straw biochar rate of 40 t ha^{-1}. However, the highest rate of 100 t ha^{-1} tended to have a negative effect on sesame in this study, which is consistent with the finding of Chan et al. (2007). The decrease in the seed yield in the 100 t ha^{-1} compared to 50 t ha^{-1} suggested the biochar addition rate had exceeded the beneficial amount. At this rate, the possible release of toxic substances like heavy metals and polycyclic aromatic hydrocarbons (PAHs) from biochar could have suppressed plant growth (Kuppusamy et al., 2016). In addition, the low yield in the 100 t ha^{-1} is likely as a result of nutrient imbalances and N immobilization (Borchard et al., 2014; Persaud et al., 2018). The decrease in the seed yield at 100 t ha^{-1} could be partly explained by the increased adsorption of available inorganic N at this high biochar addition rate. For instance, research shows that biochar addition improves the retention capacity of NH_4^+-N through enhanced CEC (Clough et al., 2013). In this study, the increased CEC could have increased ammonium adsorption in the first cropping with 100 t ha^{-1} rice husk biochar rate. However, the adsorption of inorganic N onto biochar surfaces could decrease ammonia and nitrate losses from soil, but could as well potentially lead to the slow release of these nutrients to plants (Haider et al., 2017).

Although, the leaf tissue N concentration tended to increase non-significantly with biochar rates in the first cropping, the increase in growth and yield could be entirely attributed to an increase in the leaf tissue K concentration in sesame plant. This study agrees with a meta-analysis of biochar research showing that biochar addition treatments performed better than fertilizer at increasing plant tissue K concentration and plant tissue N is unaffected by biochar addition thereby influencing yield (Biederman and Harpole, 2013). On the other hand, these results indicated that leaf K concentration was significantly reduced by continuous monocropping, but with the biochar addition, the concentration of K significantly increases. Therefore, the higher seed yield, number of seeds per plant and plant height compared to the control in the second cropping is attributed to this increased leaf K suggesting seed yield decline under continuous monocropping could be recovered by adding more K fertilizer. This also suggests future research should consider comparing and contrasting biochar addition with more K fertilizer rates for sesame cropping. The increased seed yield was due to this increased K concentration due to the rice husk biochar addition. Moreover, the leaf tissue K concentration was above the adequate level of 2.4% required for sesame growth since K plays a significant role in increasing the internodes lengths consequently increase in sesame plant height (Mitchell et al., 1974). However, the lower leaf tissue K concentrations in the second than first cropping accompanied by the low soil exchangeable K suggested a decrease in availability of K could have led to the lack of positive effect of rice husk biochar rates on sesame yield in the second cropping. Similar effects of lack of positive effect of biochar on crop yields have been attributed to decreasing nutrient contents in biochar addition after its addition (Steiner et al., 2014).This could suggest that the benefit of K addition from rice husk biochar could decrease over time affecting sesame yield. Therefore, the lack of non-significant effect on leaf tissue K concentration in the second cropping suggested K was the most determinant factor on growth and seed yield.

Furthermore, the non-significant effect on K concentration in sesame in combination with factors that affected the rice husk biochar properties in the second cropping could have contributed to low yields in the second cropping. For instance, the first cropping field had fresh biochar applied whereas the second cropping field had old biochar that could have influenced sesame yield due to biochar aging effect on the temperate soil. The meta-analyses by Biederman and Harpole (2013) shows that the effect of biochar addition is less pronounced on temperate climate soils and freshly added biochar could perform better than the old or aged biochar. In this study, the seed yield did not show significant differences between biochar rates in the second cropping which also agrees with Persaud et al. (2018) who reported no beneficial effect of rice husk biochar in on yield in the second cropping. The authors observed increase in above ground biomass of pak choy (*Brassica rapa* subsp. *chinensis*) by 32.81% in the 25 t ha^{-1} application rate (0, 5, 25 and 50 t ha^{-1}) to acidic Tabela sandy soil of Guyana in the first cropping compared with the control and attributed to increasing soil pH, exchangeable cations, CEC, and decrease in soil bulk density that is also consistent with these results. Therefore, improvement in the soil chemical properties and physical properties by the rice husk biochar enhanced sesame productivity especially in the first cropping. However, these benefits could deteriorate with an increase in the number of cropping as the biochar becomes old. For instance, changes in the physico-chemical properties as a result of aging when incorporated into the soil have been reported (Sohi et al., 2010). In this study, the porosity tended to decrease in the second cycle suggesting biochar particles had been crushed or broken down as a result of continuous cropping during tillage operation. The particles of biochar may also break and become smaller with time due to the physical interaction with drainage water (Spokas et al., 2014). With tillage, the particles could have been degraded and the ashes contents of the biochar increased leading to easy leaching in drainage water affecting soil pH that depends on the ash content. Thus, the lack of significant effect of the rice husk biochar in the second cropping could partly be due to leaching of the alkaline ashes (Glaser et al., 2002; Lehmann and Rondon, 2006). Furthermore, the no effect on the second cycle could be attributed to the loss in the properties of biochar to adsorption and immobilization of heavy metals, polycyclic aromatic hydrocarbons (PAHs), phthalates etc. from the soil (Hagemann et al., 2017). A study found that fresh rice husk biochar had a higher adsorption capacity for toxic compounds than aged one (Khorram et al., 2016). In addition, rice husk biochar addition up to 30 t ha^{-1} is reported to have a high affinity to removed cadmium from aqueous solutions when mixed in soil, attributed to high surface charge (net negative charge) of the bio-sorbents, thereby eliminating inhibition effect of cadmium (Cd^{2+}) on plant growth and improve yield (Mahmoud et al., 2011). Therefore, the fresh biochar as observed in the first cropping could have absorbed toxic heavy metals and other compounds that would hinder sesame growth and yield. The decrease in ability to increase pH, adsorb potential heavy metals, and overall changes in the physical properties of the rice husk biochar could be possible factors that led to the low yield in the second cropping.

Nonetheless, the rice husk biochar addition to sesame improved growth and yield at increasing rates; it could be recommended to apply rates not exceeding 50 t ha^{-1}. Although sesame is considered a high value oilseed crop, higher rates than 50 t ha^{-1} are not economically feasible under field conditions. The higher biochar rates are not economically feasible in most farming systems due to high costs (Woolf et al., 2010; Clare et al., 2015). Given the temperate climate where soils have favorable properties for plant production than tropical soils, large quantities of biochar may be required to achieve significant positive effects on yield as observed in this study (Borchard et al., 2014). However, further studies are needed to determine the optimal rice husk biochar rate while considering the cost and benefits of sesame cultivation on upland fields converted paddy.

7.5. Conclusions

The results demonstrated that sesame seed yield, plant growth and mineral content are improved with the biochar addition on upland field converted paddy. The biochar addition increased plant height with significant interaction in both cropping fields whereas the seed yield was only significantly influenced in the first cropping. The higher rate of biochar addition significantly improved the seed yield of sesame in the first cropping whereas in the second cropping field, biochar addition did not significantly influence seed yield and the number of seeds per plant. Among the seed mineral nutrients, K content was most increased by biochar. The overall improvement in the sesame growth, seed yield and mineral contents especially K was attributed to mainly increased K availability, soil pH, CEC, improved porosity and bulk density. This study also suggests that rice husk biochar addition may not have a long lasting effect on sesame yield on upland field converted paddy since the positive effect of biochar tended to fade in the second cropping as its biochar aged suggesting one-time application would not be sufficient. However, further investigations are still required to clarify the non-significant influence of biochar addition on seed yield and growth of sesame in the second cropping when biochar had been incorporated in the first cropping to uncover the mechanisms underlying these processes with biochar addition in long-term field trials.

CHAPTER EIGHT

General discussion, conclusions and further research

8.1. General discussion

This study found that sesame suffers a continuous monocropping obstacle on upland field converted paddy fields while using four different cultivars. Although, there were several factors which caused the obstacle, the two major factors included, indirect autotoxicity and imbalance of mineral nutrients in continuous monocropping.

Results showed that autotoxicity of phenolic compounds from decomposing roots and rhizosphere soils in the short duration of continuous monocropping could contribute to continuous monocropping obstacle of sesame on the field (Chapter Three). The high phenolic compounds detected in the short duration of continuous monocropping could proliferate disease incidences in continuous monocropping apart from directly affecting sesame germination and seedling. Furthermore, continuous monocropping obstacle related to autotoxicity depended on the cultivar type. Usually, the response to continuous monocropping obstacles varies with plant species, cultivars, and soil conditions (Chen et al., 2015). For instance, the cultivars such as 'Gomazou' and 'Masekin' showed tolerance to the negative influence of continuous monocropping when compared to both 'Maruhime' and 'Nishikimaru'. Whereas it is reported that some cultivars produce more allelochemicals than others and hence selection of genotypes with less phytotoxic potential is necessary (Wu et al., 2007), this study showed that cultivars released more autotoxicity compounds such as phenolics in the rhizosphere soils from the roots tended to have high yield under field conditions, suggesting it is cultivar tolerance to autotoxins rather than quantity of allelochemicals released that confers resistance to continuous monocropping obstacles of sesame. In chapter three, the quantity of all phenolic compounds released were significantly highest in the short duration of continuous monocropping including Year 0 (non continuous monocropping) with 'Gomazou' and 'Masekin' showing higher contents of soil phenolic compounds when compared to that of 'Maruhime' and 'Nishikimaru' indicating their tolerance to autotoxicity of phenolic compounds. It could also be suggested that low autotoxicity activities could exists since the content of phenolic compounds was higher in the short than in the long duration of continuous monocropping.

The problem of soil fertility related to decreasing available N and limited K nutrition caused by imbalances in soil cations was another factor causing continuous monocropping obstacle. The declining nutrients especially decrease in total C and N were related with soil phenolic compounds. This study showed that the decrease in total C and N resulting from low residues (organic materials) in long continuous monocropping significantly decreased soil phenolic contents. Autotoxicity compounds are often released from decomposing crop residues and root exudates. Organic matter especially from decomposing residues plays a role in the fate of phenolic compounds (Inderjit, 1996). It was reported that during decomposition of plant residues, phenolic compounds, including ferulic and coumaric acids were released and their concentrations positively correlated with organic carbon content since phenolic compounds are involved in the soil stabilization and carbon cycling (Martens, 2002). Moreover, Autotoxicity compounds including phenolic compounds make nutrients available through chelation increasing plant nutrient availability and absorption by plants (Inderjit et al., 2011). The observed high nutrient in the short duration of continuous monocropping suggested that soil phenolic compounds played a role in nutrient availability, apart from predicting the soil organic C content. Furthermore, phenolic compounds are sources of substrates for the multiplication of several groups of soil microorganism and are linked to increase in soil-borne diseases in continuous monocropping systems (Kefeli et al., 2003; Badri et al., 2013; Zhou et al., 2017). Hence, this study indicated that direct autotoxicity from phenolic compounds could not be the cause of continuous monocropping obstacles since bioassay results showed significant inhibition of germination and growth but growth and yield of the two cultivars ('Gomazou' and 'Masekin') were still high under field conditions. Instead, soil phenolic contents could possibly increase disease incidences and severity under continuous monocropping. 'Maruhime' showed the highest disease incidence and severity indicating its susceptibility to diseases especially *Fusarium* wilt in continuous monocropping.

In addition to yield and growth decline, seed mineral quality was decreased, especially N, P, Fe, Zn and Mn that were attributed to changes in soil nutrient status in continuous monocropping of sesame (Chapter Two). For instance, depletion of available N, and low absorption of P led to significant decrease in seed total N and crude protein content rendering poor quality of sesame seeds in terms of minerals. This result is consistent with several researches that show that continuous monocropping caused decrease of seed N including P affecting seed quality (Riedell et al., 2009; Bellaloui et al., 2010; Stepien et al., 2017). This could occur due to synergistic relationship between N and P in which a low accumulation of N leads to less of P. Furthermore, this study indicated a trade-off between sesame yield and quality of fatty acids. The seed yield and 1000-seed weight were significantly decreased in the long duration of continuous monocropping, but oleic, linoleic and linolenic acids significantly increased suggesting fatty acid quality could be enhanced through this system of management at the expense of yield (Chapter Four). According to Chapter Five, the sesame yield significant decreased attributed partly to decrease in available N and uptake of K resulting from erroneous fertilization including large amount of dolomite that increased Ca and Mg in the soil leading to competitive ion effect. The competitive ion effect occurred in the absorption of Ca, Mg and K in which decreased leaf tissue K concentration was observed indicating decrease in its uptake in the plants whereas leaf tissue Mg concentration was maintained. Hence, the ratio of Mg/K in plant tissue increased enhancing efficient utilization of Mg for synthesis of fatty acids in sesame plant. On the other hand, the significant increase in oleic, linoleic, and linolenic acids is attributed to increased soil magnesium (Mg) it regulates fatty acid synthesis in higher plants (Singh, 1998; Hodges, 2010).

On the other hand, the decreased leaf tissue K concentration significantly led to the decrease in 1000-seed weight, plant height, height of first/lowest capsule indicating that K is important in maintaining sesame growth and yield on upland field converted paddy fields. In addition to low K uptake, significant decrease in N uptake indicated by low leaf tissue N concentrations in the long continuous monocropping durations (Years 4, 5 and 6) compared to non-continuous monocropping fields suggested both K and N require proper management to sustain growth and yield of sesame. The decrease in leaf tissue N concentration was due to the decrease in available N resulting from its depletion from continuously monocropped soil through annual crop removal. Results also showed that continuous monocropping significantly decreased soil enzyme activities. Soil dehydrogenase, urease and catalase activities were lower in long than short durations of continuous monocropping indicating loss in soil quality of the upland field converted paddy. Soil enzymes are involved in nutrient cycling and therefore, low enzyme activities are related to declining soil fertility (Schloter et al., 2003). The decrease in soil enzyme activities suggested lack of substrates such as glucosides, urea etc. for enzymes. Organic matter inform of crop residues are broken during decomposition by soil microorganisms that involve several enzymes. However, results showed decrease in total N and C that is due to lack of residue return in continuous monocropping fields. Total C and N directly relate with organic matter content in the soil and this indicated decrease in organic matter as results showed low C and N in long duration of continuous monocropping. Similar findings that continuous monocropping decreases soil total organic carbon and nitrogen pools thus lowering the soil quality and crop yields (Al-Kaisi et al., 2005; Van Eerd et al., 2014; Buchi et al., 2017). It is known that high organic matter content is indicator of good soil fertility.

Hence, with low total N and C, enzyme activities that consequently lead to poor sesame growth and yield, continuous monocropping of sesame under this management practice is not sustainable. Therefore, increase soil N availability while ensuring proper K nutrition is paramount to maintaining average sesame yield. Overall, maintaining and increasing sesame growth and yield under continuous monocropping could be achieved through increasing availability and uptake of K and N while using high yielding cultivars. This study also showed that balancing soil cation ratios is one way of ameliorating imbalance in cation. The increase in soil exchangeable Ca and Mg that consequently affect uptake of K could be eliminated by either adding more K into continuous monocropping soil or avoiding large application of dolomite. In the green house experiment (Chapter Six), results showed that reducing Ca/K and Mg/K could significantly increase uptake of K demonstrating that sesame is susceptible to wide Ca/K and Mg/K ratios (Loide, 2004; Kumar and Sharma, 2013). The dry weight and plant height of sesame seedlings significantly increase in balancing soils with addition K from inorganic. Furthermore, the Ca/Mg ratio did not influence sesame growth suggesting that high exchangeable Mg and Ca originating from chemical fertilizer as dolomite does not have significant negative effect on sesame under continuous monocropping. This also suggested that high application of Ca and Mg to raise soil pH is not economically beneficial in addition to the negative effect of high Ca and Mg on K nutrition in sesame. Therefore, much emphasis should focus on increasing K instead of increasing Ca and Mg.

Generally, potassium is an essential nutrient for crop growth and yield and decrease in potassium leaf tissue concentrations could result into deficiency. Earlier research reported that adequate K supply in plants is required to maintain N metabolism (Leigh and Wyn Jones, 1984). It is also required for protein synthesis, which directly determines tissue growth (Leigh and Wyn Jones, 1986). On the other hand, low K uptake in low-K soils decreases photosynthesis in crops as a result of sucrose accumulation in leaves (Hermans et al., 2006; Romheld and Kirkby, 2010). Research showed that under K deficiency, the synthesis of starch and cellulose is suppressed and low-molecular-weight organic compounds accumulate; K can promote development of thick epidermal cells, which prevent pathogen attacks (Dordas, 2008). It is reported that a decreased uptake of K could increase susceptibility of plants to fungal diseases (Cao et al., 2016). In this study, disease incidence and severity showed higher percentages in the years 1–5 suggesting increase in soil pathogens and increased susceptibility due to poor K nutrition since K offers plants resistance to diseases. On the other hand, the year 6 field showed low disease symptoms despite low K uptake possibly due to increase in the bio control agents that naturally developed under continuous monocropping (Cook, 2003). However, more detailed research on soil borne pathogens and beneficial microorganism under continuous sesame is needed to support this hypothesis.

This study also demonstrated increasing K availability for sesame under continuous monocropping through biochar application could significantly increase sesame productivity on upland field converted paddy mitigating continuous monocropping obstacles (Chapter Seven). Rice husk biochar at 50 t ha^{-1} could significantly increase sesame yield by 35.0% in the non-continuous monocropping and 20 t ha^{-1} by 25.1% in the continuous monocropping (Year 1). In addition, sesame growth was improved with higher rate of rice husk biochar. Furthermore, mineral quality of crude protein, Ca, K and Mg were improved suggesting sesame respond well to biochar addition. The increase in the yield and growth of sesame was mainly attributed to increase in K including N indicated by high soil exchangeable K, soil total N and total C. In addition to increasing soil mineral nutrients, rice husk biochar might have adsorbed allelochemicals including phenolic compounds released by sesame on the second cropping field. For instance, it was reported that maize yield increased by 11-55% in the first year of application of biochar that was attributed to increase nutrient availability and absorption phytotoxins from crop residues of maize by the biochar alleviating growth inhibit due to allelopathy that causes continuous monocropping obstacle (Rogosvkas et al., 2014). Since sesame exhibit allelopathic activity (Kumar and Varshney, 2008; Hussain et al., 2017), there is a possibility that phytotoxins from decomposing root residues were detoxified by the biochar in the soil thereby increasing sesame growth. Thus application and use of biochar in a continuous monocropping system would be highly recommended in recovery of yield lost due to continuous monocropping obstacles. Hence, apart from alleviating potential autotoxicity from allelochemicals, rice husk biochar could restore the decreasing total N and C content of the upland field converted paddy under continuous monocropping of sesame increasing growth and yield. Although 50 t ha^{-1} rice husk biochar showed the highest yield, it is important to apply after economic benefits of using biochar for sesame are considered. Rice husk biochar could mitigate continuous monocropping obstacles of sesame related to limited soil nutrient availability and depletion.

The exact cause or combination of factors involved in the decline is not clear in the field conditions because of interaction among many factors (Bennett et al., 2012; Perez-Brandan et al., 2014). Several factors including loss in soil quality indicated by decrease in available N, total N, C, soil enzyme activities (dehydrogenase, urease and catalase), competitive ion effect among Ca, Mg and K leading to decrease uptake of K, and increase in disease incidence and severity possibly due to phenolic compounds including autotoxicity compounds such as ferulic, *p*-hydroxybenzoic, *p*-coumaric, caffeic and vanillic acids contributed to continuous monocropping obstacle of sesame (**Figure 36**). Therefore, to mitigate continuous monocropping of sesame, a combination of strategies focused towards increasing available N, K uptake achieved by both biochar addition and additional fertilizers, and use of tolerant cultivars such as ‘Gomazou’ and ‘Masekin’ could be suggested.

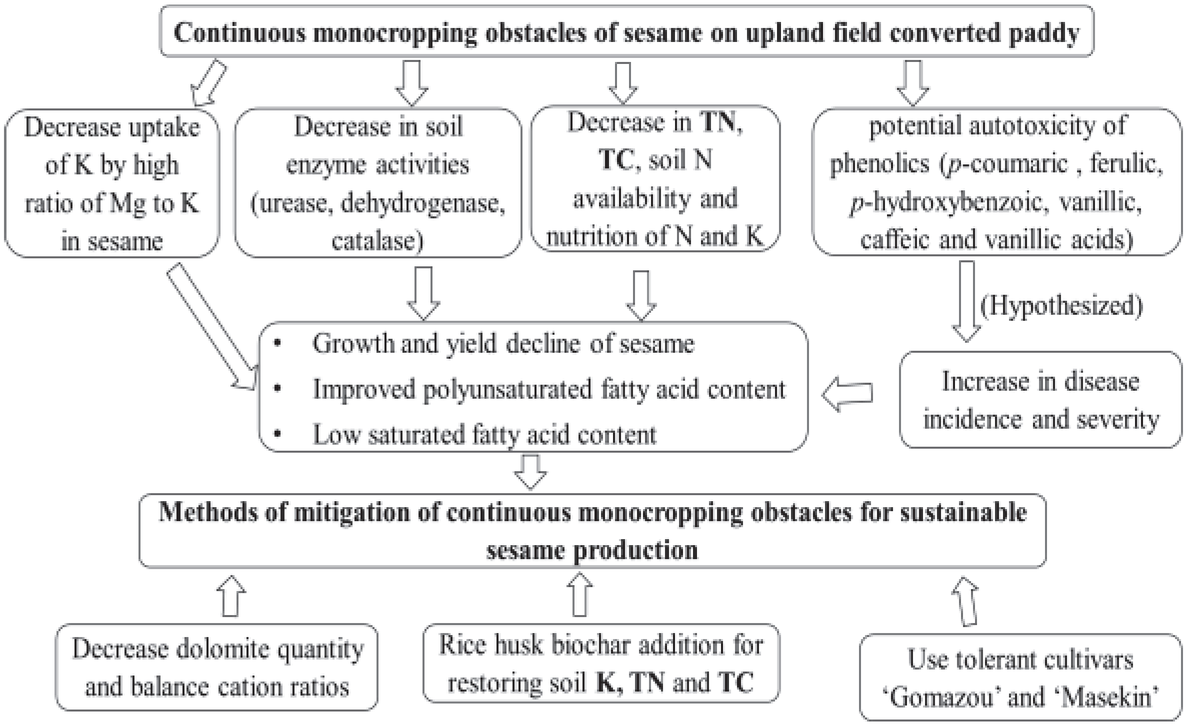


Figure 36. A diagram of the causes and mitigation of continuous monocropping obstacles of sesame on upland field converted paddy.

8.2. Conclusions

This study demonstrated that continuous monocropping of sesame on upland field converted paddy was detrimental to sesame growth, yield and seed mineral contents. However continuous monocropping under this management practice improved sesame fatty acid quality in terms of high oleic, linoleic and linolenic contents that are important fatty acids for health benefits. The growth and yield declines depended on the cultivars indicating the magnitude of continuous monocropping obstacles vary with sesame cultivars attributed to the resistance towards autotoxicity of phenolic compounds accumulated in soil and disease occurrence under field conditions. The high content of phenolic compounds could possibly promote occurrence of diseases under continuous monocropping evidenced by increase in disease incidence and severity. It could be recommended to select and adopt cultivation of 'Gomazou' and 'Masekin' that are tolerant cultivars to continuous monocropping obstacles for considerably high seed yield and quality on upland fields converted paddy. In addition, decrease in the uptake of K due to imbalance in soil cations such as K, Ca and Mg, and decreasing soil available N and its uptake negatively affected sesame growth and yield. Therefore, balancing soil cations in fertilizers to increase availability of K and use of rice husk biochar amendment to enhance K and N nutrition along with cultivating tolerant cultivars could boosts sesame productivity on upland field converted paddy.

In this study, it could be recommended to select and adopt cultivation of 'Gomazou' and 'Masekin' that are tolerant cultivars to continuous monocropping obstacles for considerably high seed yield and quality on upland fields converted paddy.

8.3. Further research

Although, there was decrease in phenolic compounds in long duration of cropping, phenolic compounds could play a significant role in modifying microbial population leading to increased sesame diseases. This study did not investigate continuous monocropping diseases and causative pathogens into details. More research is required to uncover the mechanism of disease pathogens interacting with phenolic compounds released from sesame in continuous monocropping on upland field converted paddy.

Research should also focus on increasing N availability and establishing appropriate use of dolomite lime in continuous sesame cropping on upland field converted paddy for sustainable sesame production. Despite the application of large quantity of dolomite (1000 kg ha^{-1}) to increase soil pH and supply Mg, the growth and yield is higher in non-continuous monocropping (year 0) than long duration cropping. This suggests dolomite could limit sesame growth and yield if continuously applied in each year of cropping. Hence, dolomite should be limited to one application (first cropping) and not added again as long as soil pH is in the acceptable range for sesame growth (5.0-8.0) on upland field converted paddy. Alternatively, identifying different Mg sources to maintain fatty acid quality while maintaining sufficient K and N for sustainable yield could be suggested.

Furthermore, rice husk should be adopted at an optimal rate while considering economic benefits of sesame on upland field converted paddy to replenish the depleted N and for increasing K availability. Although 50 t ha^{-1} increased sesame yield, more research is required to identify the best combinations of rice husk biochar for optimal sesame production on upland field converted paddy.

Future research could also be emphasized on returning the abandoned paddy fields to rice production after significant sesame yield declines in the long duration of continuous monocropping occur. Alternatively, a rotation of sesame with spring or autumn crops to temporarily break the continuous cropping obstacles before sesame is cultivated in the summer could be suggested.

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SUMMARY

Sesame (*Sesamum indicum* L.) is an oilseed crop cultivated throughout the tropical and subtropical regions of the world. Although Japan's sesame production is still low, its cultivation could be promoted through utilization of abandoned paddy fields. Given the profitability of sesame production, it is likely to increase from the utilization of these abandoned paddy fields and continuous monocropping is expected to become more popular.

The objectives of this study were to; (1) determine the influence of continuous monocropping (obstacles) of sesame on upland field converted paddy on seed yield and mineral nutrient contents of sesame cultivars, (2) determine the autotoxicity potentials of sesame cultivars, (3) determine the seed fatty acid compositions in relation to yield of sesame, (4) identify potentially limiting mineral nutrients, (5) determine influence of additional nutrients on the seedling growth of sesame on continuously monocropped soils and (6) determine influence of rice husk biochar addition on the growth, seed yield, and seed mineral nutrient contents of sesame.

In Chapter two, a field experiment was conducted from 2012 to 2014 to determine the effect of continuous monocropping on seed yield, crude protein and mineral nutrient contents of four sesame cultivars ('Maruhime', 'Nishikimaru', 'Gomazou', 'Masekin') and identify cultivars adaptable to continuous monocropping obstacle. Seed yield, crude protein and mineral nutrient contents were negatively affected in the second cropping: however, the level of response differed among the cultivars. Averaged over years, seed yield was significantly lowest in 'Maruhime' and 'Nishikimaru' compared with 'Gomazou' (588.3 kg ha^{-1}) and 'Masekin' (450.3 kg ha^{-1}). Averaged across cultivars, seed the crude protein and N, P, Fe, Zn and Mn decreased by 7.5, 10.0, 19.4, 14.7, and 13.6% in second cropping compared with first cropping. The variation in the seed yield, crude protein and mineral nutrient contents in the second cropping reflected differences in the cultivar response to continuous monocropping that influence the seed composition.

In chapter three, a field experiment was conducted with four sesame cultivars in 2018 on fields of 0, 1, 2, 3, 4, 5 and 6 years under continuous monocropping to analyse and identify phenolic compounds as allelochemicals in rhizosphere soils and decomposing roots of four sesame cultivars to understand the mechanisms of cultivar differences in responses towards continuous monocropping obstacle. Results indicated that decomposing sesame roots contained ferulic, *p*-hydroxybenzonic, caffeic, *p*-coumaric and vanaillic acids as the dominant

phenolic compounds with ‘Maruhime’ showing significantly highest caffeic acid content compared to all cultivars and the total phenolic compounds was highest in ‘Nishikimaru’. Phenolic compounds in the rhizosphere soil tended to decreased with increase in the duration of continuous. Although ‘Gomazou’ and ‘Masekin’ showed high phenolic contents in rhizosphere soils and high inhibition of germination and radicle growth in bioassay, their growth and yield are high under continuous monocropping in the field suggesting the allelochemical concentrations in the field are not sufficient to cause autotoxicity.

In chapter four, a field study evaluated the fatty acid compositions in relation to yield decrease of from four fields A, B, C and D with sesame cropping history of 0, 1, 2 and 3 continuous monocropping years respectively from 2015 to 2016. Compared with 2015, all fields in 2016 had higher contents of MUFA (mono-unsaturated fatty acids) and oleic acid while, in 2016, lauric, myristic and palmitic acids were lower in all fields. The high in the oleic, linoleic and linolenic acids were attributed to high soil Mg in field D whereas 1000-seed weight, lauric and myristic acids due to the high soil K in field A.

In chapter five, sesame growth and yield, nutrient concentration and soil chemical properties were investigated on five fields with continuous monocropping history: non-continuous monocropping (Year 0) and durations of two, four, five and six years fields. Plant height significantly decreased by 18.8%, 15.2%, and 13.6% in the Year 4, Year 5 and Year 6 fields, respectively, compared to Year 0. Plant leaf tissue N concentration significantly decreased in the Year 2, Year 4 and Year 6 fields compared to Year 0, whereas leaf tissue K concentration decreased in the Year 6 field. The increase in duration of continuous monocropping years gradually altered soil chemical properties. Soil pH, exchangeable Ca and Mg and CEC gradually increased in the long duration of continuous monocropping, whereas total N and C, exchangeable NH_4^+-N, urease, dehydrogenase and catalase activities decreased. This study suggested that the decrease in soil available N and enzyme activities, and decrease in K nutrition due to competitive ion effect as a result of increase in soil Ca and Mg could possibly contribute to the growth and yield decline of continuous sesame on upland field converted paddy.

In chapter six, a pot experiment was conducted under greenhouse condition of to determine effect of balancing cations of continuously monocropped soils of 1, 2 and 4-yrs on sesame growth. Results showed that balancing of soil cation ratios improved the soil chemical properties increasing nutrient concentration K, K uptake and growth of sesame plants more in the 4-yr soils than the 1 and 2-yr soils suggesting balancing the soil cations is beneficial in long term continuous monocropping. Decreasing the Ca/K and Mg/K ratios led to significant increase in the soil K saturations indicating that increased growth of sesame in continuous monocropping can be achieved when soil cation ratios are balanced bringing the K saturations above 5% to increase its availability that could be achieved through adding more K fertilizer or rice husk biochar.

Finally chapter seven was designed to assess the effect of biochar addition on sesame performance, with a specific emphasis on growth, yield, leaf nutrient concentration, seed mineral nutrients, and soil physicochemical properties in a field experiment. Rice husk biochar was added to sesame cropping at rates of 0, 20, 50 and 100 t ha^{-1} and combined with NPK fertilization in a first cropping and a second cropping field in 2017. Biochar addition increased plant height, yield and the total number of seeds per plant more in the first cropping than in the second cropping. The F+50B significantly increased seed yield by 35.0% in the first cropping whereas the F+20B non-significantly increased seed yield by 25.1% in the second cropping. At increasing biochar rates, plant K significantly increased while decreasing Mg whereas N and crude protein, P and Ca were non-significantly higher compared to the control. Soil porosity and bulk density improved with biochar addition while pH, exchangeable K, total N, C/N ratio and CEC significantly increased with biochar, but the effect faded in the second cropping. Conversely exchangeable Mg and its plant tissue concentration decreased due to competitive ion effect of high K from the biochar. Biochar addition is effective for increasing nutrient availability especially K for sesame while improving soil physicochemical properties to increase seed yield, growth and seed mineral quality.

This study demonstrated that continuous monocropping of sesame on upland field converted paddy is detrimental to sesame growth, yield and seed mineral contents but improved seed fatty acid quality in terms of high oleic, linoleic and linolenic contents. Growth and yield declines depend on cultivars indicating the magnitude of continuous monocropping obstacles vary with sesame cultivars attributed to resistance to autotoxicity of phenolic compounds and disease occurrence under field conditions. In addition, decreasing soil available N and uptake of K negatively affected sesame growth and yield. Therefore, balancing soil cations to increase availability of K and use of rice husk biochar amendment to enhance K and N nutrition could mitigate and boosts sesame productivity on upland field converted paddy. However, further research should focus on increasing N availability and appropriate use of dolomite lime without causing competitive ion effect and research to uncover the mechanism of disease pathogens interacting with phenolic compounds is necessary.

SUMMARY IN JAPANESE (要約)

ゴマ(*Sesamum indicum* L.)は，世界の熱帯から温帯にかけて広く栽培されている重要な油料作物の1つである．しかし日本の生産量は低く，日本政府はゴマの生産量を上げるために水田転換畑でのゴマ栽培を奨励してきている．しかし，ゴマ栽培は連作が困難で生産量をあげることができない．本研究では，1)水田転換畑におけるゴマの連作障害の確認，2)ゴマの品種における潜在的な自家中毒の有無，3)連作におけるゴマ子実中の品質の変化，4)連作における生育阻害を引き起こす無機成分の調査および5)その軽減に向けた栽培技術において大きく分けて5つの実験を行い下記の新たな知見を得た．

第2章では，異なる年数における新作と2年連作ほ場に4品種を栽培し，収量および子実品質について調査した．この結果，新作での4品種の子実収量は453.1～937.3kg ha^{-1}であったが，2連作目で各品種の子実収量は221.7～588.3kg ha^{-1}と明らかに収量が低下することを確認した．また，2連作目の子実の1000粒重，粗タンパク質および各無機成分は，新作に比べて明らかに低くなった．しかし，連作によるこれらの減少度合いは品種によって異なり，4品種の中で‘ごまぞう’は他の品種に比べて連作障害に対して耐性を有していた．

第3章では，新作から6連作ほ場に4品種を栽培し，各品種の子実収量および根圏土壌と残根からアレロパシー候補物質であるフェノール化合物の同定および定量を行い，各物質に対するゴマの根長および胚軸長の阻害率を調査した．この結果，連作年数が多くなると，‘まるひめ’の収量および1000粒重は他の品種に比べて少なかった．各品種の残根からは，フェラル酸，p－ヒドロキシ安息香酸，コーヒー酸，p－クマル酸およびバニリン酸を同定した．これらの化合物はある濃度でゴマの発芽率および根長を阻害させた．分散分析によってゴマの根長阻害にはコーヒー酸およびバニリン酸の濃度と品種に有意な関係性を明らかにした．しかし，‘ごまぞう’や‘真瀬金’の根圏土壌には高いフェノール化合物量が認められたが，連作年数が増えても，他の品種に比べ高い成長量と子実収量を示したことから，ゴマの連作障害に関与するアレロパシー物質は上記のフェノール化合物でない可能性が考えられた．

第4章では，異なる年数における連作年数の違い（0年～3年）が子実収量および脂肪酸組成の変化を調査した．実験1および2同様に，連作すると子実収量は明らかに減少した．子実中の脂肪酸組成の変化は，連作年数よりも栽培したほ場に依存していた．つまり，子実中のオレイン酸，リノール酸およびリノレン酸含量は土壌中のMg含量に，ラウリン酸およびミリスチン酸，そして1000粒重は土壌中のK含量に大きく影響することを明らかにした．

第5章では，異なる連作年数（0，2，4，5および6年）における品種‘ごまぞう’の生育と収量，葉の無機成分および土壌中の化学的特性を調査した．この結果，連作年数が増えるほど‘ごまぞう’の収量は減少せず，連作5年目が最も低い値（約70%減）であった．また，ゴマの葉へのN吸収は連作に伴って低下し，同時にK吸収も減少する傾向を示した．土壌pH，交換性CaおよびMgとCECは連作年数が多くなるに従って増加したが，可給態Nと土壌酵素の一種であるウレアーゼ，デヒドゲナーゼおよびカタラーゼ活性は減少した．このように，ゴマ連作土壌では可給態Nと酵素活性の減少，土壌CaとMg増加によるK吸収を阻害させ，それがゴマの成長と収量低下に寄与したと推察した．

第6章および第7章では，新作，2および4年連作土壌を供試し，ゴマの成長と土壌中のK，CaおよびMgバランスを調査した．この結果，特に連作によって元肥として施肥している苦土石灰が土壌中で蓄積することでKの植物体への吸収が低下することを明らかにし

た．今後，Ca/K 比と Mg/K 比を調整し，ゴマ自体に交換性 K を効率的に吸収させることで，連作でも成長阻害を軽減できる可能性を示唆した．K 源としては，塩化カリウムやカリウムを多く含むもみ殻炭化物の利用が有効であることも明らかにした．特にもみ殻炭化物の利用は，土壌の間隙率，かさ密度，土壌 pH，交換態 K，全 N，C/N 比および CEC など土壌物理化学性を改善させる効果を示したが，同時に交換態 K 量が多いことから Mg の吸収を若干阻害させる傾向も示した．

以上から，水田転換畑におけるゴマの連作は生育，収量，1000 粒重および子実中のミネラルを低下させた．しかしながら，連作によって収量などは低下したものの，脂肪酸組成は大きく変化し，オレイン酸，リノール酸およびリノレン酸の高い品質の良いゴマ生産ができることが確認された．連作によって生育および収量の低下は，品種に依存し，フェノール性化合物の自家中毒や土壌病害に対する耐性に寄与していると考えられた．さらに，連作によって有効態 N と K の低下が大きくゴマの生育および収量に大きく影響していた．以上から，連作障害を回避させる栽培技術は，主に連作に強い品種の導入，土壌中のカチオン（K，Ca および Mg）比の調整のための苦土石灰の施用量の適量および塩化カリウムやもみ殻炭化物のような K 資材の導入の必要性を明らかにした．

今後は，連作時の土壌中のカチオン（K，Ca および Mg）比の調整のための苦土石灰の施用量の適量と N 吸収の相互作用をより調査し，フェノール性化合物と土壌病害の発生の相互作用の解明も必要である．

LIST OF CONFERENCE PRESENTATIONS

1. **Wacal, C**., Ogata, N., Basalirwa, D., Sasagawa, D., Handa, T., Ishigaki, T., Acidri, R., Yamamoto, S., Nishihara, E. Growth and nutrient uptake of sesame as affected by amelioration of soil cation imbalance of continuously monocropped soils of an upland field converted paddy. The 246th Meeting of the Crop Science Society of Japan (Poster). Hokkaido, Japan (Sep, 2018).

2. **Wacal, C**., Acidri, R., Basalirwa, D., Handa, T., Sasagawa, D., Ishigaki, T., Ogata, N., Nishihara, E. Cultivar rotation to mitigate continuous monocropping obstacle of sesame (*Sesamum indicum* L.). The 245th Meeting of the Crop Science Society of Japan (Oral). Tochigi, Japan (Mar, 2018).

3. **Wacal, C**., Basalirwa, D., Sasagawa, D., Acidri, R., Ogata, N., Nishihara, E. Seed yield and fatty acid composition of sesame (*Sesamum indicum* L.) seeds as influenced by continuous monocropping on fallow paddy fields. The 32nd Meeting of The Sesame Society of Japan (Oral). Ibaraki, Japan (Oct, 2017).

4. **Wacal, C**., Basalirwa, D., Sasagawa, D., Acidri, R., Ogata, N., Nishihara, E. Inhibition of mineral nutrient uptake on seed yield decline in sesame (*Sesamum indicum* L.) grown in different continuous monocropping years. The 244th Meeting of the Crop Science Society of Japan (Oral). Gifu, Japan (Sept, 2017).

5. **Wacal, C**., Basalirwa, D., Sasagawa, D., Acidri, R., Fukuda, S., Nishihara, E. Role of allelopathy in growth and yield decline of sesame (*Sesamum indicum* L.) in continuous monocropping. The 243rd Meeting of the Crop Science Society of Japan (Oral). Tokyo, Japan (Mar, 2017).

LIST OF PEER REVIEWED JOURNAL PUBLICATIONS

1. **Wacal, C**., Ogata, N., Sasagawa, D., Handa, T., Basalirwa, D., Acidri, R., Ishigaki, T., Yamamoto, S., Nishihara, E., 2019. Seed yield, crude protein and mineral nutrient contents of sesame during a two-year continuous monocropping on upland field converted from a paddy. Field Crops Research. Accepted 8 June 2019. (**Partly covers Chapter Two**)

2. **Wacal, C**., Ogata, N., Basalirwa, D., Sasagawa, D., Ishigaki, T., Handa, T., Kato, M., Tenywa, M. M., Masunaga, T., Yamamoto, S., Nishihara, E., 2019. Imbalanced Soil Chemical Properties and Mineral Nutrition in Relation to Growth and Yield Decline of Sesame on Different Continuously monocropped Upland Fields Converted Paddy. Agronomy 9, (4), 184. https://doi.org/10.3390/agronomy9040184 (**Partly covers Chapter Five**)

3. **Wacal, C**., Ogata, N., Basalirwa, D., Handa, T., Sasagawa, D., Acidri, R., Ishigaki, T., Kato, M., Masunaga, T., Yamamoto, S., Nishihara, E., 2019. Growth, Seed Yield, Mineral nutrients and Soil Properties of Sesame (*Sesamum indicum* L.) as influenced by Biochar Addition on Upland Fields Converted From Paddy. Agronomy 9, (2), 55. https://doi.org/10.3390/agronomy9020055 (**Partly covers Chapter Seven**)