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Repellent Effect of Neem against the Cabbage Armyworm on Leaf Vegetables

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Abstract. We investigated the pest repellent effect of azadirachtin formulation and neem seed kernel oil cake. In laboratory tests, the repellent effect of komatsuna and spinach treated with azadirachtin formulation or neem seed kernel oil cake for 7 days on the feeding cabbage armyworm were evaluated. The feeding repellent effect of azadirachtin formulation treatment was equivalent to that of commercial biological pesticide, while the effect of neem seed kernel oil cake treatment was higher. This result clarified that neem seed kernel oil cake has a high feeding repellent effect against cabbage armyworms. In field tests, although the feeding percentage for komatsuna and spinach controls was 70%, that for komatsuna and spinach treated with azadirachtin formulation and neem seed kernel oil cake was about 40% and 30%, respectively. These laboratory and field test findings demonstrated that despite having an affect less than that of azadirachtin, neem seed kernel oil cake is a high effective feeding repellent.
Keywords: neem seed kernel oil cake, azadirachtin formulation, biological pesticide, cabbage armyworm, feeding repellent

INTRODUCTION:

Recently, there have been doubts on the use of chemical pesticides and fertilizers due to concerns regarding environmental pollution, adverse effects on human health, disruption of natural biological control, and evolving resistance of pests to pesticides. Both consumers and producers of crops are interested in chemical-free vegetables and organic farming. As consumer interest in the safety of food and agricultural products has risen in recent years, producers are increasingly focused on pesticide-free and organic farming methods that take both human health and the environment into account. In Japan, about 600 agricultural pesticides have been included in the “Positive List”, which since its introduction in 2006 has established maximum limits for chemical residues that can remain in food products. Without the use of chemicals, however, such farming methods increase the risk of disease and insect damage to crops (Perry et al., 1998; Isman, 2006). The development of neem materials is focused on the modern paradigm for the development of botanical insecticides (Isman, 2006; Thompson and Kreutzweiser, 2007).

The neem tree (*Azadirachta India* L.) is a fast growing hardy and evergreen tropical and subtropical plant belonging to Meliaceae. Neem is well known in India and its neighboring countries where for 2000 years it was one of the most versatile medicinal plants, having a wide spectrum of biological activity (Alves et al., 2009; Atawodi and Atawodi, 2009). Highly concentrated azadirachtin is the main active ingredient in neem and is the starting point for neem extracts.

There are two types of harvestable neem materials. The first is azadirachtin which is extracted from the neem oil of compressed neem seeds, concentrated, and purified. Azadirachtin is a pest repellent sprayed onto leaves diluted with water. The second material is neem seed kernel oil cake, simply called neem cake, which is divided into fruit cake, seed cake and neem seed kernel oil cake, and is used primarily as an amendment and growth-promoting agent.

The neem tree synthesizes compounds for chemical defense to protect against herbivorous insects (Atawodi and Atawodi, 2009). These compounds function on the basis of interfering with insect hormones (Mordue and Blackwell, 1993, Anibal, 2007). Nine limonoid compounds with pest control properties have been extracted from neem seeds and shown to inhibited pest growth, and the most effective of main compound is azadirachtin (Koul et al., 1990; Schmuttere, 1990; Alves et al., 2009). Azadirachtin induces a physiological effect on insects by interfering with the synthesis and release of ecdysteroids which disrupts larval moulting in hemi- and holometabolous insects, interferes with pupation and/or eclosion of adults, and interferes with reproduction (Mordue and Blackwell, 1993). Salannin is another pest management component that is reported to have a strong repellent effect. Meliantriol is an insect feeding deterrent effective at low temperatures. The other limonoids comprise ninbin and nimbidin, which have antivirus activity, ninbiol, which has antiprotozoal and antitubercular activity, gedunin, which has antimalarial activity, sodium nimbinate, which has diuretic and spermicidal activity, and quercetin, which has antioxidant and antimicrobial activity (Dai et al., 1999; Subapirya and Nagini, 2005). On insect larvae, these compounds have growth regulatory effects which include disruption of moulting, growth inhibition, and malformation and may contribute to mortality. Their effect can be attributed to disrupting endocrine events by downregulating the haemolymph ecdysteroid level through blocking the release of prothoracicotropic hormone (PTTH) from the brain-corpus cardiacum complex or delaying the appearance of the last ecdysteroid peak completely inhibiting molting. These compounds also affect allatoropin and juvenile hormone levels (Gill and Lewis 1971; Koul et al., 1990; Schmutterer 1990; Ascher 1993). Neem-based insecticides are known for their pesticidal activity against more than 400 species of insects (Siddiqui et al., 2003). However, they are not toxic to humans or many beneficial arthropods, and targeted pests are unlikely to develop resistance; therefore, these insecticides have been advocated to replace synthetic insecticides as it become the more sensible to be used in most pest management programs (Schmutterer, 1990; Ascher, 1993; Mordue and Blackwell, 1993; Schmutterer 1995; Mordue et al., 1998; Isman, 2006; Irigaray et al., 2010). Thus, neem oil-based pesticides are available for use against many pests and have been evaluated as an alternative to synthetic pesticides (Walter, 1999; Anibal, 2007).

The cabbage armyworm (*Mamestra brassicae* Linnaeus), which is a considerable pest to many economically important vegetable crops, emerges 2 or 3 times per year. Larvae are grown from the first to the sixth star before emerging from their pupae. In addition, the cabbage armyworm feeds on various plants, except those of Poaceae (Bonnemaison, 1965), and it causes extensive damage on a global scale, and in particular, to a field of Meiji University, Japan.

In this study, we investigate whether or not the cabbage armyworm is repelled when feeding on komatsuna (*Brassica rapa* var. *peruviridis*) and spinach (*Spinacia oleracea* L.) treated with azadirachtin and neem seed kernel oil cake, in both the laboratory and field, and we evaluate the utility of neem formulation.

MATERIALS AND METHODS:

Materials

Komatsuna 'Akisai' and spinach 'Okame' were purchased from Tohoku Seed Co. (Tohoku Seed Co. Ltd., Utsunomiya, Japan) and Takii Seed Co. (Takii Seed Co. Ltd., Kyoto, Japan). Azadirachtin formulation, 'AZ green N' (1.2% azadirachtin and 2.8% of neem extract), and neem seed kernel oil cake 'Rikunomegumi' (0.3~0.5% azadirachtin) were purchased from OM Science

(Osaka, Japan).

Pests

Two- and three-star larvae of the cabbage armyworm were collected from a field at Meiji University. The larvae were reared on cabbage in a plastic case (10 cm × 20 cm × 7 cm) at 25 °C under a L12:D12 photoperiod until reaching 4-star stage when they were used in the experiments.

Cultivar condition

Komatsuna and spinach for the feeding repellence test were seeded at 5-cm intervals in a 30 cm × 240 cm patch in a field at Meiji University on August 27, 2009 and were harvested on October 7. Similarly, komatsuna and spinach for the feeding damage test were seeded at 5-cm intervals in a 30 cm × 240 cm patch on August 27 and harvested on October 13, 2009. Fertilization conditions were according to the common used concentration.

Neem treatment

For the feeding repellence test, komatsuna and spinach were treated with azadirachtin formulation diluted 500-fold with tap water (a commonly used concentration), and 2 ml of solution was subsequently applied to the leaf surface. One hundred milligrams of neem seed kernel oil cake was also used to treat the leaf surface. As a positive control, commercial chemical pesticide 'ST Aquateric' (Sumitomo Chemical Garden Products Inc., Tokyo, Japan) and biological pesticide 'Esmark DF' (Sumitomo Chemical Co. Ltd., Tokyo, Japan) were diluted 750-fold and 1000-fold, respectively, in tap water as per commonly used concentrations, and 2 ml of each solution was applied to the leaf surface. As a control, 2 ml of tap water was applied to the leaf surface. All cultivars were performed in ten replicates.

For the feeding damage test, komatsuna and spinach were treated with azadirachtin formulation diluted 500-fold with tap water (a commonly used concentration), and 2 ml of the solution was applied to the leaf surface after germination. Three grams of neem seed kernel oil cake per plant was also applied. Treatment with azadirachtin formulation and neem seed kernel oil cake was once a week. As a positive control, commercial chemical pesticide 'ST aquateric' and biological pesticide 'Esmark DF' were diluted 750-fold and 1000-fold in tap water as per commonly used concentrations, and 2 ml of these solutions were applied 4 times to the leaf surface before harvest. The control was not treated, and experiments for each cultivar were performed in ten replicates.

Feeding repellency test

After harvest, komatsuna and spinach leaves were cut into 4 cm^2 squares, and 2 4-star cabbage armyworm larvae were placed in a 9 cm plastic petri dish and reared at 25 °C under a L12:D12 photoperiod for 7 days. Feeding percentage was evaluated to calculate the surviving (uneaten) leaf area.

Feeding damage test

Feeding damage of harvested komatsuna and spinach (measured on September 25 and November 24, 2009) were visually evaluated using a 5-point scale as follows: 100%: only stem or firing, 80%: prototype leaves absent, 60%: leaves of no commercial value, 40%: leaves of slight commercial value, 20%: almost no feeding damage.

Statistical analysis

Statistical differences between treatments were assessed using the Turkey-Kramer test at $P<0.05$.

RESULTS AND DISCUSSION:

The feeding percentage results of the spinach treated in the laboratory are shown in Figure 1. The feeding percentage of the control was 100% on day 1 after treatment. The feeding percentage of azadirachtin formulation treatment was 75% on day 1 after treatment, 90% on day 2, and 98% on day 7; azadirachtin formulation did not show a feeding repellent effect. The feeding percentage of neem seed kernel oil cake treatment was 52% day 1 after treatment, 70% on day 2, and 88% on day 7; neem seed kernel oil cake maintained a higher feeding repellent effect than azadirachtin formulation throughout the experimental period. The feeding percentage of biological pesticide treatment was 67% on day 1 after treatment, 97% on day 2, and 100% on day 7; biological pesticide did not indicate a repellent effect. The feeding percentage of chemical pesticide maintained a low value of about 3% for 7 days.

The feeding percentage results of komatsuna treated by various neem compounds are shown in Figure 2. Feeding percentage of the control was 100% on day 1 after treatment. The feeding percentage for azadirachtin formulation treatment was 57% on day 1 after treatment, 87% on day 2, and 94% on day 7. The feeding repellent effect of azadirachtin formulation increased in line with days of treatment, but was lower than that of spinach. The feeding percentage of neem seed kernel oil cake treatment was 52% on day 1 after treatment and 68% from day 2 to day 7, which was higher than that of spinach. The feeding percentage of biological pesticide was 27% on day 1 after treatment, 85% on day 2, and 100% on day 6 and increased in line with days of treatment, but did not indicate a persistent feeding repellent effect. The feeding percentage of chemical pesticide treatment was maintained at about 3% for 7 days. These results suggest that neem seed kernel oil cake contains feeding repellent compounds because feeding repellent effect of neem seed kernel oil cake was higher than azadirachtin formulation which is the topic of more reports regarding feeding repellent effects. In addition, it is suggested that compounds except for azadirachtin contribute to feeding repellency in neem seed kernel oil cake.

The feeding percentage for komatsuna and spinach treated in the field is shown in Figure 3. The feeding percentage for komatsuna treated with neem seed kernel oil cake, azadirachtin formulation, biological pesticide, and control was 36%, 44%, 51%, and 77%, respectively. Additionally, the feeding percentage for spinach treated with the neem seed kernel oil cake, azadirachtin formulation, biological pesticide, and control was 29%, 35%, 42%, and 66%, respectively. The feeding percentage for komatsuna and spinach treated by various neem materials was lower than that of the control, and all neem treatment indicated a feeding repellent effect. Moreover, when compared to the feeding repellent effect of commercial biological pesticide, that of azadirachtin formulation and neem seed kernel oil cake treatments was significantly lower than for biological pesticide, and neem formulation exhibited a high feeding repellent effect. In particular, the study revealed that neem seed kernel oil cake has an extremely high feeding repellent effect. Furthermore, we compared the feeding repellent effect between laboratory and field tests. In the laboratory, which is a closed system; pest behavior and food selection is impossible, and feeding conditions are poor. In contrast, the field is an open system, and pest behavior and food selection is possible; therefore, pests can seek out places with a rich food supply if food quality in a particular location is poor. It is thought that the feeding percentage in the field decreases more than in the laboratory.

To date, many studies exist on oviposition and the feeding repellence effect of neem seed extract and neem oil-based insecticides on lepidopterans (Blaney et al., 1990; Schmutterer, 1990; Simmonds et al., 1990; Mordue and Blackwell, 1993; Mordue and Davudson, 1995; Schmutterer, 1995; Meadow and Seljasen, 2000; Liang et al., 2003). Seljasen and Meadow (2006) reported that a commercial extract of neem (NeemAzal-T) is used to treat against the egg and 1-star larvae of *M. brassicae* L. in the laboratory; the surviving larvae after hatching on neem-treated plants decreased within days and were 0 by day 14. Newly hatched larvae on neem-treated plants are highly sensitive to neem and showed inhibited growth before death due to feeding directly on the treated cabbage leaves. It is though that the feeding repellent effect against larvae of *M. brassicae* was decreased because the larvae of *M. brassicae* used in this study were 4 star and had developed a low sensitivity to neem. It was also found that the lepidopterans *Spodoptera littoralis*, *Spodoptera frugiperda*, *Heliothis virescens* (F.), and *Helicoverpa armigera* (Hub.) responded to low concentrations of azadirachtin and were repelled by treatments of 0.1–10 ppm of azadirachtin (Blaney et al., 1990; Simmonds et al., 1990; Mordue et al., 1998). However, Liang et al. (2003) suggests that other chemicals also contribute to the feeding repellent effect since the biological activity of neem-based insecticides cannot be judged solely on its azadirachtin content. The repellent effect of the three neem-based insecticides were indicated by the pattern of *Plutella xylostella* larval development and foliage consumption of neem-based insecticide-treated leaves, and no significant difference in larval development among the three neem-based insecticide treatments were found. Moreover, Perera et al. (2000) reported that the feeding percentage for cabbage leaves treated with two neem preparations and fed on by *P.*

xylostella was 70–83% with 1 ppm of azadirachtin, 46–61% with 10 ppm of azadirachtin, and 37–40% with 20 ppm of azadirachtin. The concentration of azadirachtin in this study was 24 ppm, and therefore the percentage of larvae feeding on *M. brassicae* was 57–75%, which was slightly high compared with *P. xylostella*. Mordue and Davidson (1995) described a high feeding repellent effect at 50 ppm of azadirachtin in small-scale field trials. The concentration of azadirachtin formulation used in this study (24 ppm) was half that used by Mordue and Davidson (1995), who found a feeding percentage of 40% and a high feeding repellent effect. It is thought that the main factor is the difference of content due to the difference in neem formulations as well as cultivated crops and environment. The feeding compounds showing repellency against lepidopterans in neem oil were azadirachtin, azadirachtol, 22,33-dihydroazadirachtin, and 2',3',22,23-tetrahydroazadirachtin (Blaney et al., 1990). The difference in concentration and component ratio of these compounds in neem formulations is thought to influence their repellent effect. Moreover, it was suggested that neem seed kernel oil has compounds that affect adjunctively, or other compounds with high repellence, because the repellent effect of komatsuna and spinach treated with neem seed kernel oil cake was higher than that of azadirachtin formulation in the field test. To date, although there are many studies on the repellent effect of neem oil, seed extracts, and azadirachtin against feeding and oviposition by pests, there has been no study on the feeding repellent effect of neem seed kernel oil cake. The present study is the first to have found that neem seed kernel oil has an extremely high repellent effect against pests. These results suggest that neem seed kernel oil cake can be used as a new biological pesticide. In future, we need to identify the repellent compounds in neem seed kernel oil cake and attempt to determine its effect against other pests using other vegetables.

CONCLUSIONS:

The feeding repellent effect of komatsuna and spinach treated with azadirachtin formulation and neem seed kernel oil cake were demonstrated in both the laboratory and the field. In the laboratory, the feeding repellent effect of azadirachtin formulation was higher or the same as that of biological pesticide. Moreover, neem seed kernel oil cake was found to exhibit an extremely high feeding repellent effect. In the field test, the feeding repellent effects of azadirachtin formulation and neem seed kernel oil cake was higher than that of commercial biological pesticide, and in particular, neem seed kernel oil cake exhibited an extremely high feeding repellent effect compared with azadirachtin. Neem seed kernel oil cake has been used as a soil conditioner and fertilizer in the past, but the present findings suggest that neem seed kernel oil cake might be useful as a new biological pesticide.

References

[1] Alves P.D., Brandão M.G.L., Nunan E.A. and Vianna-Soares, C.D. 2009. Chromatograhic evaluation and antimicrobial activity of neem (*Azadirachta indica* A. Juss., Meliaceae) leaves hyroalcoholic extracts. Brazilian Journal of Pharmacognosy, 19: 510-515.

[2] Anibal F, Condor, G. 2007. Effect of neem (*Azadirachta indica* A. Juss) insecticides on parasitoids. The Peruvian Journal of Biology, 14: 69-74.

[3] Ascher K.R.S. 1993. Nonconventional insecticidal effects of pesticides available from the neem tree, *Azadirachta indica*. Archives of Insect Biochemistry and Physiology, 22: 433-449.

[4] Atawodi S.E. and Atawodi J.C. 2009. *Azadirachta indica* (neem): a plant of multiple biological

and pharmacological activities. Phytochemistry Reviews, 8: 601-620.

[5] Blaney W.M., Simmonds M.S.J., Ley S.V., Anderson J.C. and Toogood P.L. 1990. Antifeedant effects of azadirachtin and structurally related compounds on lepidopterous larvae. Entomologia Experimentalis et Applicata, 55: 149-160.

[6] Bonnemaison L. 1965. Insect pests of Crucifers and their control. Annual Review of Entomology, 10: 233-256.

[7] Dai J., Yaylayan V.A., Raghavan G.S.V. and Paré J.R. 1999. Extraction and colorimetric determination of azadirachtin-related limonoids in neem seed kernel. Journal of Agricultural and Food Chemistry, 47: 3738-3742.

[8] Gill J.S. and Lewis C.T. 1971. Systemic action of an insect feeding deterrent. Nature, 232: 402-403.

[9] Irigaray F., Javier S.D.C., Fernando M.G., Vicente M. and Ignacio P.M. 2010. Acute and Reproductive Effects of Align®, an Insecticide Containing Azadirachtin, on the Grape Berry Moth, Lobesia botrana, Journal of Insect Science, 10: 1-11.

[10] Isman M.B. 2006. Botanical insecticides, detterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology, 51: 45-66.

[11] Koul O., Isman M.B. and Ketkar C.M. 1990. Properties and uses of neem, Azadirachta indica. Canadian Journal of Botany, 68: 1-11.

[12] Liang G.M., Chen W. and Liu T.X. 2003. Effects of three neem-based insecticides on diamondback moth (lepidoptera: plutelidae). Crop Protection, 22: 333-340.

[13] Meadow R. and Seljasen R. 2000. Neem seed extract against mamestra brassicae in field-grown cabbage. Vegetable Crops Research Bulletin, 52: 63-67.

[14] Mordue A.J. and Blackwell A. 1993. Azadirachtin: an Update. Journal of Insect Physiology, 39: 903-924.

[15] Mordue A.J. and Davidson G. 1995. Observation on azadirachtin for management of cabbage

caterpillar infestations in the field. BCPC symposium Proceedings, 63: 371-378.

[16] Mordue A.J., Simmonds M.S.J., Ley S.V., Blaney W.M., Mordue W., Nasiruddin M. and Nisbet A.J. 1998. Actions of azadirachtin, a plant allelochemical, against insects. Pesticide Science, 54: 277-284.

[17] Perera D.R., Armstrong G. and Senanayake N. 2000. Effect of antifeedants on the diamondback moth (*Plutella xylostella*) and its parasitoid *Cotesia plutellae*. Pest Management of Science, 56: 486-490.

[18] Perry A.S., Yamamoto I., Ishaaya I. and Perry R.Y. 1998. Retrospects and Prospects. Springer-Verlag, Berlin.

[19] Schmutterer H. 1990. Properties and potential of natural pesticides from the neem tree, Azadirachta indica. Annual Review of Entomology, 35: 271-297.

[20] Schmutterer H. 1995. The neem tree *Azadirachta indica* A. Juss. and other meliaceous plants: sources of unique natural products for integrated pest management, medicine, industry and other purposes. VCH Weinheim, New York.

[21] Seljasen R. and Meadow R. 2006. Effects of neem on oviposition and egg and larval development of *Mamestra brassicae* L: Dose response, residual activity, repellent effect and systemic activity in cabbage plants. Crop Protection, 25: 338-345.

[22] Siddiqui B.S., Afshan F., Gulzar T., Sultana R., Naqvi S.N. and Tariq R.M. 2003. Tetracyclic triterpenoids from the leaves of *Azadirachta indica* and their insecticidal activies. Chemical & Pharmaceutical Bulletin, 51: 415-417.

[23] Simmonds M.S.J., Blaney W.M., Ley S.V., Anderson J.C. and Toogood P.L. 1990. Azadirachtin: stuructural requirements for reducing growth and increasing mortality in lepidopterous larvae. Entomologia Experimentalis et Applicata, 55: 169-181.

[24] Subapirya R. and Nagini S. 2005. Medicinal properties of Neem leaves: a review. Current Medicinal Chemistry - Anti-Cancer Agents, 5: 149-156.

[25] Thompson D.G. and Kreutzweiser D.P. 2007. A Review of the Environmental Fate and Effects of Natural "Reduced-Risk" Pesticides in Canada. In: Crop Protection Products for Organic Agriculture, ed. S.F. Allan and D.R. Kenneth, 245–274. Washington, DC: American Chemistry Society.

[26] Walter J.F. 1999. Commercial experience with neem products. In: Method in Biothechnology 5: Biopesticides, ed. E.R. Hall and J.J. Menn, 155-170. Totowa: Human Press.

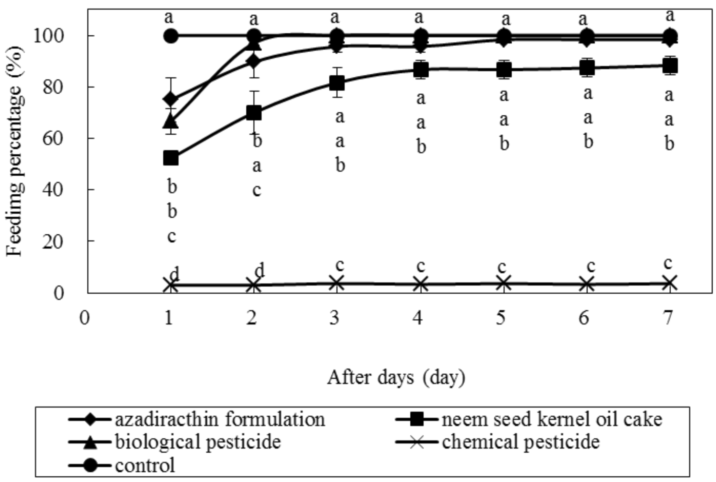

Figure 1. Feeding percentage for spinach in the laboratory

Means in the same day followed by the same letter did not differ significantly by the Turkey-Kramer test (P>0.05).

Vertical bars represent standard error of mean (n=10).

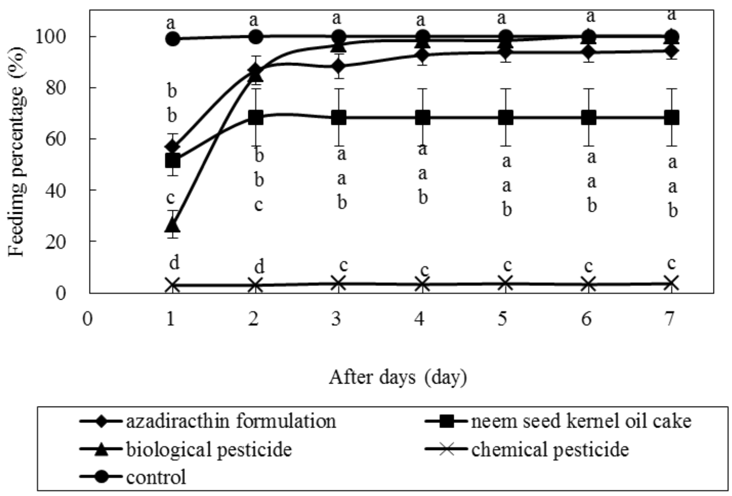

Figure 2. Feeding percentage for komatsuna in the laboratory

Means in the same day followed by the same letter did not differ significantly by the Turkey-Kramer test (P>0.05).

Vertical bars represent the standard error of mean (n=10).

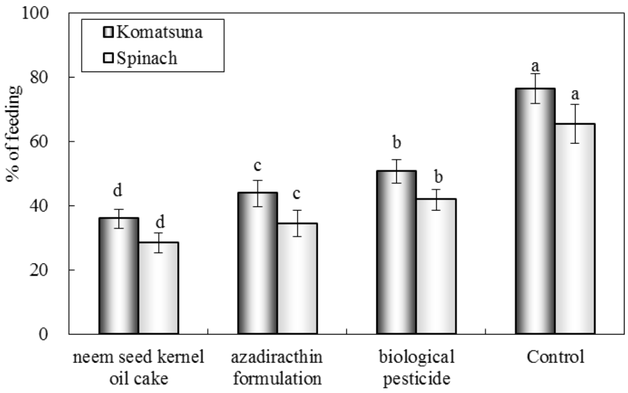

Figure 3. Feeding percentage for komatsuna and spinach in the field

The same letters are not significantly different at $p<0.05$ by the Turkey-Kramer test.

Vertical bars represent the standard error of mean (n=20).