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Repellency and Residual effect of Neem or Mineral Oil on the Distribution and Oviposition of Maize Weevil, *Sitophillus zeamais* Motsch

[1]Masood Khan Khattak, [2]Alberto B. Broce and [3]Barry Dover
[1]Department of Entomology, Faculty of Agriculture, Gomal University, D.I. Khan, Pakistan
[2]Livestock Entomology, Kansas State University, Manhattan, Kansas 66506, USA
[3]Stored Grain Insect Pest Section, Kansas State University, Manhattan, Kansas 66506, USA

Abstract: The Oviposition rate of the maize Weevils was greatly reduced when they were held with corn kernels treated with 1,000 and 10,000 ppm neem oil in acetone. The cleteramental effect of the 1,000 ppm neem oil treatment was lost by 30 days after treatment, but the 10,000 ppm retained its effect for 60 days. Doses of 1,000 and 10,000 ppm of neem oil repelled weevils from corn kernels treated 24 h before the test, however, repellency disappeared after 15 days in 1,000 ppm, but not the 10,000 ppm.

Key words: Repellent and residual effect of neem oil on maize weevil

Introduction

Main biological factors causing losses to stored food grains consist of insects, rodents and molds. Majority of the farmers in developing countries can not afford the costly modern grain protectants. The fumigants, besides, its risky nature are effective only in the airtight structures, where the storages in the farm level in Pakistan are not airtight. The insecticides can protect snood grains against insect pests but its harmful residues can cause health lazards, mamalian toxicity and developement of resistant insect strains. People have also been practicing plant derivatives for the last few centeries, Feinstein (1952) described species of atleast 46 families of flowering plants having insecticidal activities. The most important of these plants are tobacco, chrysanthemum, sabadilla, derris, camphor and neem etc. Neem, *Azadirachta indica* A. Juss being native to India and Pakistan, is found in abundance in these countries, especially in Pakistan (Ahmad and Grainage, 1988). The use of neem derivatives can play a vital role for the control of insect pests. Besides, its safe nature also make it superior to the synthetic chemicals. The neem tree can effectively protect stored grains from insect infestation because, besides, other compounds in neem, the most important ones are azadirachtin, salannin and maliantriol which have feeding and ovipositional deterrent, repellent, growth regulating and ovipositional inhibition activities against a great variety of insects (Jacobson, 1988). The insecticidal activities (repellent, residual, deterrent, anti-ovipositional, growth regulating and toxic) of neem have also been studied by Jilani *et al.* (1988), Sharma and Ansari (1994), Awan (1994), Khattak (1994), Sharma and Dhiman (1993), Pathak and Krishna (1991) and Okonkwo and Okoye (1996).

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 to 14% moisture contents in a controlled environment at $27 \pm 1^{\circ}C$ and $65 \pm 5\%$ RH with a 12:12 light and dark cycles. Weevils used in all these studies were 1 to 2 wk old unless otherwise noted. Neem oil was obtained from C.M. Ketkar, Neern Mission, 471 Shanwar Peth Pune 411030. Both neem and mineral oils were diluted in acetone.

Repellency: An olfactometer similar to one described by Ullah (1990) was used in these studies (Fig. 2). The apparatus consisted of a stainless steel olfactory chamber ($101.6 \times 203.2 \times 203.2$ mm) supported by 4 stainless steel legs. Internally the lower half of the chamber was divided into two equal sections by a vertical (101.6×76.2 mm) partition, In the center of each section, a 2.5 cm long copper tribe was fitted to pass the flow of air from treated and control kernels to the respective sections. Both sections were filled with marbles to evenly distribute the air entering the chamber. Marbles were washed with acetone after each trial and allowed to dry. The olfactory chamber had an opening in one side, through which a small steel tray ($6.4 \times 16.5 \times 3.8$ cm) was fitted to hold insects during repellency trials. The bottom of this tray was made of a fine 40 mesh brass screen; its walls were treated with Teflon^{R} solution to prevent weevils escaping during trials. The copper tube at the bottom of the olfactory chamber were connected to two glass tube with Tygon^{R} tubing. The glass tubes (15.2 cm) contained treated and untreated corn kernels. The tubing was extended from the glass tubes to a pair of flow meters and these connected to a 2,000 ml erlenmeyer flask containing distilled water to humidify the air. The olfactory chamber was covered with a plexiglass lid fitted with a partition attached perpendicularly. This lid divided internally the tray into two halves, leaving a space to allow for free movement of the weevils during trials. Two 2.5 cm copper tubes were fitted in each half of the lid and connected to another two flowmeters. The flowmeters were connected to vacuum pumps by Tygon tubing. Valves on each vacuum pump regulated the air pulled through the olfactory chamber. The experimental setup minimized the mixing of the air from treated and untreated corn kernels in the two halves of the tray. Compressed air (from a cylinder) was passed through two (15.2 cm) glass tubes containing charcoal to remove any odor from the air, prior to entering the tubes containing treated and untreated corn kernels. Air flow through these tubes was regulated by flowmeters. Acetone solution of 100, 1,000 and 10,000 ppm neem and mineral oil were applied at the rate of 2.5 ml/100 whole corn kernels. An acetone treatment served as control. Solvent was allowed to completely evaporate from the kernels before use. Before releasing weevils in the olfactometer tray, a constant air flow of 315 ml/min from the compressed air cylinder was pushed through each glass tube containing the kernels. The vacuum pumps pulled a constant air flow of 200 ml/min from the testing chamber. The amount of air pulled by the vacuum pumps was kept lower than the amount of air pushed, to avoid creating a vacuum and pulling room air into the olfactory chamber.

For each trial, 20 adult maize weevils (1 to 2 wk old) were

released in the middle of the small tray. The top of the chamber was than covered with the plexiglass lid. After 5 min, the number of weevils in each section of the chamber were counted and recorded. This experiment was replicated 5 times for each treatment. All trials were run in dark and counted in light. The data obtained in this experiment were converted to percent repellency (Ullah 1990) as follows:

Percent repellency = (Nc-Nt/NT)X100
where
Nc = Number of insects found in untreated (control) section
Nt = Number of insects found in treated section
NT = Total number of insects used
The repellency data were then analyzed by an analysis of variance (ANOVA) and the mean values were compared by LSD (p = 0.05).

Residual effects of Neem or mineral oil: Weevils were placed with corn kernels treated with 100, 1,000 and 10,000 ppm neem oil or 10,000 ppm mineral oil solutions at different time intervals to determine wether neem or mineral oil had residual effect on the distribution and oviposition of the weevils. Four samples of corn kernels (1,000 kernels/sample) were treated with 25 ml neem or mineral oil solutions. An acetone treatment served as control, Solvent was allowed to completely evaporate from the kernels and the kernels were placed into 0.473 l wide mouth glass jars.
At three time intervals, 24 h, 30 d and 60 d post-treatment, weevils were exposed to treated kernels in both choice and no-choice situations.

Distribution and oviposition
Choice test: Weevils were presented with the choice of dispersing and ovipositing onto either treated or untreated corn kernels in an arena (Fig. 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. Each treatment was replicated 5 times. 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. The data for percent adult distribution were tested with Chi-Square test and those of eggs laid/female/day were analyzed by ANOVA. The mean values of the treatments were compared by LSD (p = 0.05).

No-choice test: Fifty kernels from each treatment (mentioned above) were placed in 0.473 1 wide mouth glass jar and 6 female weevils were released in each jar. After 24 h weevils were removed from the kernels and the kernels were stained with acid fushcin solution. The kernels were then examined under the microscope and the number of egg-plugs in the kernels for each treatment were recorded.
In all these experiments, there were 5 replications for each treatment and the results were analyzed either by Chi-Squere and or by an analysis of variance (ANOVA) and the mean values of the treatments were compared by LSD (p = 0.05).

Results and Dicussion

Repellency: When neem oil was applied to corn kernels 24 h before testing, repellency by volatile from the 1,000 and 10,000 ppm neem oil doses was significantly greater (26% and 48%) than that of 100 ppm neem oil and 10,000 ppm mineral oil ($p < 0.001$; Table 1). The significant effect of 1,000 ppm neem oil dose at 24 h was not observed 15 d after treatment. The 10,000 ppm dose also showed a decline in its repellency after 15 d but significantly more weevils (26%) were found in the control half of the tray. As before, neem oil at 100 ppm and mineral oil at 10,000 ppm did not show any repellency. Earlier, Roomi and Atiquiddin (1977) found that neem seed and leaves applied to bags and eathern pots protected stored grains by repelling stored grain insect pests. Jilani *et al.* (1988) found that the termeric oil. sweetfiag and neem at 100, 500 and 1,000 ppm significantly repelled *Tribal'um castaneum* Everst. from rice grain. Sharma and Ansari (1994) demonstrated that neem oil mixed with kerosene oil and burnt in lamps repelled mosquitoes from living rooms. Sharma and Dhiman (1993) noticed significant repellent effect of neem oil against Sand Fly, Mosquitoes were also significantly repelled by Neem oil mats Sharma *et al.* (1993).

Residual effects of neem oil Distribution and oviposition
Choice test: Neem oil applied to corn kernels at 1,000 and 10,000 ppm, significantly reduced oviposition of the maize weevil, since only 0.7 and 0.4 eggs/female/day, respectively, were laid 24 h after the treatment (Table 2). These numbers were significantly lower ($p < 0.001$) than the 1.6 and 0.8 eggs/female/day on the control of the same arena. Similar to previous tests, neem oil at 10,000 ppm not only reduced the weevil's oviposition on treated kernels but also inhibited oviposition on the untreated kernels in the same arena. In this case, weevils laid 0.8 eggs/female/day in the control kernels, a significantly lower number ($p < 0.05$) than the 1,6, 1.6 and 1.6 eggs/female/day in controls for the 100, 1,000 ppm neem oil and 10,000 ppm mineral oil treatments, respectively (Table 2).
Neem oil at the 1,000 ppm rate, lost its inhibitory effect on oviposition by 30 d after treatment. Although, 60 d after treatment, the 10,000 ppm neem oil treatment still significantly reduced oviposition, it had lost its deterrent effect on weevil distribution on treated kernels. At the 10,000 ppm dose, 0.8 and 0.8 eggs/female/day were laid on the treated kernels 30 and 60 d after treatment, respectively, which were significantly lower than 1.4 and 1.4 eggs/female/day laid on the respective controls. In this case the proportion of the weevils found on the kernels 60 d after treatment (51%) was similar to those in the control (49%).

No-choice test: When maize weevils were confined on either treated or untreated kernels, their oviposiition was similar to that observed under choice conditions. Neem oil at 1,000 and 10,000 ppm greatly inhibited the oviposition, but over time, its

Table 1: Mean percent repellent response[a] of maize weevil at different time intervals and different dose levels of neem or mineral oil[b]

Treatment	Repellency after 24 hours	Repellency after 15 hours
Control	2.0 ± 13.0A	-4.0 ± 11.4A
Neem oil		
100 ppm	4.0 ± 15.2A	2.0 ± 8.4A
1,000 ppm	26.0 ± 11.4B	2.0 ± 8.4A
10,000 ppm	48.0 ± 8.4B	26.0 ± 8.9B
Mineral oil		
10,000 ppm	-2.0 ± 19.2A	4.0 ± 18.5A

a: Each value is a mean ± SE of 5 replications. Means within a column followed by the same letters are not significantly different at α = 0.05. The data was tested both with Chi-square and ANOVA (SAS, 1988).

b: Twenty adult maize weevils were released in repellency chamber.

Table 2: Effect of neem or mineral oil residues on distribution and oviposition of maize weevil. Choice tests[a]

Treatment	Percent distribution		Eggs/female/day	
	Control	Treated	Control	Treated
		After 24 hours		
Neen oil				
100 ppm	49.0 ± 10.8A	51.0 ± 10.8A	1.6 ± 0.2A	1.5 ± 0.2A
1,000 ppm	52.0 ± 5.7A	48.0 ± 5.7A	1.6 ± 0.2A	0.71 ± 0.2B
10,000 ppm	75.0 ± 6.1A	25.0 ± 6.1B	0.8 ± 0.1A	0.4 ± 0.1B
Mineral oil				
10,000 ppm	51.0 ± 9.6A	49.0 ± 9.6A	1.6 ± 0.2A	1.6 ± 0.3A
		After 30 days		
Neem oil				
100 ppm	48.0 ± 12.5A	52.0 ± 12.5A	1.7 ± 0.2A	1.7 ± 0.3A
1,000 ppm	51.0 ± 9.6A	49.0 ± 9.6A	1.6 ± 0.2A	1.6 ± 0.3A
10,000 ppm	61.0 ± 4.24	39.0 ± 4.2A	1.4 ± 0.1A	0.8 ± 0.1B
Mineral oil				
10,000 ppm	50.0 ± 10.0A	50.0 ± 10.0A	1.6 ± 0.2A	1.6 ± 0.1A
		After 60 days		
Neem oil				
100 ppm	49.0 ± 9.6A	51.0 ± 9.6A	1.6 ± 0.2A	1.6 ± 0.2A
1,000 ppm	50.0 ± 106A	50.0 ± 10.6A	1.7 ± 0.3A	1.6 ± 0.2A
10,000 ppm	49.0 ± 6.5A	51.0 ± 6.5A	1.4 ± 0.1A	0.8 ± 0.1A
		Mineral oil		
10,000 ppm	49.0 ± 11.4A	51.0 ± 11.4A	1.6 ± 0.2A	1.5 ± 0.1A

a: Each value is a mean ± SE of 5 replications. Paired means within a row followed by the same letters are not significantly different at α = 0.05

Table 3: Effect of neem or mineral oil residues on oviposition of maize weevils[a] No-choice test[b]

Treatment	Eggs/female/day 24 hours post-treatment	Eggs/female/day 30 days post-treatment	Eggs/female/day 60 days post-treatment
Control	2.8 ± 1.0.34	2.7 ± 0.2A	2.7 ± 0.2A
Neem oil			
100 ppm	2.7 ± 0.2A	2.7 ± 0.3A	2.6 ± 0.9A
1,000 porn	1.6 ± 1.6B	2.5 ± 0.3A	2.4 ± 0.2A
10,000 pprn	1.21 ± 0.2C	1.4 ± 0.3B	1.5 ± 0.3B
Mineral oil			
10,000 ppm	2.6 ± 0.4A	2.6 ± 0.5A	2.5 ± 0.3A

a: Six female maize weevils were placed with 50 treated corn kernels

b: Each value is a mean ± SE of 5 replications. Means within a column followed by the same letters are not significantly different at α = 0.05

effect gradually declined (Table 3). Although, 1,000 ppm of neem oil at 24 h significantly reduced weevil oviposition (1.6 vs 2.8 eggs/female/day for control), this treatment lost its effect after 30 d (2.5 vs 2.7 eggs/female/day for

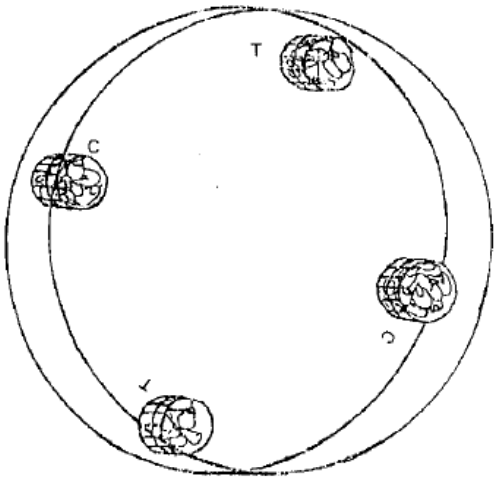

Fig. 1: Arena used for choice studies T = Treated, C = Control

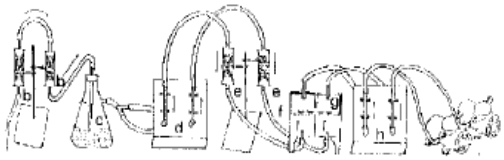

Fig. 2: Apparatus used in repellency studies (a) Compressed air (b) glass tube containing charcoal (c) flask with distilled water (d) flow meter with metering valves (e) glass tubes containing treated and untreated kernels (f) olfactory test chamber (g) testing tray (h) flow meters (i) vacuum pumps with metering valves

control). Neem oil at 10,000 ppm significantly reduced ($p < 0.001$) weevil oviposition for up to 60 d, as weevils laid significantly fewer eggs (1.5 eggs/female/day) than in the controls (2.7 eggs/female/day). In both situations (choice and no-choice test), mineral oil at 10,000 and neem oil at 100 ppm neither inhibited oviposition nor affected weevil distribution.

Many workers have rewieved residual effect of the neeni derivatives against different insects. Observed that neem seed powder mixed with wheat grains at 1 or 2% protected wheat against *Sitophilus oryzae, Rhizopertha dominica* (F.) and *Trogoderma granarium* Everst. for ca. 269, 321 and 379 days, respectively. Devi and Mohandas (1982) found that against *R. dominica,* neem seed extracts at 0.5 and 1% gave good protection to stored rice for up to 6 months. Awan (1994) demonstrated that diferrent neem extracts significantly reduced the percent infestation of cotton by cotton insect pests complex upto 17 days. Khattak (1994) Observed that neem oil at 10,000 ppm significantly affected the feeding and oiposition of the maize weevil upto 60 days. Results of the present studies indicated that in choice test, 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 test insect.

References

Ahmad, S. and M. Grainage, 1988. The use of Indegious Plants Resources in Rural Development: Potential of Neem Tree. In: Focus of Phytochemical Pesticides, Volume1. The Neem Tree, Jacobson, M. (Ed.). CRC Press, Boca Raton, Florida, USA., pp: 178.

Awan, M.N., 1994. Evaluation of some insecticidal combinations and neem extracts againts insect pests of cotton. M.Sc. Thesis, Agriculture Faculty, Gomal University, D.I. Khan, Pakistan.

Devi, D.A. and N. Mohandas, 1982. Relative efficacy of some antifeedants and deterrents against insect pests of stored paddy. Entomon, 7: 261-264.

Feinstein, L., 1952. Insecticides from Plants. In: Insects: The Yearbook of Agriculture, USDA (Ed.). United States Department of Agriculture, Washington, DC., pp: 222-229.

Jacobson, M., 1988. Focus on Photochemical Pesticides, Volume1. The Neem Tree. CRC Press, Boca Raton, Florida, USA., pp: 178.

Jilani, G., R.C. Saxena and B.P. Rueda, 1988. Repellent and growth-inhibiting effects of turmeric oil, sweetflag oil, neem oil and Margosan-O on red flour beetle (Coleoptera: Tenebrionidae). J. Econ. Entomol., 81: 1226-1230.

Khattak, M.K., 1994. Effect of neem oil on maize weevil, *Sitophilus zeamais* Motsch and its parasitoid, *Anisopteromalus calandrae* (Howard). Ph.D. Thesis, KSU, Manhattan, KS, USA.

Okonkwo, E.O. and W.I. Okoye, 1996. The efficacy of four seed powders and the essential oils as protectants of cowpea and maize grains against infestation by *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) and *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in Nigeria. Int. J. Pest Manage., 42: 143-146.

Pathak, P.H. and S.S. Krishna, 1991. Post-embryonic development and reproduction in *Cocyra Cephalonica* Stainton (Lepidoptera: Pyralidae) on exposure to eucalyptus and neem volatiles. J. Chem. Ecol., 17: 12-12.

Roomi, M.W. and M. Atiquiddin, 1977. Observations on the repellency of 'Neem' plant, *Melia azadirachta* L., on some stored grain pests from Pakistan. J. Applied Entomol., 84: 124-129.

SAS., 1988. SAS User's Guide: Statistics. Release 6.03. SAS Institute Inc., Cary, NC., pp: 1028.

Sharma, V.P. and M.A. Ansari, 1994. Personal protection from mosquitoes (Diptera: Culicidae) by burning neem oil in kerosene. J. Med. Entomol., 31: 505-507.

Sharma, V.P. and R.C. Dhiman, 1993. Neem oil as a sand fly (Diptera: Psychodidae) repellent. J. Am. Mosquito Control Assoc., 9: 364-366.

Sharma, V.P., B.N. Nagpal, and A. Srivastava, 1993. Effectiveness of neem oil mats in repelling mosquitoes. Trans. Royal Soc. Trop. Med. Hyg., 87: 626-626.

Ullah, N., 1990. White sagebrush, *Artemisia ludoviciana* Nutt.(Asteraceae) flower extract as a protectant for wheat against stored-product insects. Ph.D. Thesis, Kansas State University, USA.