EVALUATION OF THE EFFECTIVENESS OF PROPOLIS AND GARLIC IN THE MANAGEMENT OF MAIZE WEEVIL (*SITOPHILUS ZEAMAIS*) IN STORED MAIZE (*ZEA MAYS*) GRAINS

W. A. Adeyemi* and A. A. Osipitan*

* Department of Crop Protection, Federal University of Agriculture, P. M. B. 2240, Abeokuta, Ogun State, NIGERIA. E-mail: flourishinggold@rocketmail.com

[**Adeyemi, W. A. & Osipitan, A. A.** 2014. Evaluation of the effectiveness of propolis and garlic in the management of Maize Weevil (*Sitophilus zeamais*) in stored Maize (*Zea mays*) grains. Munis Entomology & Zoology, 9 (1): 117-124]

ABSTRACT: The study evaluated the efficacy of garlic powder and propolis extracts at controlling *Sitophilus zeamais* infestation in stored maize grains. One hundred grammes of maize grains were weighed into 250 cm^3 jars. The grains were seperately mixed with 1 g, 3 g and 5 g garlic powder and 5 %, 10 % and 15 % propolis extract. The treated grains were infested with 5 pairs of 1-10 day old *S. zeamais* and left for 90 days. Each treatment was replicated four times and arranged on work table in the laboratory using Complete Randomized Design. The untreated grains (control) had a significantly ($p < 0.05$) higher number of *S. zeamais*, % weight loss, % grain damage and % weight of grain powder. All the treated grains had 100 % adult mortality of *S. zeamais* at all treatment levels. The % grain damage, % grain weight loss, weight of grain powder and number of *S. zeamais* were significantly ($P < 0.05$) lower in the garlic powder and propolis extract -treated maize grains compared to the untreated ones. Propolis extract at 15 % concentration had the lowest grain weight loss followed by maize grains treated with 3 g garlic powder. Garlic powder and propolis extract could be used to control infestation in maize grains to overcome draw-backs associated with the use of synthetic insecticides.

KEY WORDS: Garlic, propolis, infestation, *Sitophilus zeamais*, synthetic insecticide.

Maize (*Zea mays)* is an important cereal grain widely cultivated and consumed in Africa. The crop is cultivated as staple food in Meso America and it is a good source of carbohydrates, protein, vitamin B and mineral. Maize is a staple food with great nutritional value and is one of the main sources of calories in the major producing areas (Abebe et al., 2009). The crop is highly yielding, matures easily, easy to process, readily digestible and cost less than other cereals. It is the third most important cereal crop grown in Sub-Saharan Africa after rice and wheat (CIMMYT, 1994). The crop is used for animal and human consumption and also for ethanol production and about 100 million people in the world consume maize in the form of thin, round cake or as porridge (Compton, 1999).

Post-harvest insect pest of maize such as Angoumois grain moth – *Citotroga cerealella*, lesser grain borer *Rhizorpertha dominica*, larger garin borer – *Prostephanus truncatus*, rice weevil – *Sitophilus oryzae* and Maize weevil *Sitophilus zeamais* among others have been recognised as increasing problem to maize production in Africa (Gwinner et al., 1990; Giga et al., 1991; Bekele et al., 1995; Abebe et al., 2009). Maize weevil *S. zeamais* is a very serious insect pest of stored maize grains that is cosmopolitan and greatly constrains post-harvest storage of maize grains (Longstaff, 1981). Initial infestations of maize grains by maize weevil occur in the field just before harvest, and the insects are carried into the store where the population builds up rapidly and lion share of damage done to the maize (Appert, 1987; Demissie, 2008). Addis-Teshome (2008) reported that stored insect pests are capable of inflicting serious damage to stored commodities due to very rapid capacity to increase in number, migrate, infest and thus spreading the infestation. Giga et al. (1991) reported maize grain loss of 20 % - 90 % worldwide due to infestation by maize weevils.

Farmers have largely depended on the use of synthetic insecticides as insect pest control measures against *S. zeamais*, however, these chemicals have some limitations, which include development of resistance in the pest organisms, hazardous effect in the environment, high persistence, high toxicity, residues accumulation in food and feed, negative effects on non-target organisms and high cost for resource poor farmers (Dhuyo & Ahmed, 2007).

Propolis is a resin-like material from the buds of poplar and cone-bearing trees. It is the dark-brown or black sticky plant derived 'glue' found around wounds on plant and sometimes around buds and is used by bees for sealing, lining, strengthening of their hives and serve as repellent materials inside the hives and around the entrance (Banskota et al., 2001). Propolis is a veritable cascade of aromatic nutrient that possess anti-fungal, anti-bacterial anti-viral properties due to its remarkable properties. Orsi et al. (2005) reported that the flavonoids, organic, phenolic and aromatic acids coumarins, in the presence numerous mineral elements and vitamins have strong anti-oxidant, anti-inflammatory, antiseptic and pain killing effect. Torreblance et al. (1983) reported that honeybees gather propolis and combine the resin with nectar, creating a mix of wax, pollen and bee bread.

Garlic (*Allium sativum*) of the Family: Alliceae, is a close relative of onions, shallot, and leek, chive and rakkyo. It is a cosmopolitan plant grown in the temperate, tropics and the sub-tropics and used for both culinary and medicinal purposes (Block, 2010). It has a pungent hot flavour that mellows and sweetens considerably with cooking. Two major constituent of garlic oil were allyl methyl disulfide and diallyl trisulfide and when crushed, garlic yields allicin, a powerful anti-fungal and anti-biotic compound (Block, 2010). Garlic was believed to have evolved a defensive mechanism that deterred animals like birds and insects from eating the plant. Grainge et al. (1985) and Kain (1999) reported the ability of garlic to protect crops against a variety of insects such as aphids, mites and thrips. Grainge et al. (1985) reported that bulb of garlic has insect controlling properties with repellant, antifeedant, bactericidal, nematicidal and fumigant mode of action. Anwar et al. (2009) reported that garlic act as nematicide and insecticide and has been used to control cabbage root fly and red mite in poultry. *S. zeamais* is a very serious pest of stored maize grains that is highly destructive. Control of the insect is mostly done with the use of insecticides. These insecticides are however, enmeshed in controversies of drawbacks such as high cost, toxicity, hazard, adulteration, development of resistance and pollution of environment among others. Propolis and garlic are organic products with no reported cases of such drawbacks. This study therefore evaluates the use of propolis and garlic for the management of maize weevils in maize grains.

MATERIALS AND METHODS

Location of study site

This study was conducted at the Entomological Research Laboratory of the Department of Crop Protection, College of Plant Science and Crop Production, Federal University of Agriculture, Abeokuta (UNAAB).

Source of *Sitophilus zeamais* culture

The maize weevil *Sitophilus zeamais* used for the study was obtained from Federal University of Agriculture, Abeokuta (FUNAAB) and cultured in glass jars in the laboratory. The maize dust was sieved periodically in other to prevent the growth of mould which may lead to the caking of grains and ultimate death of the insects.

Source of maize grains

The maize kernels used for the study (SUWAN- 1) was procured from FUNAAB. The damaged kernels were picked and the clean ones disinfested to eliminate eggs, larvae, pupae and adult insects by subjection to aluminium phosphide (Phostoxin) for 24 hours. Dead insects were sieved out of the kernels and they were aired for 72 hours prior to use. The moisture content of the grain (13%) was determined to ensure that the kernels moisture content is suitable for the feeding of the maize weevils.

Preparation of garlic powder

The garlic *Allium sativum* used for the study was procured from kuto market, Abeokuta, Ogun State, Nigeria. They were peeled and chopped, then sun-dried for 7 days, after which they were ground into granules using an electric blender and sun-dried for 48 hours before pounding it into powder form using a mortar and a pestle. The powder was left to dry at room temperature for 48 hours.

Preparation of propolis extract

Propolis was collected from the flight entrance and openings between the top bars of bee hives located at an apiary in FUNAAB. One hundred grammes raw propolis was cut into small bits of about 5-10 mm and placed in a 250 ml conical flask. 150 ml of ethanol was poured into the conical flask to submerge the propolis. The outlet of the flask was covered with a foil paper and held tightly with rubber bands; the mixture was vigorously shaken for an hour using the IKA Orbital shaker to allow for extraction of the active ingredients in the mixture (Obasa *et al.*, 2007). The resultant extract was filtered through a Whatman No. 1 filter paper into a 250 ml conical flask. The sticky crude extract was thereafter serially diluted with ethanol to prepare 5 %, 10 % and 15 % ethanolic extracts of propolis (EEP).

Admixture of garlic powder and propolis with grains

One hundred grammes of maize grains were weighed into 250 cm^3 Kilner jars using Mettler weighing balance (Mettler Toledo). The grains were mixed with 5 %, 10 % and 15 % of ethanolic extracts of propolis and 1 g, 3 g and 5g powder of garlic in 4 cm x 4 cm sized perforated envelope and were separately inserted into maize grains in each of the jars. The treated grains were seperately infested with 5 pairs of 1-2 day's old *S. zeamais*. Each treatment was replicated four times and arranged on work tables in the laboratory using Complete Randomized Design (CRD). Four control glass jars contained 100 g maize grains and was infested with 5 pairs of 1-2 days old *S. zeamais*, but were not treated with propolis and garlic. 100 g clean disinfested grains were weighed into the jars to monitor change in weight of grains as a result of moisture loss or gain (Hurlock, 1967). At 90 days post-infestation of the maize grains, the powder and frass in each replicate sample was sieved out; the grains were sorted into damaged and undamaged and insects into dead and living. Insects that did not move or respond to three probings with a blunt probe were considered dead (Obeng-Ofori & Reichmuth, 1997). The following data were taken:

i. Number of adult *Sitophilus zeamais*.
ii. Number of adult mortality.
iii. Weight of powder/frass (g).
iv. Number of damaged and undamaged grains.
v. Total number of grains.
vi. Final weight of grains.

Percentage weight loss and percentage damage respectively were calculated using the formulae according to Baba-Tierto (1994).

$$\% \text{ Grain weight loss} = \frac{\text{Weight of control sample} - \text{Final weight of grain X 100}}{\text{Weight of control sample}}$$

$$\% \text{ Grain damage} = \frac{\text{Number of damaged grains}}{\text{Total number of grains}} \text{ X 100}$$

The weight of grain dust was determined from the composite weight of dust sieved from the grains using the formulae:

Weight of grain dust = Weight of treatment powder and grain dust – Weight of treatment powder

Weights of treatment powder are 1 g, 3 g and 5 g.

Statistical analysis

Statistical analysis of data was based on SAS's general linear models procedure (SAS, 1998). The data were subject to analysis of variance (ANOVA). Significant means were compared using Student's Newman-Keuls Test (SNK) at $P < 0.05$

RESULTS

Mortality of *Sitophilus zeamais* in maize grains treated with garlic powder and propolis

The mean mortality of *S. zeamais* in maize grains treated with garlic powder at 1g, 3g and 5g treatment levels and propolis extract 5%, 10% and 5% concentration levels shows that all the treatment caused 100 % mortality of the introduced *S.zeamais*. The mortality induced by these treatments were not significantly ($P > 0.05$) different from each other. In the control, none of the introduced *S. Zeamais* died and it was significantly ($P > 0.05$) different from the mortality in treated grains (Table 1).

Weight of grain powder and number of adult *Sitophilus zeamais* in maize grains treated with garlic powder and propolis extract.

The mean weight of grain powder and number of adult *S. zeamais* in maize grains treated with garlic powder and propolis extract is shown on Table 2. The highest grain powder (0.19g) was generated from the untreated maize grains (control) and it was significantly ($P < 0.05$) different from what obtains in treated grains that has no grain powder. The mean number of adult *Sitophilus* (34.75) was from the untreated maize grains (control) and it was significantly ($P < 0.05$) different from what obtains from other treatments. All the insects introduced to grains treated with garlic powder and propolis extract at all levels died.

Grain weight loss and damage in propolis and garlic-treated maize grains infested with *Sitophilus zeamais*.

The untreated grains (the control) had a significantly ($P < 0.05$) higher grain weight loss and grain damage relative to the treated maize grains. Of the treatments, maize grains treated with 5 % propolis ethanoic extract had the lowest grain weight loss (0.67). However, it was not significantly ($P > 0.05$) different from the % weight losses in maize grains treated with extract of propolis at 10 % (0.54), garlic at 1g (0.51), garlic at 3g (0.29) and garlic at 5g (0.37).

The maize grains treated with 15 % ethanolic extract of propolis had the lowest % grain weight loss. It was however, not significantly (P > 0.05) different from all the other treated maize grains except untreated maize grains (control) and maize grains treated with 5 % ethanolic extract of propolis. A significantly higher (P < 0.05) % grain damage (8.04) was recorded in the untreated maize grains (control). All the treated maize grains were not visibly damaged by the introduced *S. zeamais* and they were not significantly (P > 0.05) different from each other.

DISCUSSION

The results of this study revealed the potency of propolis extracts and garlic powder at managing the population of *Sitophilus zeamais*. In this study, extract from propolis and garlic powder demonstrated great potential at reducing the population of *S. zeamais* in the laboratory. The result of study corroborated the findings of Yan Huang et al. (2000) that tested the two constituents of garlic against *S. zeamais* and *T. castenum* for antifeedant activity, contact and fumigant toxicity and reported that the two constituents reduced egg hatch emergence of larva and adults. Similarly, Osipitan & Mohammed (2008) reported the ability of garlic to manage the population of larger grain borer, *Prostephanus truncantus* in maize grains. Osipitan et al. (2010) tested propolis for the management of LGB in maize grains and suggested that the product could be explored singly or integrated with other control management options to manage the population of LGB in infested maize grains. Kain (1999) also reported the ability of garlic to protect crops against variety of insect pests. Likewise, Grainge et al. (1985) reported that garlic has insect controlling properties that repel and make the host less favourable and less prone to attack and infestation by insects. Sforcin et al. (1995) and Obasa et al. (2007) reported that propolis has biological properties such as antibiotics, antifungal, anti-inflammation, anesthetic, healing, antioxidant and cacinostatic properties.

In this study, there seems to be no direct relationship between the level at which the treatments were applied and their effectiveness on *S. zeamais*, because the effect of the treatments at different levels on the *S. zeamais* were not significantly different from each other. This is a good development as it indicates the effectiveness of the treatments at minimal treatment level. The entire introduced insect died, suggesting that garlic and propolis have high insecticidal property.

Saxena (1987) reported that botanical insecticides are generally pest-specific and are relatively harmless to non-target organisms including man. They are also biodegradable and harmless to the environment. Furthermore, unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise an array of chemical compounds which act concertedly on both behavioural and physiological processes. Thus, the chances of pests developing resistance to such substances are less likely. Botushanov (2001) reported that propolis consist of more than 200 constituent in its waxes and resins that made it a “veritable cascade of aromatic nutrient” remarkable for combating all type of pathogens such as bacteria, virus, parasites and fungi. Orsi et al. (2005) analysed propolis from the province of Henan in China and reported sinapic acid, isoferulic acid and caffiec acid as compounds showing anti-bacteria properties.

Insect pests have been mainly controlled with the use of synthetic insecticides. However, problems of pesticide resistance and negative effects on non-target organisms, including man and the environment has negated wide spread acceptance of their use. Rembold, (1994) and FAO (1992) reported that the indiscriminate use of chemical pesticides has given rise to many well-known and serious problems, including genetic resistance of pest species, toxic residues in stored products, increasing costs of application, hazards from handling, environmental pollution and so on. Since garlic powder and propolis extract in this study were effective at managing the population of *S zeamais*. The extract from the products may be utilized as natural products in the management of *S. zeamais*.

LITERATURE CITED

Abebe, F., Tefera, T., Beyene, Y. & Vidal, S. 2009. Resistance of maize varieties to the maize weevil *Sitophilus zeamais* (Motsch.) (Coleoptera:Cucurlionidae). Afr. J. Biotechnol., 8: 8937-5943.

Addis Teshome, K. 2008. Evaluation of fungal entomopathogen, *Beauvera bassiana* and *Metarhizium anisopliae* against *Sitophillus zeamais* Mostch (Coleoptera: Curculionidae) on maize. M. Sc. Thesis, Haramaya University, pp. 1-6.

Anwar A., Groom, M. & Sadler-Bridge, D. 2009. Garlic from nature's food to nematicide. Pesticide News, 84: 18-20.

Appert, J. 1987. The storage of food grains. The Tropical Agriculturist CTA, Macmillian Publishers LTD., London. Pp. 146.

Baba-Tierto, N. 1994. Ability of powders and slurries from ten plants species to protect stored grains from attack *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) and *Sitophilus oryzae* L. (Coleoptera: Curculionidae). J. Stored Prod. Res. 30: 297-301.

Banskota, A. H., Tezuka, Y. & Kodota, S. 2001. Recent progress in pharmacological research in propolis. Phtother. Res., 15 (17): 561-571.

Bekele, J. A., Obengofori, D., Hassanali, A. A. & Nyamasyo, G. H. N. 1995. Products derived from the leaves of *Ocimum kilimandscharicum* as post harvest grain protectants against the infestation of three major stored product insect pests. Bull. Entomol. Res., 85: 361-367.

Block, E. 2010. Garlic and other alliums: The Lore and the Science. Royal Society of Chemistry. Pp. 197.

Botushanov, P. I., Grigorov, G. I. & Aleksandrov, G. A. 2001. A clinical study of silicate toothpaste with extract from propolis. Folia Med. (Plovdiv), 43 (1-2): 28-30.

CIMMYT, 1994.World maize facts and trends. Maize seeds industries revisited: Emerging roles of the public and private sectors. Mexico City.

Compton, J. A. F. 1999. Rapid assessment method for stored maize cobs, weight losses due to insect pests. Journal of Stored Products research, 35 (1): 77-78.

Demissie, G., Tefera, T. & Tadese, A. 2008. Importance of husk covering on field infestation of maize by *Sitophilus oryzae* L. (Coleoptera: Curculionidae) at Bako, Western Ethiopia. Afr. J. Biotechnol., 7: 3774-3779.

Dhuyo, A. R. & Ahmed, S. 2007. Evaluation of the fungus *Beavaria bassiana* (Bals.) infectivity to the larger grain borer *Prostephanus truncatus* (Horn). Pak. Entomol., 29: 77-82.

FAO 1992. Pesticide Residues in Food. Report, 116: 146.

Giga, D. P., Matemerewa, S., Maya, G. & Neeley, D. 1991. Assessment and control of losses caused by insect pests in small farmer's stores in Zimbabwe. Crop Prot., 10: 287-292.

G**rainge, M. A., Mitchell, W. C & Hylin, J. W.** 1985. Plant species reportedly possessing pest-controlling properties- An EWC/UN database. Resource system institute. EWC Honolulu College of Tropical Agriculture and Resources, University of Hawaii.

Gwinner, J., Harnish, R. & Muck, O. 1990. Manual on the prevention of post harvest grain losses. Postharvest project, pickvben 4, D-200. Hamburg. 11. FRG. Pp. 294.

Hurlock, E. T. 1967. Some observation on the amount of damage caused by *Oryzaephilus surinamensis* (L) (Coleoptera:Silvidae) to wheat. J. Stored Prod. Res., 3: 75-78.

Kain, D. 1999. Green movement. Scaffolds fruit Journal, Cornell University, New York State Agricultural Experiment, 7(18).

Longstaff, B. C. 1981. Biology of the grain pest species of the genus *Sitophilus* (Coleoptera Curculionidae): A critical review: Journal Protection Ecology, 3: 283-130.

Obasa, K. C., Adeoti, A. Y. A., Enikuomehin, O. A. & Bodunde, J. G. 2007. Efficacy of bee-propolis in the control of *Collectotrichum lindemuthianum* (Sacc. and Megn) Briosi and cav. in vitro. Res. J. Microbiol., 2 (2): 175-179.

Obeng-Ofori, D. & Reichmirht, C. H. 1997. Bioactivity of engenol, a major component of essential oil of *Ocimum Suave* (Wild) against four species of stored products Coleoptera. International Journal of Pest Management, 43 (1): 89-94.

Orsi, R. O., Sforcin, J. M., Rall, V. L M., Funari, S. R. C., Barbosa, L. & Fernades, JR. A. 2005. Susceptibility profile of Salmonella against the antibacterial activity of propolis produced in two regions of brazil. J. Venom. Anim Toxins, 11 (2): 109-116.

Osipitan, A. A. & Muhammed, I. G. 2008. Evaluation of the potential of Garlic – *Allium sativum* for the management of the larger grain borer - *Prostephanus truncatus* (Horn) in maize – (*Zea mays* L.). ASSET Series A, 8 (1): 51-60.

Osipitan, A. A., Ogunbanwo, I. A., Adeleye, I. G. & Adekanmbi, D. I. 2010. Propolis production by honey bee *Apis mellifera* (Hymenoptera:Apidae) and its potential for the management of the larger grain borer *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) on maize grains. Journal of Plant Protection Research, 50 (1): 61-66.

Rembold, H. 1994. Secondary plant compounds in insect control with special reference to Azadirachtin. Adv. Invertebrate Reprod., 3: 481-491.

Saxena, R. C. 1987. Antifeedants in tropical pest management. Insect Sci. Appl., 8: 731-736.

Sforcin, J. M., Fernandes, J. A., Lopes, C. A. M., Bankova, V. & Funari, S. R. C. 1995. Seasonal effect of propolis antibacterial activity. J. Ethanopharmac., 73: 243-249.

Torreblanca, R. A., Adem, C. E. & Bourges, R. H. 1983. Losses caused by *Prostephanus truncatus* (Horn) in maize stored under controlled condition. P. 87-89. In: Memorias del coloquio International sobre Conservacion de Semmillas y Granos Almancenados" (Moreno-Martinez, Retimerez-Martinez, eds) 20-25 October 1980, Oaxtepec, More los, Institute de Biologia, Universidad Nacional autonona de Mexico, 51 p.

Yan, H., Shao, X. C. & Shuit, H. H. 2000. Bioactivities of Methyl Allyl Disulfide and Daily trisulfide from essential oil of garlic to two species of stored-product pest, *Sitophilus zeamais* (Coleoptera:Curculioniadae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae). Journal of Economic Entomology, 93 (2): 539-543.

Table 1. Mortality of *Sitophilus* in maize grains treated with garlic powder and propolis.

TREATMENTS	NUMBER OF ADULT MORTALITY ± SE
CONTROL	0.00 ± 0.00^{b}
GARLIC 5g	10.00 ± 0.00^{a}
GARLIC 3g	10.00 ± 0.00^{a}
GARLIC 1g	10.00 ± 0.00^{a}
PROPOLIS 15%	10.00 ± 0.00^{a}
PROPOLIS 10%	10.00 ± 0.00^{a}
PROPOLIS 5%	10.00 ± 0.00^{a}

Means followed by the same letter are not significantly different from each other at $P < 0.05$ using Student Newsmanskeul Test.

Table 2. Weight of powder and number of adult *sitophilus* adult *Sitophilus* in maize grains treated with garlic powder and propolis extract.

Treatments	Weight of grain powder ± SE	Number of adult *Sitophilus* ± SE
CONTROL	0.19 ± 0.02^{a}	34.75 ± 5.27^{a}
GARLIC 5g	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
GARLIC 3g	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
GARLIC 1g	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
PROPOLIS 15%	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
PROPOLIS 10%	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
PROPOLIS 5%	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}

Means followed by the same letter are not significantly different from each other at $P < 0.05$ using Student Newsmanskeul Test.

Table 3. Percentage grain weight loss and percentage grain damage in maize grains treated with garlic powder and propolis extract.

TREATMENTS	% Weight loss ± SE	% Grain damage± SE
CONTROL	1.95 ± 0.10^{a}	8.04 ± 0.17^{a}
GARLIC 5g	0.37 ± 0.04^{bc}	0.00 ± 0.00^{b}
GARLIC 3g	0.29 ± 0.03^{bc}	0.00 ± 0.00^{b}
GARLIC 1g	0.51 ± 0.03^{bc}	0.00 ± 0.00^{b}
PROPOLIS 15%	0.22 ± 0.01^{c}	0.00 ± 0.00^{b}
PROPOLIS 10%	0.54 ± 0.02^{bc}	0.00 ± 0.00^{b}
PROPOLIS 5%	0.67 ± 0.01^{b}	0.00 ± 0.00^{b}

Means followed by the same letter are not significantly different from each other at $P < 0.05$ using Student Newsmanskeul Test.