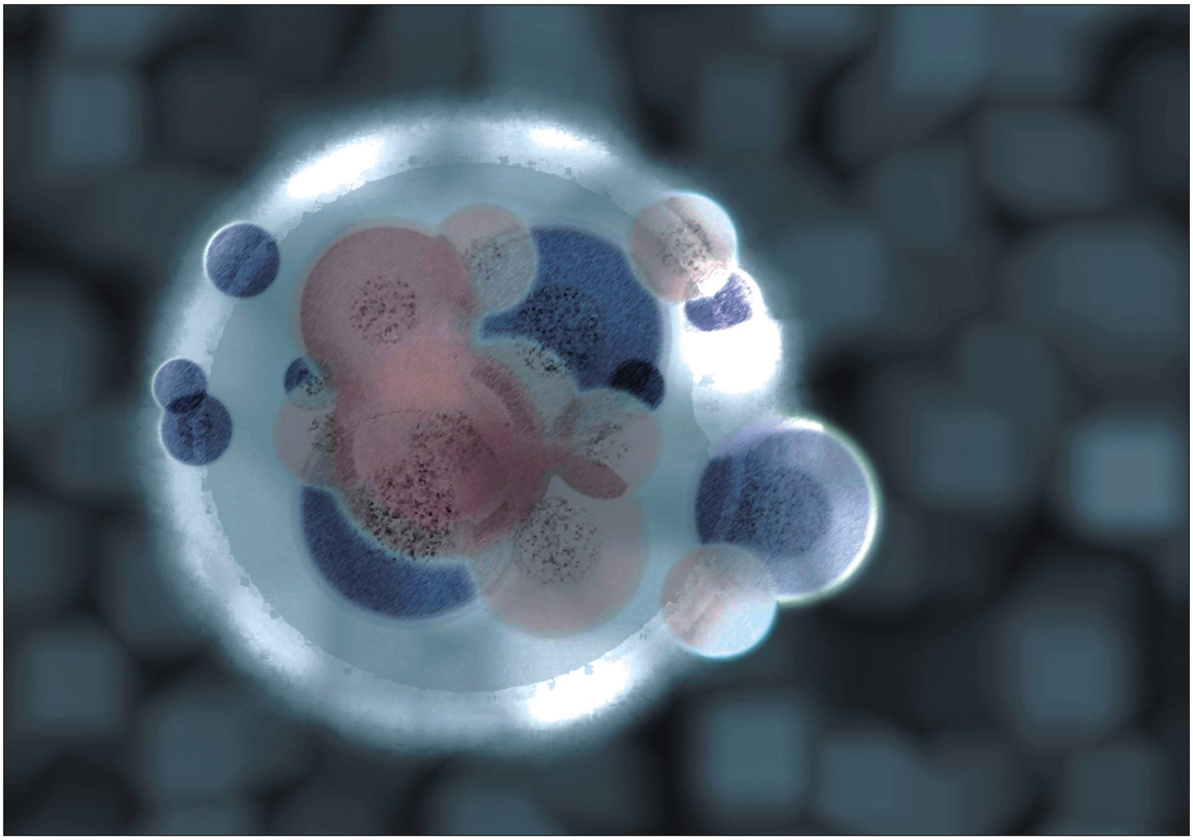

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Comparative Effects of Neem or Mineral Oil on Maize Weevil, *Sitophilus zeamais* Motsch. And its Parasitoid, *Anisopteromalus calandrae* (Howard).

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Abstract: Crude neem oil diluted in acetone was tested for its toxic effect on the reproductive ability of the *S. zeamais* and its parasitoid *A. calandrae*. Neem oil at 100, 1,000 and 10,000 ppm on corn kernels did not affect the survival of adult weevils. Although, the oviposition rate was greatly reduced with 1,000 and 10,000 ppm neem oil, the eggs hatching and the F1 progeny reproduced normally. Neem oil at all doses (100-10,000 ppm) did not show any detectable effect on the biology of *A. calandrae*. However, at 10,000 ppm neem oil showed a significant effect on the reprodutive potential of the parasitoid as measured by adult emergence and normal reproduction by F1 progeny.

Key words: Effect of Neem oil on Maize Weevil and its Parasitoid

Introduction

Insect pests cause heavy losses to stored grains, specially in humid and warm area of the world. At least 15 (primary) species of the insects are well adapted to stored grain (Pederson *et al*., 1977). Nature keeps a partial check on these insect pest by the action of predators, parasites, parasitoids and pathogens. Man uses mostly synthetic insecticides for the control of these stored grain insect pests. These insecticides carry potential health hazards and affect non-target organisms, Therefore, search for chemicals from natural products is in progress which is safer to man and useful insects.

The neem tree, *Azadirachta indica* A. Juss has successfully been used to protect stored grain from the attack of stored grain insect pests. Almost every part of the tree (root, trunk, bark, leaves, flowers, fruits and seeds) is known to have some use in the countries where neem is found (Radwaski, 1981). The most important compounds of neem (azadirachtin, salannin and maliantriol) have feeding and ovipositional deterrent, repellent, growth regulating and ovipositional inhibition activities against a great variety of insects (Jacobson, 1989). The insecticidal properties of the neem have also been reviewed by Tanzubil (1991), Becker (1994) and many others. Besides other insecticidal activities (feeding and ovipositional deterrent, insect growth regulator and repellent), neem has also shown some toxic effect against some insects; however, all these effects are dose dependant, vary from insect to insect and affect different stages of the same insect differently. Awan (1994) found the significant toxic effect of diferrent neem extracts against cotton insect pests. Ethanolic extracts of neem were more toxic than neem in the powder form to *S. zeamais* confined for 72 h on treated grains (Kossou, 1989). Neem seed extracts are non-toxic to warm blooded organisms and show little or no side effects on natural enemies of insect pests (Schmutterer, 1985). The injection of azadirachtin into *Menduca sexta* L., when used prior to the first larval ecdysis of the wasp, *Cotesia congregata* (Say) adversely affected their subsequent development (Beckage *et al*., 1988).

Materials and Methods

The maize weevils And *Anisopteromalus calandrae* were obtained from cultures maintained in the Entomology Department, Kansas State University. Weevils were reared on whole commercial corn kernels and the parasitoid were reared on 3rd and 4rth instar larvae of maize weevil in the infested corn kernels. The cultures and experiments were maintained in a controlled environment at 27±1EC and 65±5% RH with a 12:12 ligth and dark cycle. Weevils and *Anisopteromalus calandrae* used in all these experiments were 1-2 wk and 1-3 days old respectively,unless otherwise noted.

The use of acid fuchsin solution for egg-plug detection: Acid fuchsin staining solution was used in this studies to facilitate identification of the weevil's egg-plugs on corn kernels. In some test, kernels were held for a period of time to determine treatment effect on egg viability. However, it was not known wether the staining solution had any deleterious effect on egg viability. For this a test was conducted using an acid fuchsin solution prepared according to Frankenfeld (1948). A sample of 440 whole corn kernels was placed in a 0.946 l small mouth glass jar and ca. 60 pairs of maize weevils were released in the kernels. After 48 h, weevils were removed from the kernels and the kernels were randomly divided into 4 equal subsamples. Kernels of the three subsamples were soaked for 5, 5 and 2 min in distilled water; distilled water + glacial acetic acid; and distilled water + glacial acetic acid + acid fuchsin, respectively. The kernels of the fourth subsample were held as untreated controls. The kernels of the first three subsamples were dried and transferred to 0.473 l wide mouth jars. After 40 d the newly emerged maize weevils were counted in each jar. Five replications were used for each treatment in this experiment.

Effects of neem or mineral oil on the maize weevil

Toxicity: Neem oil was diluted in acetone to obtain 100, 1,000 and 10,000 ppm solutions. A sample of 100 whole corn kernels was placed in 115 ml screw cap glass bottle and 2.5 ml of the neem or mineral oil solution was added to the kernels. The bottle contents were shaken for a few minutes to get a uniform application of the solution on the surface of the kernels. One set of 100 kernels was treated with acetone alone and used as control. Each of these treatments was replicated 5 times. The solvent was completely evaporated from the kernels. Kernels were taken in 0.473 l wide mouth jar and 30 adult maize weevils were released in each jar (each treatment). After 30 d, the number of dead and live individual in each treatment was recorded.

Oviposition on treated and untreated corn kernels, subsequent adult emergence and oviposition of F1 progeny:

No-choice test: The effect of neem or mineral oil on the oviposition of the maize weevil was evaluated under no-choice experimental situation. Acetone solutions of 100, 1,000 and 10,000 ppm neem and mineral oil were applied to corn kernels as mentioned earlier to get uniform application of the solution. Seventy five kernels from each treatment were placed in 0.473 l wide mouth jars and ten female weevils were placed in each jar. After 24 h weevils were removed from the kernels and the kernels were stained with the acid fuchsin solution. The kernels were examined under the microscope and the number of egg-plug in the kernels for each treatment recorded. These kernels with the egg-plugs were held for 40 d to determine wether neem or mineral oil had affected the viability of the eggs. After 40 d, the number of adults emerged from the kernels in each treatment was recorded and expressed as percent adult emergence. Subsequently, three mated females (7-10 d old) of the F1 progeny were exposed to 21 untreated corn kernels to determine wether neem or mineral oil had adversely affected the reproduction of the F1 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.

Effects of neem and mineral oil on *Anisopteromalus calandrae*
Longevity of untreated *A. calandrae:* Before testing neem and mineral oil on *A. calandrae*, an experiment was conducted to determine the longevity of this parasitoid. Twenty (one d old) female parasitoids were placed with ca. 100 corn kernels in a 0.473 l wide mouth glass jar. In a parallel experiment, an equal number parasitoids were placed in a jar with ca. 100 corn kernels mixed with 20 raisin pulps. The jars were covered with screened lids and kept in a controlled environment at 27±1EC and 65±5% RH. Each treatment was replicated three times. Parasitoids were regularly inspected in both experiments for dead and live individuals and the observations recorded and plotted.

Toxicity: The possible toxic effect of neem and mineral oil on *A. calandrae* was studied by using doses of 100, 1,000 and 10,000 ppm neem or mineral oil, applied to corn kernels as described before. The kernels were placed in 0.473 l wide mouth glass jars; ten female (one d old) parasitoids were added to the kernels and the jars were covered with screened lids. After 48 h, lives and dead insects were recorded. Each treatment was replicated 5 times and the results were analyzed by an analysis of variance (ANOVA). The mean values of the treatments were compared by LSD (p = 0.05; SAS, 1988).

Reproductive ability of *A. calandrae:* The effect of neem and mineral oils on *A. calandrae* reproduction was studied by assessing reduction of adult emergence from infested corn kernels exposed to the parasitoids. For this, a sample of 750 whole corn kernels was placed in a 0.946 l small mouth glass jar. Maize weevil (ca. 40 pairs) were released in the jar and allowed to oviposit in the kernels. Jar was kept in the controlled environment as mentioned before. After 96 h, weevils were removed from the kernels and the kernels were kept in the same environmental conditions. After 17-20 d, when the kernels were expected to have late 3rd or 4th instar weevils, they were randomly divided into five sub-samples. Four of these sub-samples were treated with 100, 1,000 and 10,000 ppm neem oil or 10,000 ppm mineral oil solutions in acetone. The 5th sub-sample was treated with acetone alone and used as control. The kernels were transferred to 0.473 l wide mouth glass jars after the solvent was allowed to completely evaporate from the kernels. Five pairs (two d old) of the parasitoid were released in the jars. After 16-17 d, the number of emerged parasitoids in each treatment was recorded.
Five pairs of F1 progeny of the parasitoid from each treatment were placed with 100 untreated infested corn kernels in 0.473 l wide mouth glass jars to evaluate wether neem or mineral oil had affected reproductive ability of the parasitoid. After 16-17 d, the number of adult parasitoids emerged was recorded. In both these tests each treatment was replicated 5 times. Results of all the experiments in the present study were analyzed 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

The use of acid fuchsin solution for egg-plugs detection: Acid fuchsin solution did not affect the viability of maize weevils eggs, as the mean number adult emerged in different treatments were not significantly different to the controls (Table 1). This most probably was due to the body fluid of weevils which seals the cavity when the eggs are deposited. The sealing of the cavity protect the egg from excessive drying, change in temperature, attack of parasitoids and against gases used in fumigation (Lathrop, 1914).

Effects of neem and mineral oil on the maize weevil
Toxicity: Neem and mineral oils were found to be non-toxic to the maize weevil. Concentration as high as 10,000 ppm did not cause any mortality when weevils were exposed to treated kernels for 30 d (Table 2). Different insects are affected differently by different neem derivatives. Ethonalic extracts of neem were more toxic than neem in powder form to *Sitophillus zeamais* confined for 72 hours on treated grains (Kossou, 1989). Awan (1994) found some significant effect of neem extracts against cotton insect pests. In the present studies the non-toxic effect of the neem may be due to the biology of this insect where all immature stages (egg-pupae) remain inside the kernel.

Oviposition on treated and untreated corn kernels, subsequent adult emergence and oviposition of F1 progeny
No-choice test: When weevils were confined with treated kernels, neem oil at 100 ppm did not reduced their oviposition. The 1,000 ppm solution had 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 3). This negative effect on oviposition was further observed with weevils on kernels treated with 10,000 ppm of neem oil, which laid 1.1 eggs/female/day. The percent adult emergence of the F1 progeny was similar in all concentrations, as the eggs hatched normally, when they 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 from the F1 progeny from all concentrations oviposited equally on untreated corn kernels (Table 3). Jilani *et al*. (1988) observed that *Tribolium castaneum* adults fed on wheat flour treated with 0.4% neem oil failed to reproduce, possibly due to an effect on oviposition or reproductive physiology. Pathak and Krishna (1991) investigated some growth regulating effect of neem volatiles.

Effects of neem or mineral oil on *Anisopteromalus calandrae*
Longevity of untreated *A. calandrae:* Parasitoids (one d old)

lived, an average, 4-6 d when placed with uninfested corn kernels. Only 3 out of the total of 60 of the adult parasitoids survived up to day 7 (Figure 1). However, adult parasitoids survived up to 44 days when released in untreated uninfested corn kernels mixed with raisin pulp.

Table 1: Effect of acid fuchsin solution on the viability of maize weevil eggs

Treatment[a]	Mean adult emergence[b]
Control	44.4 ± 3.4A
Distilled water	41.8 ± 3.6A
Distilled water + Glacial acetic acid	41.2 ± 3.3A
Distilled water + Glacial acetic acid + Acid fuchsin	45.4 ± 3.7A

a/One hundred and ten equally infested corn kernels were used in each treatment.
b/Each value is a mean ± SE of 5 replications. Means followed by the same letters are not significantly different at ∝ = 0.05.

Table 2: Toxicity of neem or mineral oil to maize weevil[a]

Treatment	Mean percent survival[b]
Control	95.3 ± 0.8A
Neem oil	
100 ppm	93.3 ± 0.7A
1,000 ppm	94.0 ± 1.5A
10,000 ppm	89.3 ± 0.8A
Mineral oil	
100 ppm	96.7 ± 0.7A
1,000 ppm	91.3 ± 1.1A
10,000 ppm	88.7 ± 0.9A

a/Thirty weevils were placed with 100 treated corn kernels for 30 days.
b/Each value is a mean ± SE of 5 replications.
Means followed by the same letters are not significantly different at ∝ = 0.05.

Table 3: Oviposition of maize weevils held on corn kernels treated with neem or mineral oil[a], subsequent adult emergence and their oviposition[b] on untreated kernels. No-choice test[c].

Treatment	Eggs/female /day on treated kernels	Percent adult emergence	Eggs/female /day of F1 on untreated kernels
Control	3.0 ± 0.3A	91.2 ± 7.1A	3.7 ± 0.6A
Neem oil			
100 ppm	2.8 ± 0.2A	90.2 ± 7.0A	4.1 ± 0.4A
1,000 ppm	1.5 ± 0.2B	83.9 ± 10.6A	3.5 ± 0.5A
10,000 ppm	1.1 ± 0.6C	84.2 ± 13.3A	3.9 ± 0.7A
Mineral oil			
10,000 ppm	2.7 ± 0.4A	92.5 ± 5.5A	4.1 ± 0.3A

a/Ten female maize weevils were placed with 75 treated corn kernels.
b/Three female maize weevils of F1 were placed with 21 untreated corn kernels.
c/Each value is a mean ± SE of 5 replications.
Means with in a column followed by the same letters are not significantly different at ∝ = 0.05.

Toxicity: The survival of *A. calandrae* did not appear to be affected either by neem or mineral oil. When this parasitoid was held for 48 h in uninfested corn kernels treated with 100, 1,000 and 10,000 ppm neem oil, the mean percent number of parasitoid surviving after 48 h was not significantly different ($p > 0.05$; Table 4) from the controls. Similar to neem oil, mineral oil did not have any effect on the survival of *A. calandrae.* These results and that of Chatterji (1955) indicate that in addition to raison palp, access to host may provide necessary nutrients for an extended life span.

Table 4: Toxicity of neem or mineral oil to *Anisopteromalus calandrae*[a].

Treatment	Mean percent survival[b]
Control	72.0 ± 0.8A
Neem oil	
100 ppm	78.0 ± 1.1A
1,000 ppm	76.0 ± 0.6A
10,000 ppm	74.0 ± 1.4A
Mineral oil	
10,000 ppm	80.0 ± 0.7A

a/Ten (one day old) female *A. calandrae* were placed with uninfested treated corn kernels for 48 hours.
b/Each value is a mean ± SE of 5 replications. Means followed by the same letters are not significantly different at ∝ = 0.05.

Table 5: Effect of neem or mineral oil on the emergence of *Anisopteromalus calandrae*[a] and the reproduction of its F1 progeny[b].

Treatment	Adult emergence	Adult emergence from F1 on infested untreated corn kernels
Control	48.6 ± 5.2A	30.6 ± 2.8A
Neem oil		
100 ppm	46.6 ± 3.4A	31.3 ± 2.7A
1,000 ppm	44.0 ± 4.1A	30.0 ± 1.8A
10,000 ppm	31.4 ± 2.5B	30.0 ± 2.2A
Mineral oil		
10, 000 ppm	47.4 ± 5.6A	31.2 ± 1.3A

a/Five pairs of parasitoids were placed with 150 treated infested corn kernels.
b/ Five pairs of parasitoids were placed with untreated infested corn kernels.
c/Each value is a mean ± SE of 5 replications.
Means within a column followed by the same letters are not significantly different at ∝ = 0.05.

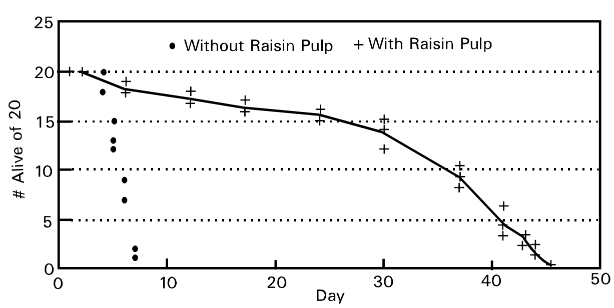

Fig. 1: Longevity of *Anisoptromalus calandrae* with/without access to raisin pulp

Reproductive ability of *A. calandrae:* Emergence of adult parasitoids from treated infested kernels was considered a criterion to evaluate the effect of neem or mineral oil on the reproductive ability of the parasitoid, i. e., the reduction in the parasitoid's emergence from any treatment relative to that of the control would be indicative of the effect of neem or mineral oil on the reproductive ability of the parasitoid. When parasitoids were exposed to infested corn kernels treated with 100 and 1,000 ppm neem oil and 10,000 ppm mineral oil, 46.6, 44.0 and 47.4 adult parasitoids emerged,respectively; these values were not significantly different from the 48.6 in the controls ($p>0.05$; Table 5). Neem oil at 10,000 ppm significantly affected the reproductive potential of the parasitoid. As compared to the control (48.6), fewer (31.4) parasitoids emerged from 10,000 ppm treated, infested kernels. However, the F1 progeny of the parasitoid from this treatment reproduced normally, as the mean number 31.0 was not significantly different from the 30.6 of the controls (Table 5). According to research results of other workers, the neem derivatives affect different parasitoids differently. Schumutterer *et al*. (1981) stated that the neem seed extracts are non-toxic to warm blooded organisms and show little or no side effect on natural enemies of the insect pests. In laboratory tests did not observe any adverse effect on the emergence of the egg prarasitoid, *Telenomus remus* before or after parasitisation when exposed to *Spodoptera litura* eggs sprayed with 2% neem seed kernels suspension. Srivastava *et al*. (1997) and Press and Mullen (1992) have also reported the non-negative effect of neem on parasitoids.

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