Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2015; 3 (1): 38-42

Received: 13-12-2014
Accepted: 29-12-2014

Ajad Kumar Bhardwaj
Jagatpur Inter College, Jagatpur, Varanasi - 221302, India.

Badre Alam Ansari
Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur - 273009 (U.P.), India.

Correspondence:
Badre Alam Ansari
Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur - 273009 (U.P.), India.

Effect of Nimbecidine and Neemazal on the developmental programming of cotton pest, *Earias vittella*

Ajad Kumar Bhardwaj, Badre Alam Ansari

Abstract
The toxic effect of Nimbecidine and Neemazal on the reproductive ability of cotton pest, *Earias vittella* was evaluated under laboratory conditions. Sufficient number of male and female insects was treated with four different doses of Neemazal T/S (0.70, 1.10, 1.50 and 1.90 µg/insect) and Nimbecidine (0.90, 1.05, 1.20 and 1.35 µg/insect). Six males and six females were used for each treated dose and were paired to mate independently. Each such pair served as one replicate. Results show significant reduction in fecundity, hatchability and adult emergence as compared to that of control. A significant decrease ($p<0.001$ for each case) in hatchability up to 44% in Neemazal T/S and 32% in Nimbecidine treated *Earias vittella* were observed. The result also shows that both the pesticides may have a significant effect on the reproduction of the cotton pest, *Earias vittella*. Therefore, less expensive natural biopesticide could be an alternative for chemical pesticides.

Keywords: Neem, Insect, Fecundity, Adult emergence, Toxicity.

1. Introduction

Plants have evolved rich sources of natural substances for protection against herbivores. These botanicals are not only biodegradable and environment friendly but also there is less likelihood for insects to develop resistance against these natural substances [1]. Biocontrol agents (natural enemies, parasitoids and predators) and neem extracts have been reported as ecofriendly options for management of insect pests [2, 3]. Neem oil produced non-toxic effects after spray and acted as antifeedant, growth inhibitor and oviposition deterrent against insect pests [4]. Different plant extracts elicit a variety of effects in insects, such as antifeedant, growth retardation, reduced fecundity, moulting disorders, morphogenetic defects, and changes of behavior [5, 6, and 7]. In addition, over use and abuse of insecticides has resulted in ecological imbalance. Therefore, to prevail over the threat of food contamination, diverse sources of bio-insecticides are being sought to replace the synthetic insecticides.

Effects of plant extracts on many insects have been already reported [8, 9]. Botanical extracts and biological control (spider, ant, lady bird beetle, *Orius*, mirid bug, *Laius, Chrysoperla, Trichogramma* etc.) should be integrated for economic management of insect pests [10]. Neem has systemic activity; it is active at low concentrations and degrades rapidly in the environment [11]. In an earlier study cotton pest, *Earias vittella* was found to be very sensitive to the oil of *Eupatorium capillifolium* and *Callistemon lanceolatus* and the effect was time-dependent [12]. Recently, it was also observed that Achook, a neem based pesticide reported to be safe for non-target animals was also toxic to zebrafish [13, 14]. In addition, *Pelargonium citrosa* [15], *Dalbergia sissoo* [16] and *Mentha piperita* [17] were shown to contain larvicidal and growth inhibitory activity against *Anopheles stephensi*. Hence, the neem based pesticides provided a good source for the control of different insect pests [11]. Cotton is the backbone of our textile industry, accounting for 70% of total fibre consumption in textile sector and 38% of the country's export. One of the very common and notorious cotton pests is *Earias vittella* Fab. In India. Therefore, the present study was undertaken to study the effect of Nimbecidine and Neemazal T/S on the fecundity, hatchability and emergence of cotton pest, *E. vittella*.

2. Material and Methods

Under laboratory conditions the stock of *E. vittella* was maintained by procuring infested okra fruits from the local fields. Freshly emerged male and female insects drawn from the stock culture of *E. vittella* were employed in this study.

Adults (1-12 h old) in sufficient number were anesthetized with ether and treated topically on the ventral thorax and abdomen with 0.70, 1.10, 1.50 and 1.90 μg/insect of Neemazal T/S and 0.90, 1.05, 1.20 and 1.35 μg/insect of Nimbecidine diluted in acetone. The control individuals were treated with equal amount of acetone without the pesticide. The neem based pesticides *viz.,* Neemazal T/S (Azadirachtin 1.0%, other limonoids 3.0%, oil fatty acids, glycerol esters 46.3%, polyethylene monosorbitol oleate 49.7%) and Nimbecidine (0.03% Azadirachtin, 90.57% neem oil, 5.0% hydroxyel, 0.50% epichlorohydrate and 3.0% aromax) used during the present study were provided by M/S EID Parry (India) Ltd. Chennai and M/S T. Stanes & Co. Ltd. Coimbatore respectively.

Six males and six females were selected for each batch. Each pair was allowed to mate inside a glass vial (7.0 cm diameter, 9.0 cm height) and served as one replicate. In this way six replicates were used for each dose of treatment. The vial was covered at its top open end with a piece of white muslin cloth bound by elastic bands and included a hanging glass capillary filled with 15% sucrose solution which served as the adult food for these moths to enable them to recover from any post operational shock that might otherwise interfere during experimentation. A small section of epicarp portion of okra fruit functioning as oviposition substrate was kept at the floor of the chamber. Oviposition was monitored daily and the total number of eggs deposited during the first four days (the period when the mated females were generally prolific in egg laying during their life time) was recorded.

Finally the eggs laid were counted and pooled together for each dose separately for further observations. Lots of 300 eggs taken from each pool (eggs laid at different doses) were arranged in linear fashion on the floor of glass petridishes of 4 inch diameter (50 eggs/petridish) for the observation of hatchability. Further, the hatched larvae were transferred to the beakers containing the food (culture medium) to observe the adult emergence. Neither the eggs nor the larvae were treated with pesticides, so there was no need of replication. Simply they were transferred to the culture medium as per convenience. All experiments were performed at 28±2 ^{0}C and 80±10% RH. The data observed for the control and treated leaves were subjected to student t-test with the help of statistical software.

3. Results

Toxicity test on reproductive parameters are the most essential part of the toxicological studies. The results are shown in tables 1 and 2. Neemazal T/S shows detrimental effects on the developmental programming (reproductive ability) of *E. vittella* (Table 1). The mean number of eggs laid by six females was recorded to be 300.50±5.06 which decreased to 229.00±5.19 at the dose of 0.70 μg/insect *i.e.,* 76% of the control (100%) (Table 1). There was further reduction in the egg yield after increase in the dose (1.10, 1.50 & 1.90 μg/insect) showing the dose-dependent response of Neemazal T/S. The maximum reduction in the eggs count was observed to be 49% of the control which was taken as 100% (Table 1). The data were statistically significant (p<0.001) when student's t-test was applied between the control and the treated (Table 1). In the second series of experiment the hatchability and the adult emergence was observed. Table 1 show that embryogenesis of *E. vittella* was affected by Neemazal T/S treated adult. There was 84% hatching and 66% emergence of the adult after 0.70 μg/insect treatment. In the eggs of the highest treated insects (1.90 μg/insect), the hatching was 44% and the emergence was recorded to be only 31% of the 300 eggs (100%). From the table 1 it is evident that the hatching and the emergence was dose-dependent (as per treated insects).

Table 1: Effect of Neemazal T/S on the fecundity, hatchability and emergence of *Earias vittella**

Treated dose (μg/insect)	**Mean egg yield (± = SE)**	**No. of eggs used (for hatching/emergence)**	**No. of eggs hatched**	**No. of adults emerged**
Control (0.00)	300.50±5.06† (100)*	300	286(100)*(95)**	249(100)*(83)**
0.70	229.00±5.19†(76)*	300	253(88)*(84)**	198(80)*(66)**
1.10	188.33±4.65†(63)*	300	214(75)*(71)**	174(70)*(58)**
1.50	166.50±3.00†(55)*	300	179(63)*(60)**	153(61)*(51)**
1.90	148.50±4.86†(49)*	300	131(46)*(44)**	92(37)*(31)**

*Sufficient number of male and female insects was treated with four different doses of Neemazal T/S. Six males and six females were used for each treated dose and were paired to mate independently. Each such pair served as one replicate. The egg yield was monitored for five days. Figures in parentheses show percent change rounded off to nearest values.
†Significant (p<0.001) when student's t-test was applied between control and treated.
*Percent values calculated with their respective controls (100%).
**Percent values when compared to the number of eggs used (300 eggs) for observation of hatching/emergence.

Table 2: Effect of Nimbecidine on the fecundity, hatchability and emergence of *Earias vittella**

Treated dose (μg/insect)	**Mean egg yield (± = SE)**	**No. of eggs used (for hatching/emergence)**	**No. of eggs hatched**	**No. of adults emerged**
Control(0.00)	326.33±6.83† (100)*	300	290 (100)*(97)**	263(100)*(88)**
0.90	250.83±6.04†(77)*	300	208(72)*(69)**	182(69)*(61)**
1.05	225.17±5.17†(69)*	300	187(64)*(62)**	138(52)*(46)**
1.20	189.17±3.90†(58)*	300	132(46)*(44)**	96(37)*(32)**
1.35	174.33±5.06†(53)*	300	96(33)*(32)**	54(21)*(18)**

*Sufficient number of male and female insects was treated with four different doses of Nimbecidine. Six males and six females were used for each treated dose and were paired to mate independently. Each such pair served as one replicate. The egg yield was monitored for five days. Figures in parentheses show percent change rounded off to nearest values.
†Significant (p<0.001) when student's t-test was applied between control and treated.
*Percent values calculated with their respective controls (100%).
**Percent values when compared to the number of eggs used (300 eggs) for observation of hatching/emergence.

Table 2 illustrates the effect of Nimbecidine on fecundity, hatchability and emergence of *E. vittella*. There was a significant reduction in the number of eggs after 1.35 µg/insect treatment, which was reduced to 53% of the control (Table 2). Here, also fecundity was dose-dependent. During the second series of experiment the hatching was 69% of the 300 eggs and emergence was 61% at the dose of 0.90 µg/insect. At the highest treated dose (1.35 µg/insect), the hatchability was 32% while the emergence remained only 18% (Table 2). From tables 1 and 2 it is evident that the Nimbecidine is more effective than Neemazal T/S for reducing the fecundity, hatchability and survival of the *E. vittella*.

4. Discussion

The results of this study indicate that plant-based pesticides such as Nimbicidine and Neemazal may be effective alternative to conventional synthetic insecticides for the control of *E. vittella*. Both the formulations (containing Azadirachtin) were found toxic to the cotton pest, *E. vittella* and significantly reduced the fecundity, hatchability and emergence of adults. The so far, rather neglected effect of neem on the fecundity was first observed by Steets and Schmutterer [18] in the Mexican bean beetle, *Epilachna varivestis*. Steets [19, 20] reported similar results with 98% reduction in fecundity in *Leptinotarsa decemlineata* after treatment with neem products. According to Steets [19, 20] that extracts from the fruit, kernel and leaf of the neem tree cause growth disturbances and decrease in fecundity of *E. varivestis* and other insects. Speckbacher [21] found the sterilizing effect of neem components in beetles such as *Agelastica alni*. Meisner *et al.* [22] investigated the effect of neem seed kernel extract (NSKE) on the development of *Earias insulana* larvae.

Similarly, azadirachtin had an impact on oogenesis and embryogenesis of *Locusta migratoria* [23]. They attributed it to be due to the inhibition of ecdysteroid synthesis in the ovary. Abdul Kareem [24] reported that azadirachtin and NSKE interfered with the larval development of *Plutella xylostella*, and also affects the pupae. Reduction in adult emergence caused by 1-3% NSKE ranged from 73.3 to 93.3%. Azadirachtin at 20 and 30 ppm completely inhibited the adult emergence. This was in agreement with the findings of Ruscoe [25] who observed that larvae of *P. xylostella, Heliothis virescens* and *Pieris brassicae* fed azadirachtin treated leaves died without further moulting. Roomi and Atiquiddin [26] reported that larvae of *Ephestia kuchniella* fed on flour impregnated with NSKE failed to pupate. Similarly, *Callosobruchus chinensis* fed with NSKE treated red gram showed reduction in egg hatching and adult emergence; after treatment a mean of 9.2 adults emerged as against 537.7 in the control [24].

Jhansi Rani [27] found that the larval development of *Corcyra cephalonica* and *Tribolium castaneum* was completely inhibited when the insects were released in wheat flour containing 2 parts of deoiled kernel per 100 parts of flour. The petroleum ether extracted kernel oil was found to be antiovipositional at 2.5% or higher concentrations against *Dacus cucurbitae* and at 20% or higher concentrations against *D. dorsalis* [28]. The kernel suspension was ovicidal against *Schistocerca gregaria* at 0.125-1.0% concentrations [29]. Recently, bio-pesticides are given for use against several insect species especially disease-transmitted vectors, based on the fact that compounds of plant origin are safer in usage, without phytotoxic and ecotoxic properties [30, 31].

Gujar and Mehrotra [32, 33] and Mehrotra and Gujar [34] reported that topical application of 10 µg azadirachtin significantly reduced adult longevity, fecundity and reproduction in *Spodoptera litura.* The probable cause as described by these workers is due to azadirachtin's interference with the neuro-endocrine system of the insects. Similarly, impaired embryogenesis in females of *Dysdercus koenigii* with 1.0 µg/insect azadirachtin treatment was reported by Koul [35, 36]. Pathak and Krishna [37] also observed a significant reduction in reproductive potential in terms of egg yield and viability of *C. cephalonica* exposed to neem oil vapours. Dorn *et al.* [38] observed a strong dose-dependent impact of azadirachtin on nymphal and adult development of *Oncopeltus fasciatus*. Azadirachtin treatment of adults causes high mortality and reduced fecundity in females. Saxena *et al.* [39, 40] studied the effect of neem seed oil on the rice leaf folder, *Cnaphalocrocis medinalis* and observed reduction in the fecundity. The hatchability of eggs laid was significantly reduced (25-50%) and showed concentration dependent effect. Rembold *et al.* [41] reported a dose dependent action of azadirachtin in *E. kuchniella.* They observed that adult emergence was reduced up to 23.3% at the dose of 5.0 µg/larvae after topical application. Studies on the insect sterility by chemicals were reviewed by Smith *et al* [42] and described by Grover *et al.* [43] and Chaudhry and Tripathi [44]. They all reported histopathological effects on the gonadal and embryonic tissues of certain insects, which induced chromatin condensation, pycnotic nuclei, cytoplasm vacuolization and histolysis of the follicular epithelium. In male insects, the effects produced by the chemicals can be attributed to dominant lethality, aspermia, loss of sperm motility, or lack of ability in the adult to impregnate the female.

The visible and measurable damage to the male gonads due to sterilizing chemicals appeared to be the reduction in the size of the testes [43]; loss of sperm motility [45]; aspermia or degeneration or resorption leading to aspermia [43]. In females the neem based pesticide may partially or completely inhibit the development of ovary, ovariole, oocyte and germarium or even complete resorption of the ovaries [46, 47].

5. Conclusion

It is concluded from the present study that the two commercial neem based formulations (Nimbicidine and Neemazal) have the potential for use in the management of *Earias vittella*. They are highly toxic to the cotton pests and Nimbicidine proved more toxic than Neemazal. However, further studies on mode of action and synergism with pesticides under field conditions are needed.

6. References

1. Erdogan P, Yildirim A, Sever B. Investigations on the Effects of Five Different Plant Extracts on the Two-Spotted Mite *Tetranychus urticae* Koch (Arachnida: Tetranychidae). Psyche 2012, Article ID 125284, 5
2. Bindu P, Bharpoda TM, Patel JR, Patel JJ. Evaluation of various schedules based on botanical and synthetic insecticides in okra ecology. Indian J Ent 2003; 65:344-346.
3. Al-eryan MAS, Zaitoon AA, Rezk HA. The use of Coccinella 11-punctata (Coleoptera: Coccinellidae) against *Aphis gossypii* (Homoptera: Aphididae) on okra plant. Alexand J Agric Res 2001; 46:107-114.
4. Ahmed H, Ali S, Khan MA, Habib A. Efficacy of neem oil and synthetic growth regulators on control of okra insect pests as compared to chemical pesticides. J Sustan Agric Environ 1995; 5:232-245.

5. Hammad AEM, Zournajian H, Talhouk S. Efficacy of extracts of *Melia azedarach* L. callus, leaves and fruits against adults of the sweet potato whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae). J Appl Ent 2001; 125:483–488.
6. Banchio E, Valladares G, Defago M, Palacios S, Carpinella C. Effects of *Melia azedarach* (Meliaceae) fruit extracts on the leafminer *Liriomyza huidobrensis* (Diptera: Agromyzidae): assessment in laboratory and field experiments. Ann Appl Biol 2003; 143:187–193.
7. Wandscheer CB, Duque JE, da Silva MAN, Fukuyama Y, Wohlke JL, Adelmann J *et al*. Larvicidal action of ethanolic extracts from fruit endocarps of *Melia azedarach* and *Azadirachta indica* against the dengue mosquito *Aedes aegypti*. Toxicon 2004; 44:829–835.
8. Schmutterer H. Side effects of neem (*Azadirachta indica*) products on insect pathogens and natural enemies of spider mites and insects. J Appl Ent 1997; 121:121-128.
9. Carpinella MC, Defago MT, Valladares G, Palacios SM. Antifeedant and insecticide properties of a limonoid from *Melia azedarach* (Meliaceae) with potential use for pest management. Agric Food Chem 2003; 15(51):369–674.
10. Arora RK, Dhillon MK, Singh H. Management of pest complex in okra research summation. Ann Agric Biol Res 1996; 1:37-45.
11. Jacobson M. Focus on phytochemical insecticides. The neem tree. CRC Inc. Boca Raton, Florida, VSA 1988; 1:178.
12. Ansari BA. Effect of essential oils of Callistemon lanceolatus and Eupatorium capillifolium against the cotton pest, *Earias vittella* Fab. (Lepidoptera: Noctuidae). Farm Sci J 2004; 13(1):68-69.
13. Sharma DK, Ansari BA. Effect of a Synthetic Pyrethroid Deltamethrin and a Neem Based Pesticide Achook on the Reproductive Ability of Zebrafish, *Danio rerio* (Cyprinidae). Arch Polish Fish 2010; 18:157-161.
14. Ahmad MK, Ansari BA. Toxicity of Neem based pesticide Azacel to the embryo and fingerlings of Zebrafish, *Danio rerio* (Cyprinidae). World J Zool 2011; 6(1):47-51.
15. Jeyabalan D, Arul N, Thangamathi P. Studies on effects of *Pelargonium citrosa* leaf extracts on malarial vector, *Anopheles stephensi* Liston. Biores Tech 2003; 89(2):185–189.
16. Ansari MA, Razdan RK, Tandon M, Vasudevan P. Larvicidal and repellent actions of *Dalbergia sissoo* Roxb. (F. Leguminosae) oil against mosquitoes. Biores Tech 2000; 73:207–211.
17. Ansari MA, Vasudevan P, Tandon M, Razdan RK. Larvicidal and mosquito repellent action of peppermint (*Mentha piperita*) oil. Biores Tech 2000; 71:267–271.
18. Steets R, Schmutterer H. Der Einffub Von Azadirachtin auf die lebensdauer und das Reproduktionsvemogen von *Epilachana varivestis* Muls. (Coleoptera: coccinellidae) z. pfl krankh Pt. Schutz 1975; 82:176-179.
19. Steets R. Die wirkung von Rohextrakten aus den meliaceen *Azadirachta indica* und *Melia azedarach* auf versehiedene Insektenarten. Z angew Ent 1976; 77:306-312.
20. Steets R. Zur Wirkung von Inhaltsstoffen aus Meliaceen und Anacardiaceen auf colepteren und Lepidoteren-Ph.D. thesis Univ. of Giessen, W. Germany, 1976.
21. Speckbacher U. Zur Wirkung Von Inhaltsstoffen aus *Azadirachta indica* A. Juss (Meliaceae). Auf einige Insektenarten. Dipl Thesis Univ of Munich, 1977.
22. Meisner J, Kehat M, Zur M, Eizick C. Respose of *Earias insulana* Boisd. Larvae to neem (*Azadirachta indica* A. Juss) kernel extract. Phytoparasiticsa 1978; 6:85-88.
23. Rembold H, Sieber KP. Inhibition of oogenesis and ovarian ecdysteroid synthesis by *azadirachtin* in *locusta migratoria* migratorioides (R&F). Z Naturforsch 1981; 36:466-469.
24. Abdul Kareem A. Neem as an Antifeedant for certain phytophagous insects and a Bruchid on pulses. Proc. Ist Int. Neem Conf. (Rattach-Egern), 1980, 223-250.
25. Ruscoe CNE. Growth disruption effects of an insect antifeedant. Nature New Biol 1972; 236:159-160.
26. Roomi MW, Atiquiddin A. Observations on the repellency of the 'Neem' plant, *Melia azadirachta* L. on some stored-grain pests from Pakistan. Z angew Ent 1977; 84:124-129.
27. Jhansi Rani B. Studies on the biological efficacy of deoiled neem (*Azadirachta indica* A. Juss) Kernel against a few insects. M.Sc. thesis, Post Graduate School, IARI, New Delhi, 1984.
28. Singh RP, Srivastva BG. Alcohol extract of Neem (*Azadirachta indica* A. Juss) seed oil as oviposition deterrent for *Dacus cucurbitae* (coq). Indian J Ent 1983; 45:497-498.
29. Bhanotar RK, Srivastava YN. Effect of Neem Kernel suspension on the development of eggs of desert locust, *Schistocerca gregaria* Forsk. Neem Newsl 1984; 1(3):30.
30. Schmutterer H. Properties and potential of natural pesticides from the neem tree, *Azadirachta indica*. Ann Rev Ent 1990; 35:271–297.
31. Senthil Nathan S, Chung PG, Murugan K. Effect of botanicals and bacterial toxin on the gut enzyme of *Cnaphalocrocis medinalis*. Phytoparasitica 2004; 32:433–443.
32. Gujar GT, Mehrotra KN. Inhibition of growth and development of the tobacco caterpillar, *Spodoptera litura* fabr. Due to Azadirachtin and other neem products. Indian J Ent 1983; 45:431-435.
33. Gujar GT, Mehrotra KN. Juvenilizing effect of Azadirachtin on a noctuid moth, *Spodoptera litura* Fabr. Indian J Exp Biol 1983; 21:292-293.
34. Mehrotra KN, Gujar GT. Neem as insect growth inhibitor. Natn. Seminar on "Neem in Agriculture", IARI, April 1983. Neem Newsl 1984; 1:6.
35. Koul O. *Azadirachtin* I. Interaction with the development of red cotton bugs. Ent mol Exp Appl 1984; 36:85-88.
36. Koul O. *Azadirachtin* II. Interaction with the reproductive behaviour of red cotton bugs. Z angew Ent 1984; 98:221-223.
37. Pathak PH, Krishna SS. Neem seed oil, a capable ingredient to check rice moth reproduction (Lepidoptera, Galleriidae). Z angew Ent 1985; 100:33-35.
38. Engelmann F. The physiology of reproduction. Pergamon Press, New York, 1970, 307.
39. Dorn A, Rademacher JM, Sehn E. Effects of azadirachtin of the moulting cycle, endocrine system, and ovaries in last instar larvae of the milweed bug, *Oncopeltus fasciatus*. J Insect Physiol 1986; 32:31-238.
40. Saxena RC, Waldbauer GP, Liquido NJ, Puma BC. Effects of Neem seed oil on the rice leaf folder, *Cnaphalocrocis medinalis*. Proc. 1st Int. Neem conf. Rottach-Egern, 1980, 189-204.
41. Rembold H, Sharma GK, Ezoppett Ch. Growth-regulating activity of Azadirachtin in two holometabolous insects. Proc. 1st Int. Neem Conf. Rottach-Egern 1980, 121-128.

42. Smith CN, LaBrecque GC, Borkovec AB. Insect chemosterilants. A Rev Ent 1964; 9: 269-284.
43. Grover KK, Pillai MKK, Dass CMS. Cytogenetic basis of chemically induced sterility in *Culex pipiens fatigans* Wiedemann, II. Cytopathological effects of certain chemasterilants on the gonodal and embryonic tissues. J Med Ent 1972; 9:451-460.
44. Chaudhry HS, Tripathi CPM. Histopathological effect of thiourea on the ovarian tissue of *Sarcophaga ruficornis* Fabr. Experientia 1976; 32:103-104.
45. LaChance LE, Leverich, AP. Chemosterilant studies on Bracon (Hymenoptera: Braconidae) sperm, I. Sperm inactivation and dominant lethal mutations. Ann Ent Soe Amer 1968; 61:164-175.
46. Smittle BJ, Schmitt JB, Burden GS. Effects of tepa on the reproductive organs and embryogeny of the German cockroach. J Econ Ent 1966; 59:1419-1423.
47. Sukumar K, Naidu MB. Inhibition of ovarian growth by tepa in *Dysdercus cingulatus*. J Econ Ent 1973; 66:20-22.