The effect of application of soy sauce cake on lettuce plants cultivated in a pot.

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Summary

Soy sauce is a traditional seasoning from ancient ages in Japan.

Soy sauce is a fermentation product from soybean seeds and wheat with a high concentration of salt, and the liquid soy sauce is filtered by cloth, and the residual waste of the filtration is called soy sauce cake. Soy sauce cake contains a high concentration of protein, so it is good for animal feeds and fertilizers. However, soy sauce cake contains about 13% of salt (NaCl), so the application of a high amount of soy sauce cake may induce salt stress in crops and salt accumulation in the field. The chemical composition of soy sauce cake contained about 4% of N, 2% of P_2O_5, and 1% of K_2O based on dry weight, respectively. By washing the cake, the P, K, and Mg concentrations were significantly decreased in the desalted cake, although the N was not washed out. The Na in the original cake was almost completely removed by washing. Pot experiments have been done for evaluating the effect of soy sauce cake on the growth of sunny lettuce (*Lactuca sativa* var. crispa) plants. In the first experiment, lettuce plant was grown in the sandy soil (about 3 L) with 0, 5, 25, 50, and 100 g of soy sauce cake in a 1/5000 a plastic pot, with 1-watering or 2-watering per day. The growth of the shoot and roots by 1-watering per day was increased by application of 5 g and 25 g of soy sauce cake but decreased with 50 g and 100 g of cake. The N concentrations and the nitrate concentrations of the shoot increased from 0 to 25 g of cake so the N may be provided by the decomposition of soy sauce cake. However, the concentration of Na was proportionally higher in the shoots to the application rates of soy sauce cake. It was 60 mgNa/g DW with 100 g cake was added. In the second experiment, a similar pot experiment was conducted to compare the application of 0, 5, 25, 50, and 100 g soy sauce cake or desalted soy sauce cake. We expected that the application of desalted soy sauce cake may give more effective than the original soy sauce cake due to the lack of salinity stress. However, the growth of lettuce was more severely depressed by the addition of desalted cake compared with the original cake. As the application rates of soy sauce cake increased, the water content in the surface soil increased. The poor growth of lettuce with desalted cake may be due to the excess water stress, which represses respiration and nutrient absorption of the roots.

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Key words : Soy sauce cake, Lettus, Ferilizer, Growth

Introduction

Soy sauce is a traditional seasoning from ancient ages in Japan to cock soup or for dipping sushi and sashimi (Ohyama et al. 2013). The annual production of soy sauce decreased from 900,000 tons in 1941 to 340,000 in 1947 during World War 2 but quickly recovered to 1,000,000 tons in 1956 (Soy sauce information center home page and statistical data). Annual production was highest around 1,200,000 tons during the 1990s, but gradually decreased about 760,000 tons in 2018. In 2018, Chiba prefecture accounted for 37% of the annual production of soy sauce, Hyogo prefecture accounted for 15% and Gunma prefecture for 6%.

Soy sauce is made from soybean seeds and wheat (Ohyama et al. 2013). Soybean seeds or the defatted soybean cake are steamed with water at 120 C for 40-60 min. Wheat seeds are parched at about 170 C. The steamed soybean seeds are mixed with parched wheat and sprayed with a suspension of koji-kabi, *Aspergillus genus*. Then it is incubated at 25-30 C for about 45 hrs under 95% moisture conditions. Koji is mixed with 20-30% saltwater under 15 C for 2 months, then salt-tolerant yeast is added to the koji, and continue to incubate at 30 C for 4-10 months. During the incubation period, some of the proteins are broken down into the amino acids or peptides and carbohydrates are degraded into sugar. At the same time, yeast ferments sugars into alcohol and fragrance. Some amino acids and sugars react to make a brown color. The product is called “moromi”. The moromi is filtered through several layers of filter cloth by pressure. The filtrate is called “ki-shoyu” raw soy sauce and usually pasteurized over 85 C to decrease microbial activity in the bottle. The residue of filtration is called soy sauce cake, and 80 g of soy sauce case is produced by 1 L of soy sauce. Then the annual production of soy sauce cake is about 60,000 tons in Japan.

The filtered residue of soy sauce cake is a wet sheet about 3 mm thickness. The taste and flavor are like soy sauce or “miso”, but it is difficult to eat as it is very salty, bitter and harsh taste with strong smell (Ito et al. 1998). Ito et al. (1998) reported the composition of soy sauce cake that

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contains 37.5% of water, 5.0% of salt, 24.3% of crude protein, 6.9% of crude fat, 25% of crude fiber, 1.6% of ashes without salt. Uehara and Yamashita reported that soy sauce cake contained 30.2% of water, 19.2% of crude protein, 10.2% of crude fat, 32.6% of carbohydrate, 7.8% of ashes with 6.6% of salt (Uehara and Yamashita, 2013). The percentage compositions are similar, and the small difference may be due to the ratio of soybean and wheat materials, or the production procedures and period.

Due to a large amount of soy sauce cake production, and the high cost of waste management such as burning, the utilization of soy sauce cake has been investigated, such as fertilizer (Uchida 1978), animal feeds (Fukuma 1978, Tanabe 2014), artificial medium for mushroom cultivation (Kadowaki 1978), and pickles production (Ito et al. 1998). By mixing soy sauce cake about 5% of the feeding, the milking cows prefer to eat and milk production increased (Tanabe and Nagao, 2014). However, a higher rate of soy sauce cake may be harmful due to the high concentration of salt. Big soy sauce company use all the soybean cake for animal feeds (Kikkoman Home Page), but it is difficult for the small producers because soy sauce cake is wet and easy to spoil.

In this research, we used the dried soy sauce cake or desalted dried soy sauce case as fertilizer in the pot cultivation of lettuce plants.

MATERIALS AND METHODS

Chemical analysis of soy sauce cake

The sheet of soy sauce cake was supplied from a soy sauce factory in Niigata. The cake contained 28% of the water in the fresh material. The soy sauce cake was dried in a ventilation oven at 60 C for three days, then ground into a fine powder by a vibration mill (Heiko Sample Mill TI-100).

In order to remove salt from the cake powder, 2 ml of water was added to the 100 mg of cake powder, and ground with a mortar and pestle. The macerate was centrifuged at 12,000 rpm for 15 min. The precipitate was washed twice by another 2 mL of water. Then washed precipitate was dried and ground by a vibration mill.

The 50 mg of cake powder or desalinated cake was digested in a test tube by the Kjeldahl digestion method using 1 mL of concentrated H_2SO_4 (Ohyama et al. 1991). The digested solution was filled up to 25 mL by water, then ammonium and phosphate concentrations in the diluted solution were determined colorimetrically by the indophenol method and ammonium molybdate method, respectively (Ohyama et al. 1991).

Another 50 mg of cake powder or desalted cake was digested by 0.5 mL of concentrated nitric acid (HNO_3) and 0.4 mL of perchloric acid ($HClO_3$) (Mizukoshi et al. 1994), and the concentrations of Na, K, Ca, and Mg were determined by an atomic absorption method (Hitachi Z-8200).

Water holding capacity of soy sauce cake

The 1 g of dried sheet or powdered soy sauce cake was incubated in 50 mL of water for 24 hrs. Then the residues were filtrated by a filter paper, and the volume of filtrate water was measured. The water holding capacity was calculated by 50 mL minus the volume of filtrate.

Pot experiment for lettuce plants

The pot experiments were conducted in the glasshouse of the Faculty of Agriculture, Niigata University (37.8701°N/138.9438°E). About 3 L of a soil from the sandy dune field of Faculty of Agriculture was put in a 1/5000 a plastic pot. The chemical fertilizers 0.338 g of ammonium sulfate, 0.388 g of superphosphate, and 0.138 g of potassium sulfate, and 5.98 g of lime were mixed in the soil. The soil in each pot was mixed with 0, 5, 25, 50, or 100 g of dried soy sauce cake. The seeds of red leaf lettuce (*Lactuca sativa* var. crispa) was planted in a vermiculite bed on 17 July 2000, and young a seedling was transplanted to each pot on 18 DAP (days after planting).

In experiment 1, watering was either once or twice a day to change the washing of salt or leaching of the fertilizer minerals. In one group the pots were watered once a day in the morning. Watering was stopped after 50 DAP, and plants were harvested on 57 DAP. In another group of pots, watering was twice in the morning and evening, and plants were harvested on 50 DAP. The harvested plant was separated into shoot and roots, and the fresh weight was measured. After plant parts were dried in a ventilation oven, the dry weight was measured and ground into a fine powder using a vibration mill (Heiko, Sample Mill TI-100). 50 mg of the dried plant powder was digested by the Kjeldahl digestion or HNO_3-$HClO_3$ digestion as mentioned before, and the concentrations of N, P, K, Ca, Mg, Na, and Fe were determined.

In experiment 2, the desalted soy sauce cake (desalted cake) was used as fertilizer. About 1 kg of soy sauce cake was put into a 4 L of water and the water was discarded at 12 hrs, and 4 L of water was added again. The washed cake was dried in a ventilation oven at 60 °C for 6 days, then ground into powder. Seeds of red leaf lettuce were planted in vermiculite bed on 10 September 2002. About 3 L of the soil from the sandy dune field of Faculty of Agriculture was put in a 1/5000 a plastic pot. The chemical fertilizers 1.428 g of ammonium sulfate, 1.143 g of superphosphate, and 0.594g of potassium sulfate, and 5.93 g of lime were mixed in the pot. Because the growth of plants was poor in Experiment 1, the amount of fertilizer was increased in Experiment 2. The soil in each pot was mixed with 0, 5, 25, 50, or 100 g of dried soy sauce cake or desalted cake. Water was supplied once a day in the evening. The watering was stopped after 50 DAP, and plants were harvested on 56 DAP. The harvested plant was separated into roots and shoot, and fresh weight was measured. After plant parts were dried, the dry weight was measured and ground into a fine powder. The dried plant powder was digested, and the concentrations of N, P, K, Ca, Mg, Na, and Fe were determined as same as above.

RESULTS AND DISCUSSION

Composition of soy sauce cake and water holding capacity

Table 1 shows the concentration of N, P, K, Ca, Mg and Na in soy sauce cake and desalted cake. The Na concentration was about 50 mg/gDW in the soy sauce cake, which is equivalent to 127 mg/gDW of NaCl. By washing soy sauce cake, the Na concentration decreased to 0.6 mg/gDW (1.5mg NaCl). The concentration of N in soy sauce cake was about 39 mg/gDW and only slightly decreased in the desalted cake (35 mg/gDW), which indicates that the most of N in the cake were water-insoluble compounds such as protein. The concentration of P, K, and Mg were about 4.5, 4.3 and 0.8 mg/gDW and the concentration decreased by washing in desalted cake P (1.9 mg/gDW), K (0.7 mg/gDW) and Mg (0.3 mg/gDW), respectively. On the other hand, Ca concentration in the desalted cake was 6.6 mg/gDW and higher than the original cake 5.2 mg/gDW, indicating that most Ca was water insoluble forms.

The chemical composition of soy sauce cake indicated that the soy sauce cake contains about 4% of N, 2.1% of P_2O_5, and 1.2% of K_2O respectively. By washing the P, K, and Mg concentration was significantly decreased in desalted cake, although N is not washed out. The Na concentration decreased to safety level by washing.

Table 1. Mineral concentrations in soy sauce cake and desalted soy sauce cake.

mg/gDW

	N	P	K	Ca	Mg	Na
Soy sauce cake	38.9	4.5	4.3	5.2	0.8	49.8
Desalted cake	35.1	1.9	0.7	6.6	0.3	0.6

Figure 1 shows the water holding capacity of the dried sheet of soy sauce cake or the powder of soy sauce cake after grinding. The one g of cake sheet held about 2 g of water, and one g of cake powder held about 6 g of water. The result showed that dried soy sauce cake has high water holding capacity especially in the form of powder.

Experiment 1

In experiment 1, watering was either once or twice a day to change the washing the salt from soy sauce cake or nutrient minerals.

Figure 2 shows the fresh weight of the shoot and roots of lettuce plants with various about of soy sauce cake watered once (50 DAP) or twice a day (57 DAP). The plant watered once showed that the application of 5g and 25g of

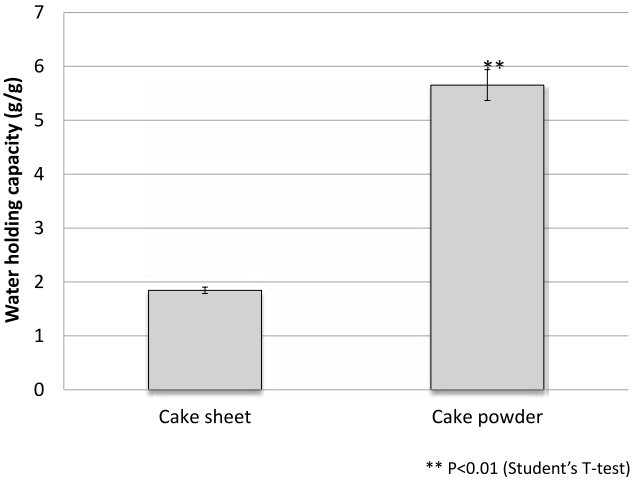

Figure 1. Water holding capacity of dried soy sauce cake.

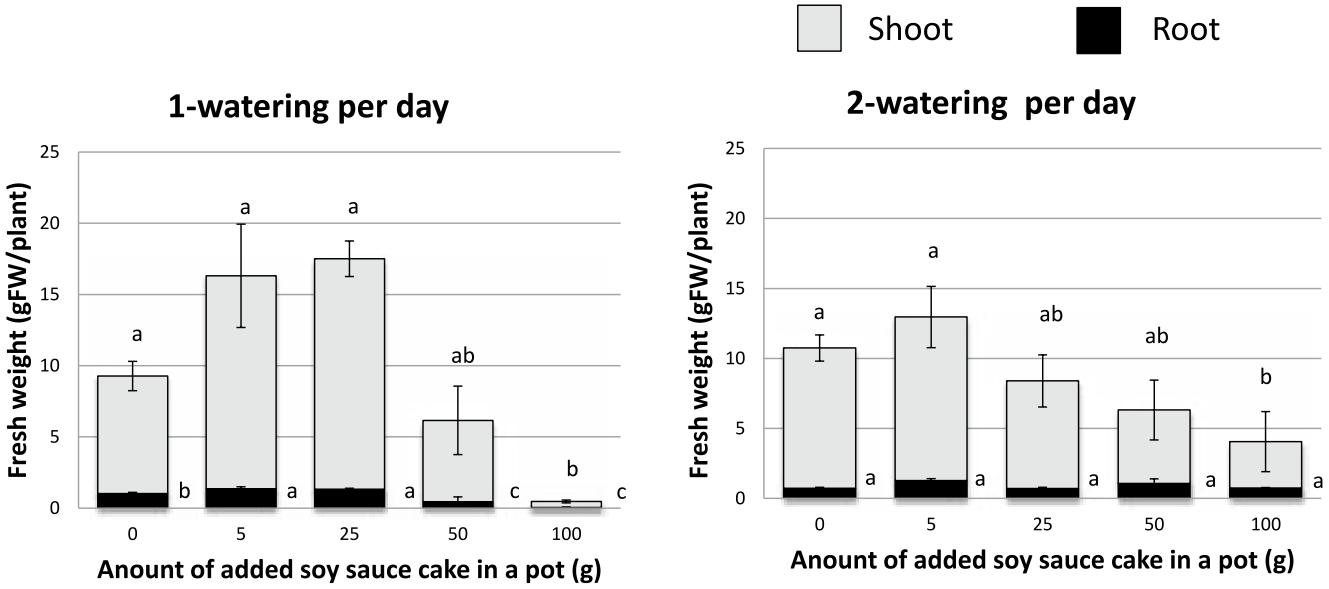

Figure 2. Fresh weight of shoot and roots of lettuce plant with soy sauce cake by one or two watering per day.
The different alphabets show the statistic difference at P<0.05 by Tukey's test.

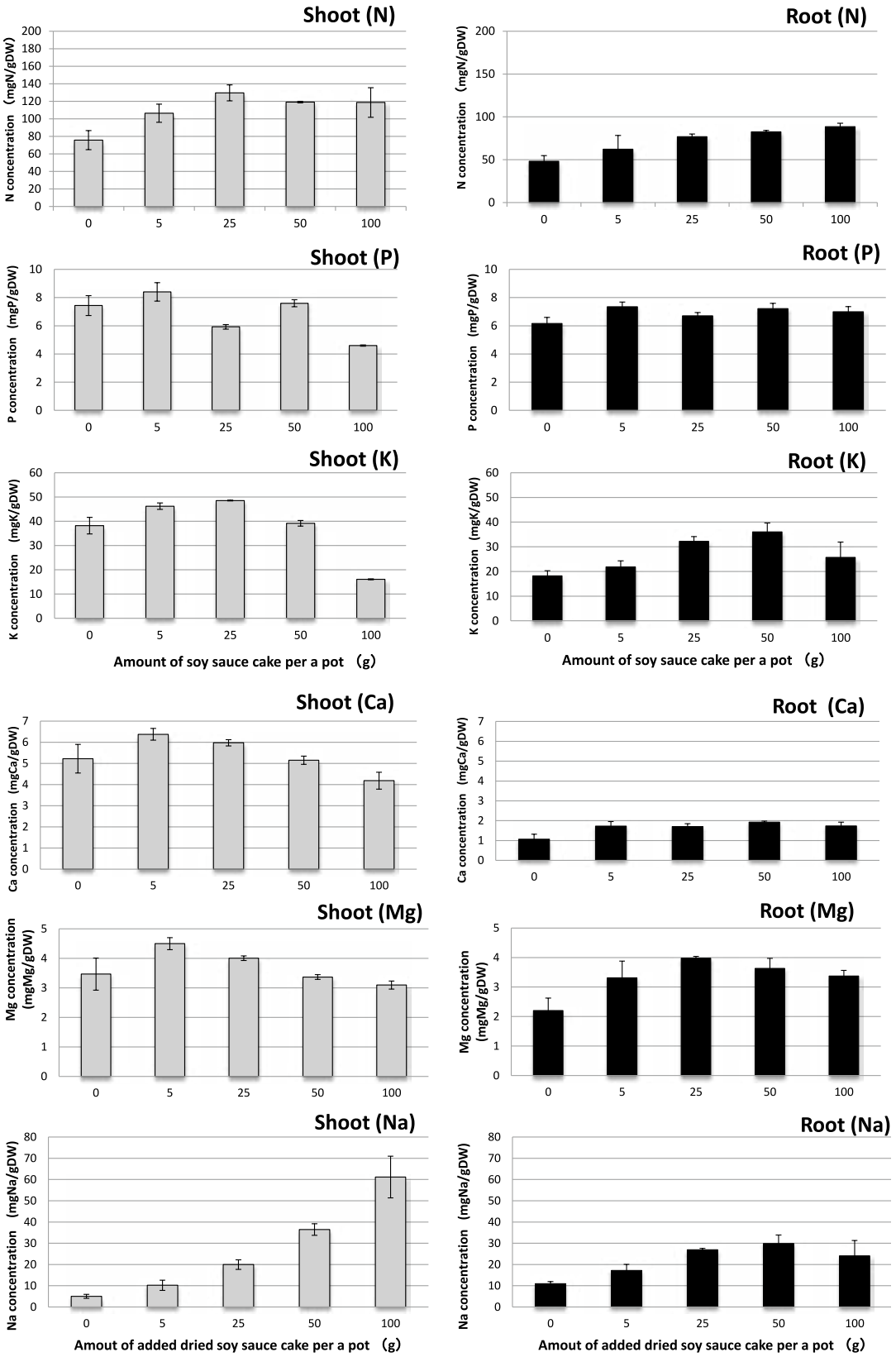

Figure 3. N, P, K, Ca, Mg, and Na concentration in the shoot and roots of lettuce plant treated with various amount of soy sauce cake supplied with 1-watering.

soy sauce cake increased the fresh weight of a shoot about 15 g and roots about 1.3 g compared without soy sauce cake (0 g) a shoot about 8 g and roots about 1.0 g. However, the application of 50 g of soy sauce cake decreased the shoot (6 g) and root (0.4 g) fresh weight, and the shoot (0.4 g) and roots (0.04 g) are very low with the application of 100 g of soy sauce cake.

By 2-watering, the fresh weight of control plants without soy sauce cake was 10 g for shoot and 0.7 g for roots. By supplying 5 g of soy sauce cake, the fresh weight of shoot (11.7g) and roots (1.3 g) was slightly increased compared with the control plant without soy sauce cake. Supplying 25 g and 50 g of soy sauce cake decreased the fresh weight of shoot and roots compared with the control. However, plant fresh weight retains the growth with 100 g cake exhibiting shoot weight (3.3 g) and root weight (0.7 g) compared with the same 100 g treatment with 1-watering.

Because the 2-watering might wash out the salt from soy sauce cake more rapidly than 1-watering, the application of 100 g of cake was better with 2-watering compared with 1-watering in which lettuce growth was almost completely inhibited. However, the application of 5 g and 25 g of soy sauce cake did not promote the lettuce growth with 2-watering although the 1-watering promoted the growth with 5 g and 25 g of soy sauce cake. This result may be due to the situations where the nutrients from chemical fertilizers and those from decomposed soy sauce cake were leached before absorbed by lettuce roots, or water excess in the pot with 2-watering.

Figure 3 shows the N, P, K, Ca, Mg, and Na concentrations in the shoot and roots of lettuce plant treated with various amounts of soy sauce cake supplied with 1-watering. The concentration of N in the shoot increased with increasing the amount of cases from 0 to 25 g per a pot from 76 to 130 mgN/gDW, then it was constant at 50 and 100g per pot. On the other hand, the N concentration in the roots consistently increased from 48 to 89 mgN/gDW with increasing the rate of soy sauce cake application.

The P concentration in the shoot tended to be decreasing by the application of soy sauce cake, although it was constant in the roots.

The K concentration in the shoot was increased by the amounts of soy sauce cake from 0 to 25 g per pot, then decreased to 100 g per pot. The concentration of K in the roots increased to 50 g per a pot, then decreased at 100g.

The Ca and Mg concentrations in the shoot and roots changed similar to K, but those were relatively constant among application of soy sauce cake compared with N, P, and K.

The Na concentration in the shoot was proportionally increased by the amount of the addition of soy sauce cake. This may be due to the origin of Na is mostly from cake with high concentration of Na (50 mg/gDW). The Na concentration in the roots increased from 0 to 50 g/pot but slightly decreased by 100 g application.

In this experiment, the plant growth with 1-watering

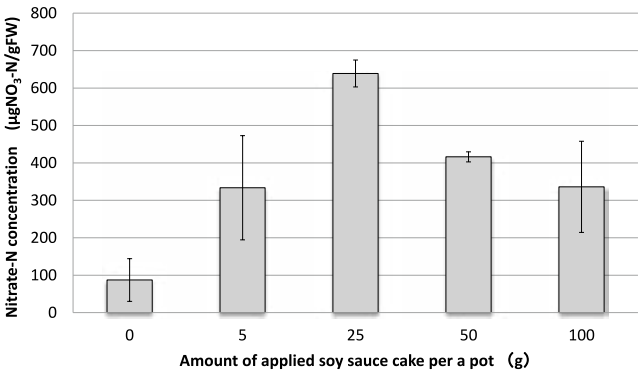

Figure 4. Nitrate-N concentration in the shoot of lettuce plant treated with various amount of soy sauce cake supplied with 1-watering.

was better than 2-watering judged from the fresh weight (Figure 2). Concerning the optimum application rate of soy sauce cake was about 25 g per pot under these conditions (Figure 2,3). The higher application of soy sauce cake inhibited the plant growth (Figure 2) and nutrients absorption of P, K, Ca, and Mg (Figure 3). In addition, the concentration of Na increased in the shoot when 50 g and 100 g of the soy sauce cake were applied, which might strongly inhibit plant growth.

Figure 4. shows the nitrate-N concentration in the shoot of lettuce plant treated with various amounts of soy sauce cake supplied with-watering. When no cake was applied at 0 g per pot, the NO_3^- concentration was only 87 μmgN/gFW, but increased to 639 μmgN/gFW by application of 25 g soy sauce cake per a pot. This suggests that the N is efficiently supplied from the soy sauce cake by the decomposition in the soil. The higher application rate of soy sauce cake at 50 and 100 g decreased the NO_3^- concentration in the shoot, possibly due to inferior growth and nitrate absorption activity in the roots by salt stress.

Experiment 2

Photo 1 and Photo 2 show the photographs of the lettuce plant in a pot with soy sauce cake (Photo 1) and desalted cake (Photo 2) in the experiment 2. The plant growth at 0 g soy sauce cake was much higher than that in Experiment 1, because the higher amounts of chemical fertilizers were applied. Therefore, by application of 5g of soy sauce cake the lettuce growth was similar to the control without soy sauce cake. By the application of 25 g and 50 g of soy sauce cake, some plants grow well but some was severely repressed. By the application of 100 g of soy sauce cake, most of the plants grow poorly. By the application of desalted cake, the growth decreased by increasing the application rate, and it was very poor with 50 g and 100 g of desalted cake.

Figure 5 shows the fresh weight of shoot and roots of the lettuce plant cultivated with various amount of soy sauce cake (left) or desalted cake (right). Unexpectedly, the depression of lettuce growth was more severe by the

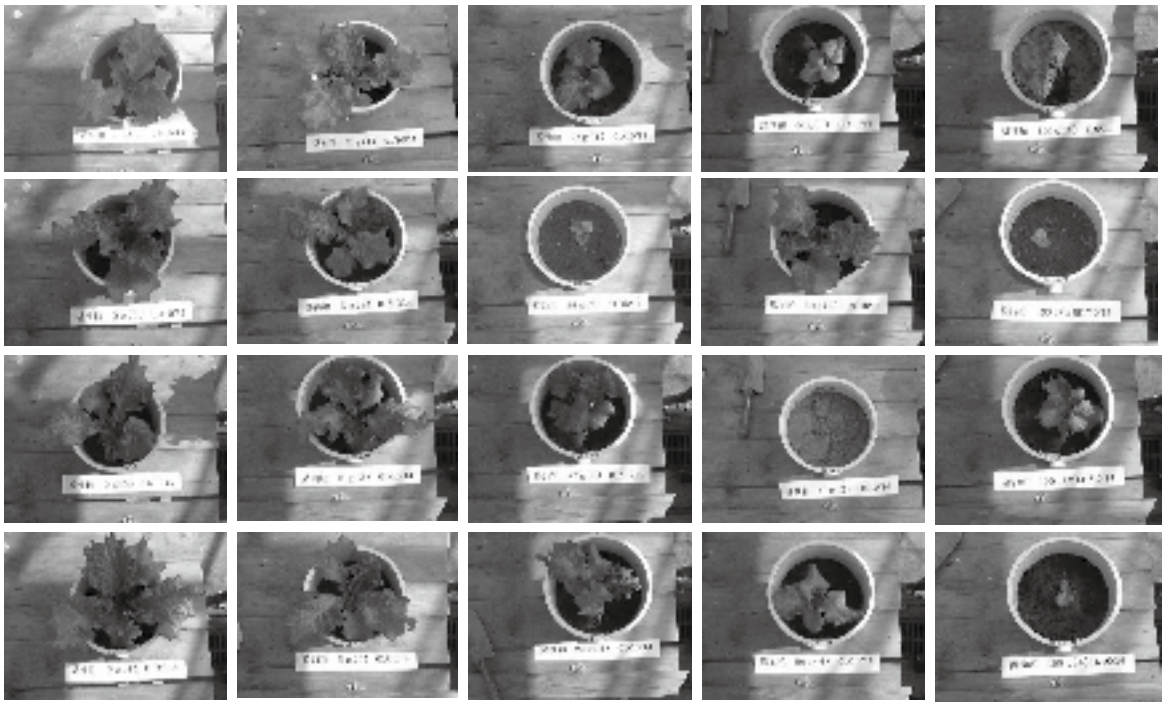

Photo 1. Photographs of lettuce in a pot with soy sauce cake.

Photo 2. Photographs of lettuce in a pot treated with desalted soy sauce cake.

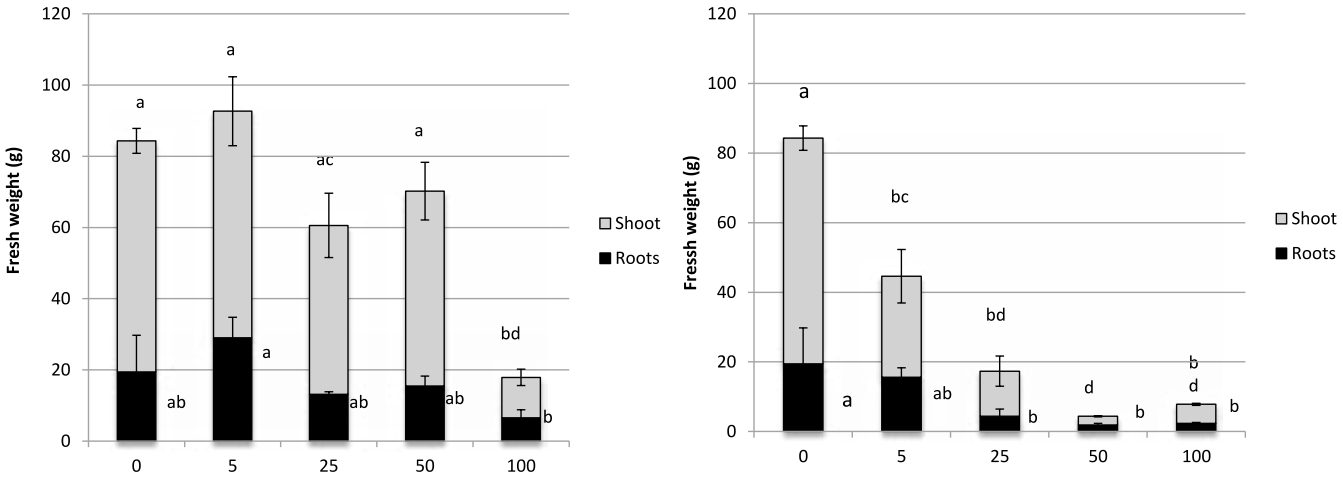

Figure 5. Fresh weight of shoot and roots of lettce caltivated with various amount of soy sauce cake or desalted soy sauce cake. The different alphabets show the statistic difference at P<0.05 by Tukey's test.

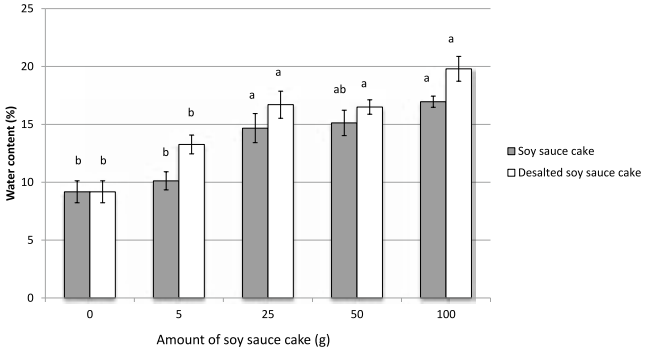

Figure 6. Water content in surface soil of pot supplied with various amount of soy sauce cake or desalted soy sauce cake. The different alphabets show the statistic difference at P<0.05 by Tukey's test.

application of desalted cake compared with original soy sauce cake with high concentration of salt. Because Na was removed in the desalted cake, so the depression of soybean growth by desalted cake is not due to Na stress. Therefore, this may be attributed to the excess water or decrease in the oxygen concentration by rapid decomposition of organic matter in the soil.

The surface soil at the depth between 0-5 cm was taken from the pot after plant sampling, then the water content was determined (Figure 6). When the amount of soy sauce cake was higher, the water content became higher from 9% (0 g cake) to 17% (100 g cake). The application of desalted cake was always higher than the original cake, and the highest water content was 20% with 100 g desalted cake. The high water-holding capacity may be beneficial for plant growth under dry conditions, however, under excess water conditions, this might repress root growth and nutrient absorption activity due to low oxygen concentration and or the accumulation of organic acids in the soil.

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ポット栽培レタスにおける醤油粕の施肥効果

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要　約

醤油は古来から日本の伝統的な調味料として使われている。醤油は、ダイズと小麦を高塩濃度で発酵させて製造され、液体の醤油は布でろ過して得られる。ろ過残渣は、醤油粕と呼ばれる。

醤油粕は、高濃度のタンパク質を含み、成分元素として、N 4%、P_2O_5 2%、K_2O 1% 程度を含み、家畜の餌や肥料として適している。しかしながら、塩濃度が高いため、使用には注意が必要である。肥料として施用する際には、作物の塩類ストレスや圃場の塩類集積に注意が必要である。

塩分を除去するために、醤油粕を水洗したしたところ、塩分はほぼ完全に除去された。また、脱塩醤油粕ともとの醤油粕は、窒素濃度はほぼ変わらなかったが、P、K、Mg 濃度が半分以下に減少した。

乾燥した醤油粕0、5、25、50、または100g を約3L の砂丘地土壌と混合して、1/5000a ポットに詰めてレタスの栽培試験を行った。最初の実験では、毎日朝だけ灌水する灌水１回区と、朝夕灌水する灌水２回区を設けた。灌水１回区では、醤油粕を5g または25g を施用した区で対照区（醤油粕0g）よりも倍近く生育が促進された。しかしながら、50g、100g 区では対照区よりも生育が抑制された。灌水２回区では、除塩効果により、元の醤油粕よりも生育促進効果が期待されたが、逆に、生育促進効果は低かった。灌水１回区の乾燥粉末中の元素濃度を調べたところ、茎葉部の窒素濃度は、醤油粕5g と25g 施用で増加したが、50g、100g の施用では、それ以上増加しなかった。また、地上部の硝酸濃度も25g 区で最大値を示したことから、醤油粕の分解により生じた窒素がレタスに吸収されていることを示した。一方、醤油粕の施用量が増すにつれて、茎葉部の Na 濃度は上昇し、100g 施用区では、乾物重あたり60 mg/gDW と高濃度に集積した。

第二の実験では、元の醤油粕粉末と、除塩醤油粕の施用効果を比べた。除塩醤油粕はナトリウムをほとんど含まないため、塩類ストレスが回避され、より効果が高いと期待されたが、むしろ、レタスの生育は抑制された。醤油粕の添加量が増えるにつれて、ポット表土の水分含有率が増加したため、本試験では、醤油粕や脱塩醤油粕を添加したポットは湿害による根の呼吸や養分吸収の阻害または、有機酸の蓄積の害を受けた可能性がある。

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キーワード：醤油粕、レタス、肥料、ポット栽培、生育

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