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Egg Developmental Inhibition and Ovipositional Deterrence of Neem or Mineral Oil on Maize Weevil, *Sitophillus zeamais* Motsch

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Abstract: Neem oil, a product from the neem tree (*Azadirachta indica* A. Juss) was evaluated for its insecticidal properties (ovipositional deterrent and egg developmental inhibitor) against the adult of the maize weevil, *S. zeamais.* Significantly fewer weevils (22%, $p<0.0011$ were found on kernels treated with 10,000 ppm neem oil than in the control (78%). Mineral oil had no deterrent effect on weevil distribution at any dose. Although, 1,000 ppm did not deter insects from corn kernels, it significantly affected their oviposition. Neern oil at 10,000 ppm level not only reduced weevil oviposition in the treated kernels but also inhibited their oviposition on untreated kernels in the same area. In no-choice test similar effect on oviposition was also observed. Weevils held for 7 days on corn kernels treated with 1,000 and 10,000 ppm neem oil significantly laid fewer eggs, but when the exposure increased to 16 days, oviposition in the treatment and in the control was the same. All the eggs hatched normally.

Key words: Effect of neem oil on the oviposition of maize weevil

Introduction

Stored product insects are an important group of pests that reduce the quality, quantity and viability of stored grains. Some of these insect pests can also attack crops in the field. Although, stored grains are commonly protected by insecticides or fumigants, these chemicals carry potential hazards to human health. Efforts are being made to fine safer insecticides.

The plant kingdom is a store-house of chemical substances which are used by plants in their own defense against insects, bacteria, fungi and viruses. These plant-based chemicals may be biodegradable, species-specific and less Or non-toxic to non-target organisms as compared to synthetic insecticides. Among all the known tropical plants with chemical properties, the neem tree, *Azadirachta indica A. Juss* may offer the greatest potential for commercial exploitation. Besides its medicinal values, neem has been used for the control of insect pests of stored grains, fruits, vegetables, ornamental, human and other animals. The effectiveness of different neem derivatives (neem seed powder, extracts in different solvents, oil and azadirachtin) against a variety of insect species have been reviewed by many workers.

The neem tree derivatives act as antifeedant protectant (Cobbinah and Appiah-Kwarteng, 1989; Jilani and Amir, 1987; Maredia *et al.,* 1992; Ivibijaro 1990), antiovipositional (Hellpap, 1984; Makanjuola, 1989; Pathak and Krishna, 1991; Okonkwo and Okoya 1996) and growth regulator (Koul *et al.,* 1987; Meisner *et al.,* 1987; Jhansi-Rani, 1987; Srivastava *et al.,* 1997).

In the light of the existing information we conducted an experiment to determine the antifeedant/deterrent, antiovipositional and egg development inhibiting properties of neem oil against maize weevil.

Materials and Methods

The maize weevils used in these studies were obtained from the cultures maintained in the Entomology Department, Kansas State University. These weevils were reared on whole commercial corn kernels with 13-14% moisture contents in a controlled environment at 27 ± 1°C and 65 ± 5% RH with a 12:12 light and dark cycles. Weevils used in all these studies were 1-2 wk old unless otherwise noted. Neem oil was obtained from C. M. Ketkar, Neem Mission, 471 Shanwar Peth Pune 411030. Both neem and mineral oils were diluted in acetone.

Effect of neem and mineral oil on the maize weevil distribution and oviposition on treated and untreated corn kernels

Choice test: Weevils were presented with the choice of dispersing and ovipositioning onto either treated or untreated corn kernels in an arena (Figure 1). This arena was made of a circular (150×25 mm) plastic petri dish with a filter paper (15 cm; Fisher Sci., Pittsburg, Penn.) on the bottom. Four cages were made of hardware (6.35 mm mesh). Each cage was large enough to hold 20 whole corn kernels. The bottom of each cage was covered with parafilm to hold kernels during trials. The wire openings were wide enough to allow movement in and out of the cage by weevils during the experiment.

Two cages with kernels treated with acetone solution of 100, 1,000 and 10,000 ppm neem or mineral oil and two cages with control kernels were placed on an alternating design and at equal distance to each other in each arena. The cages were also at equal distance from the center of the arena. Twenty female weevils were released in the middle of the arena. The arena was than covered with its plastic lid and tied with a rubber band to prevent weevils escaping during trials. After 24 h the number of weevils in the cages with treated and untreated kernels were recorded. At the end of each trial weevils were found. either in treated or untreated kernels. The kernels were stained with acid fuchsin solution, dried and the number of egg-plugs counted under a microscope. The distribution and oviposition of the weevils were expressed as percent adult distribution and eggs laid/female/day, respectively.

No-choice test: Seventy five kernels from each treatment (mentioned above) were placed in 0.473 l wide mouth glass

jar and 10 female weevils were released in each jar. After 24 h weevils were removed from the kernels and the kernels were stained with acid fuchsin solution. The kernels were then examined under the microscope and the number of egg-plugs in the kernels for each treatment were recorded.
Emergence and oviposition of Fl progeny: Kernels from the no-choice test were held for 40 d to determine wether neem or mineral oil had affected the viability of the eggs. After 40 day, the number of adults emerged from the kernels in each treatment was recorded and expressed as percent adult emergence. Subsequently three mated female (7-10 day old) of the Fl progeny were exposed to 21 untreated corn kernels to determine wether neem or mineral oil had adversely affected the reproduction of the Fl progeny. Weevils were removed from the kernels 24 h after the exposure, the kernels stained with acid fuchsin solution and the number of egg-plugs recorded.

Reproductive ability: Maize weevil is an autogenous insect. It gains full progeny production 42 h after emergence from the kernel (Singh and Soderstrom,1963). An experiment was conducted to determine wether neem or mineral oil had a deleterious effect on the reproductive ability of weevils exposed to treated kernels.

Acetone solution of 100, 1,000 and 10,000 ppm neem and mineral oil were applied at the rate of 4 ml/150 to whole corn kernels. An acetone treatment served as control. Solvent was allowed to completely evaporate from the kernels before use. The kernels were transferred to 0.473 l wide mouth glass jars. Ca. 25 pairs (1-20 h old) of weevils/treatment were exposed to the kernels at $27 \pm 1°C$ and $65 \pm 5\%$ RH. Seven d after exposure to the kernels, six pairs of weevils from each treatment were placed with 50 untreated kernels in similar jars. After 24 h weevils were removed from the kernels and the kernels were stained with acid fuchsin solution. The kernels were dried and the number of egg-plugs counted in other similar trials, six pairs of weevils were tested on 50 untreated corn kernels for their oviposition after they were exposed to treated kernels for 15 and 21 day. The same kernels in all these trials were held and incubated for 40 day in the controlled environment ($27 \pm 1°C$ and $65 \pm 5\%$ RH) to determine wether neem or mineral oil had affected adult emergence of the weevils. After 40 day, the percent adult emergence was recorded in each treatment.

In all these tests, each treatment was replicated 5 times and the observations obtained were analyzed either by Chi-Square test or by an analysis of variance (ANOVA) and the mean values of the treatments were compared by LSD ($p = 0.05$) (SAS, 1988).

Results and Discussion

Effects of neem and mineral oils on the maize weevil distribution and oviposition on treated and untreated corn kernels:

Choice tests: When weevils were presented with a choice of neem-treated or untreated (control) corn kernels, they distributed equally ($p > 0.05$) between controls and kernels treated with either 100 or 1,000 ppm neem oil (Table 1). However, significantly fewer weevils (22%; $p < 0.001$) were found on kernels treated with 10,000 ppm neern oil than on the controls (78%). In contrast mineral oil had no deterrent effect on weevil distribution at any dose ($p > 0.05$; Table 1). Although, neem oil did not deter weevils from the kernels treated with 1,000 ppm, it significantly affected their oviposition, as only 0.4 eggs/female/day were oviposited by the weevils on the treated kernels as compared to 1.2 eggs/female/day in the controls ($p < 0.001$; Table 1). Neem oil, at the 10,000 ppm level, not only reduced weevil oviposition on the treated kernels, but also inhibited their oviposition on untreated kernels in the same arena. In this, case only 0.5 eggs/female/day were recorded on the control kernels, a significantly lower number ($p < 0.05$) than the 0.9, 1.2, 0.9, 0.8 and 0.7 eggs/fernaleiday on the 100 and 1,000 ppm neem oil and 100, 1,000 and 10,000 ppm mineral nil, respectively. Mineral oil did not affect oviposition of the maize weevils on corn kernels, as concentrations up to 10,000 ppm did not significantly affect oviposition as compared to that in the controls. Results of the present studies indicated that weevils laid fewer eggs in all choice tests as compared to no-choice tests. Although, at the end of each trial (24 h after release) all weevils were found either in treated or untreated kernels. The reduction in oviposition may be due to the area of the arena where insects took time in settling down on treated and untreated kernels in the cages. Results of the present studies indicated that in choice tests, neem oil at 10,000 ppm not only reduced weevils oviposition on treated kernels but also on untreated kernels in the same arena. This effect may be due to the concentration of some volatile chemicals (Salannin, Meliantriol etc.) at this dose level that can affect the oviposition of the weevils. In all these experiments the adult emergence of the maize weevil was not affected. This may be the presence of normal and matured egg already present in the ovary of the test insect or the concentrations used in these studies were not enough to affect the viability of the weevils eggs.

No-choice test: When weevils were confined with only treated kernels, the effect on oviposition was similar to that observed under choice conditions (where both oil-treated and untreated kernels were offered to the weevils). Neem oil at 100 ppm did not reduced their oviposition but the 1,000 ppm solution, which did not affect weevil distribution, had a significantly negative effect on oviposition, as these weevils laid 1.5 eggs/female/day, compared to 3.0 eggs/female/day in the control ($p < 0.001$; Table 2). This negative effect on oviposition was further observed with weevils on kernels treated with 10,000 ppm of neem oil, which laid 1.1 egg/female/day. *Tribolium castaneum* (Hbrst.) when fed on wheat flour treated with 4% neem oil failed to reproduce, possibly due to an effect on oviposition behavior or reproductive physiology (Jilani *et al.,* 1988). Das (1986) noticed that the total number of egg laid by pulse beetle on chick pea seeds treated with 6, 8 and 10 ml neem oil/kg seed was significantly lower than in the controls. The significant effect of neem volatiles and Euclaptus on the post-embryonic development and reproduction of *Corcyra cephalonica* has also been noticed by Pathak and Krishna (1991).

Emergence and oviposition of F1 progeny: The percent adult emergence of the Fl progeny was similar in all concentrations, as the eggs hatched normally, when they

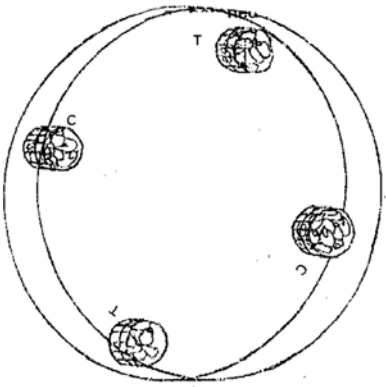

Fig. 1: Arena used for choice studies T = Treated C = Control

Table 1: Distribution and oviposition of maize weevils on corn kernels treated with either neem or mineral oil in a choice test between treated and untreated kernels[a]

Treatment[b]	Percent distribution		Eggs/female/day	
	Neem	Mineral	Neem	Mineral
100 ppm	51.0±12.9A	47.0±9.1A	0.9±0.24	0.9 ±0.1A
control	49.0±12.9A	53.0±9.8A	0.9±0.2A	0.9±0.1A
1,000 ppm	49.0±9.6A	48.0±10.4A	0.4±0.58	0.8±0.1A
control	51.0±9.6A	52.0±10.4A	1,2±0.1A	0.8 ±0.1A
10.000 ppm	22.0±9.7A	47.0±10.4A	0.2±0.1EA	0.7±0.1A
control	78.0±9.76	53.0±10.4A	0.5±0.2A	0.7±0.1A

a/Each value is a mean±SE of 2 replications. Paired values with in a column followed by the same letters are not significantly different at a = 0.05

b/Each pair represents the choice offered in an arena.

Table 2: Oviposition of maize weevils held on corn kernels treated with neem or mineral oil[a] and subsequent adult emergence and their oviposition[b] on untreated kernels. No-choice test[c]

Treatments	Egg/female/day treated corn	Percent adult emergence	Egg/female/day of Fl on untreated corn
Control	3.0±0.3A	91.2±7.1A	3.7±0.64
Neem oil			
100 ppm	2.8±0.2A	90.2±7.0A	4.1±0.4A
1000 ppm	1.5±0.26	83.9±10.64	3.5±0.5A
10000 ppm	1.1±0.6C	84.2±13.3A	3.9±0.74
Mineral oil			
10000 ppm	2.7±0.44	92.5±5.54	4.1±0.3A

a/ Ten female maize weevils were placed with 75 treated corn kernels

b/Three female maize weevils of Fl were placed with 21 untreated corn kernels.

c/ Each value is a mean±SE of 5 replications. Mean within a column by the same letters are not significantly different at $\alpha = 0.05$.

were incubated for 40 d. Despite fewer egg-plugs on the 1,000 and 10,000 ppm neem oil-treated kernels, the 83.9 and 84.2% adult emergence of the F1 progeny, respectively, was the same as the 91.2% on the control ($p>0.05$). Weevils of the F1 progeny from all concentrations oviposited equally on untreated corn kernels (Table 2).

Reproductive ability: Weevils held for 7 d on corn kernels treated with 1,000 and 10,000 ppm neem oil and subsequently held on untreated kernels for 24 h, laid 2.8 and 2.4 eggs/female/day, respectively (Table 3). These numbers were significantly lower ($p<0.05$) than the 3.3, 3.2 and 3.2 eggs/female/day from weevils held in corn kernels treated with acetone, 100 ppm neem oil and 10,000 ppm mineral oil, respectively. When the period of weevil exposure to treated grain was increased to 15 d prior to oviposition for 24 h on untreated kernels, oviposition was not significantly affected by neem or mineral oil (Table 3).

Table 3: Oviposition of maize weevil on untreated corn kernels[a] after a period of exposure[b] to corn kernels treated with neem or mineral oil[c]

Treatment	Egg/fernale/day on untreated corn kernels		
	Seven days after exposure	Fifteen days after exposure to treated corn kernels	Twenty one days after exposure corn kernels to treated corn kernels
Control	3.3±0.4 A	2.7±0.8 A	2.9±0.54
Neenn oil			
100 ppm	3.2±0.4A8	2.8±0.4 A	3.0±0.54
1000 ppm	2.8±0.36 C	2.6±0.5 A	3.2±0.5A
10000 ppm	2.4±0.3 C	2.2±0.3 A	2.8±0.7A
Mineral oil			
10000	3.2±0.5AB	2.6±0.54	3.2±0.6A

a/ Six female weevils were placed with 50 untreated whole corn kernels

b/ Weevils were placed with treated corn kernels when they were 1.20 hours old.

C/Each value is a mean SE t of 5 replications. Means within a column followed by the same letters are not significantly different at $\alpha = 0.05$.

Table 4: Subsequent percent adult emergence of maize weevil from the eggs (Table 3) laid on untreated corn kernels by maize weevil after a period of exposure to corn kernels treated with neem or mineral oil[a]

Treatment	Egg/fernale/day on untreated corn kernels		
	Seven days after exposure	Fifteen days after exposure to treated corn kernels	Twenty one days after exposure corn kernels to treated corn kernels
Control	77.1±6.8A	83.4±4.9A	81.2±7.54
Neem oil			
100 ppm	79.6±6.44	85.4±11.04	79.0±7.5A
1000 ppm	75.9±6.0A	82.0±10.7A	79.4±8.4A
10000 ppm	76.6±7.7A	85.6±11.1A	81.5±11.24
Mineral oil			
10000 ppm	77.1±5.4A	81.0±3.4A	75.8±7.1A

a/ Each value is a mean±SE of 5 replications. Means within a column followed by the same letters are not significantly different at $\alpha = 0.05$.

At 10,000 ppm neem oil weevils showed a small, but not statistically significant ($p>0.05$) reduction in oviposition. The eggs in all kernels hatched normally, as the mean percent adult emergence was the same ($p>0.05$) in all treatments (Table 4). Fewer adults emerged from the kernels exposed to the weevils treated with 1,000 and 10,000 ppm neem oil, because they had fewer egg-plugs. However, the mean percent adult emergence of Fl progeny in the control as well as in all dose levels, was the same, indicating that the eggs in all treatments hatched normally Table 4). As the maize weevil is long lived insect and need food for production and maintenance of eggs, the observed inhibition of oviposition may be due to the antifeedant property of azadirachtin, where weevils on freshly treated kernels may have not ingested enough food to produce normal number of eggs. The antifeedant effect of neem, however, may have declined overtime similarly to its effect on ovipositional behavior and weevils held for 15 and 21 d may have ingested enough food to oviposit normally. This oviposition inhibition on untreated kernels after exposure to neem for 7 d may also be due to the effect of azadirachtin (a potent growth regulator) on the endocrine system of weevils. This effect, however, may have declined overtime in its effect by 15 d post-treatment. Jacobson (1988) noted that azadirachtin affects the pars-intercerebralis which control juvenile hormone and ecdysone and therefore, may ultimately affect the process of vitellogenesis. In the present study the low oviposition rate of maize weevil may also be associated with a change in ovipositional behavior. Rembold *et al.* (1984) demonstrated that the low levels of azadirachtin "A" remaining in the insect body is sufficient to disturb many endocrine functions affecting the morphology and behavior of insect. A conclusive explanation would require the investigation of the antifeedant and growth regulating properties of azadirachtin that affect egg development in the maize weevil.

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