GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

www.scienceandnature.org

TOXIC IMPACT OF SUBLETHAL CONCENTRATIONS OF ACEPHATE (AN ORGANOPHOSPHATE) ON MORTALITY, LONGEVITY, FECUNDITY, FERTILITY, AND REPRODUCTIVE SYSTEM OF *DYSDERCUS CINGULATUS* (HEMIPTERA: PYRRHOCORIDAE)

Khowaja Jamal
Insect Physiology Laboratory, Section of Entomology, Department of Zoology, AMU, Aligarh-202 002, U.P. (INDIA)

ABSTRACT
The toxic effect of topically applied sublethal concentrations (0.0004, 0.0006, 0.0008, 0.001, and 0.002%), of acephate on 5th instar nymphs of *Dysdercus cingulatus* Fabr., was studied in laboratory under controlled conditions. The nymphal loss, survival duration, incomplete moulting as well as number of malformed adults were dose dependent and increased linearly, showing positive correlation with increasing concentrations. However, the fecundity and fertility of the adults emerged from the treated nymphs declined linearly and showed negative correlation with increasing concentrations. The ovaries of one day old affected females had no changes in their anatomical as well as histological structures; however, the ovaries of 5, 10, or even 20 days old females that emerged from 0.002 and 0.001% acephate treated nymphs showed some anomalies. Anatomically, the size of the ovaries was reduced significantly and the number of mature oocytes in the vitellarium decreased noticeably, however, in some more severely affected ovaries, the oocyte number became as low as 2-3 per ovariole as compared to normal females. The size of the egg chamber was also significantly reduced, whereas, the size of the lateral oviduct increased slightly. Histologically, the cellular organization of the germarium of some of the severely affected ovarioles lost its originality and the trophocytes of the anterior region of these germarium showed clumping of chromatin in their nuclei. The development and the maturation of the oocytes in the vitellarium were also abnormal and in few oocytes, the ooplasm was not homogeneously granular and the deposition of yolk was very thin. Some oocytes in the vitellarium showed different stages of degeneration and resorption.

KEY WORDS: *Dysdercus cingulatus*, Acephate, Mortality, Longevity, Fecundity, Fertility, Reproductive system.

INTRODUCTION

The sharp growth in agricultural production in India in the last three decades has taken place due to increasing use of pesticides. The two developments that portended possible setback to the widespread use of chemical insecticides were the development of insecticidal resistance and anxiety about environmental pollution hazards. There has been no acceptable replacement of these pesticides due to cost-benefit factors, even though they are poisonous and damage the environment if used indiscriminately and improperly. With proper monitoring of the resistance status, timely switchover to alternate insecticide as well as the use of low quantity of selective, more easily degradable insecticides in a harmonious manner, the future of insecticides still seems to be highly promising as one of the most important tools of insect pest management. According to Mahmoud *et al*., 1997 acephate is one of the ten most important organophosphorus (OP) insecticides in sales volume and is considered to be the safest of the plant systemics with an acute oral LD50 of about 360 and 900 mg/kg for mice and rats, respectively. The action of acephate on insects and its selective toxicity are attributed to more rapid bioactivation on conversion to methamidophos in insects than in mammals, offering a measure of safety.Therefore, the present investigations were undertaken with the view to assess the toxicity of organophosphorous insecticide (acephate) at sublethal dosages against the cotton bug, *Dysdercus cingulatus* under laboratory conditions, to use it alternatively with other insecticides.

MATERIALS & METHODS

A stock culture of *Dysdercus cingulatus* was maintained at 28 ± 2 ºC, 70 - 80% R.H. and 12 hrs D.L. in laboratory. Newly moulted 5th instar nymphs were isolated from the stock culture and were reared for experimental purpose. From this stock, a set of 100 newly moulted one-day-old 5th instar nymphs was treated individually with one microlitre solvent (acetone) alone to serve as control-I. Another set of one hundred untreated nymphs of the corresponding age and stage was maintained at the same controlled conditions for comparison with solvent (acetone) treated nymphs to serve as control-II. Different sets of hundred nymphs were treated individually with different concentrations of acephate to assess the Lc-50 value. Mortality of test insects was recorded 24 hrs after treatment and the data on mortality was subjected to probit analysis (Finney 1985). On the basis of this Lc-50 value, four lower concentrations *i.e.* 0.001, 0.0008, 0.0006 and 0.0004 were selected for further study. Acetone

(Analytical grade) was used as solvent. Daily mortality of the treated insects as well as the emergence of the adults from the survived nymphs was recorded to observe longevity. The females emerging from the nymphs treated with each concentration of acephate and acetone (control) were paired with males of the corresponding age obtained from untreated stock for the observations on egg production and egg viability. The response variables were subjected to one-way analysis of variance (Snedecor & Cochran, 1967; Sokal & Rohlf, 1981) to determine significance differences (P<0.001) among the treatments. The females emerged from the survived treated nymphs were dissected out in physiological saline simultaneously at the intervals of 1, 5, 10 and 20 days following emergence, to study the changes in anatomical and histological structure of the gonads. For anatomical study, the dissected material was prepared according to Pantin (1959). For histological observations, the ovaries were fixed in Duboseq-Brasil for one hour and then washed in 90% ethanol, dehydrated, cleared in methyl benzoate and benzene and finally embedded in paraffin wax (56-58 °C melting point, Merck). Serial sections of 5-8 micron thick were cut on rotary microtome. These sections were stained with Heiden Hains Iron Haematoxylin and Eosin. The staining was achieved by YSI-106 Yorko automatic staining machine having 12 stations using Culling (1974) method.

RESULTS & DISCUSSION

The mortality of the test insects was found to increase with an increase in concentrations. The application of the serially diluted sub-lethal concentrations *i.e.* 0.002, 0.001, 0.0008, 0.0006 and 0.0004% acephate on 5th instar nymphs (24 hrs old) caused respectively 39, 26, 20, 12 and 02% total nymphal mortality up to adult emergence. The regression between concentration strength and total nymphal mortality up to adult emergence showed positive correlation (Y= 1 + 20000 X, r = 0.9538, P< 0.001; Fig.1).

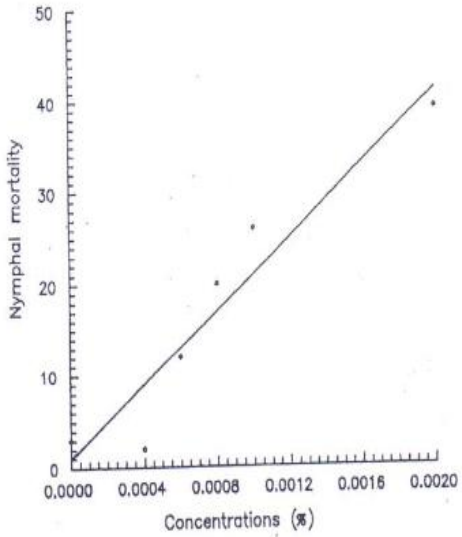

FIGURE 1: Nymphal mortality of *Dysdercus cingulatus* following application of sublethal concentrations of acephate

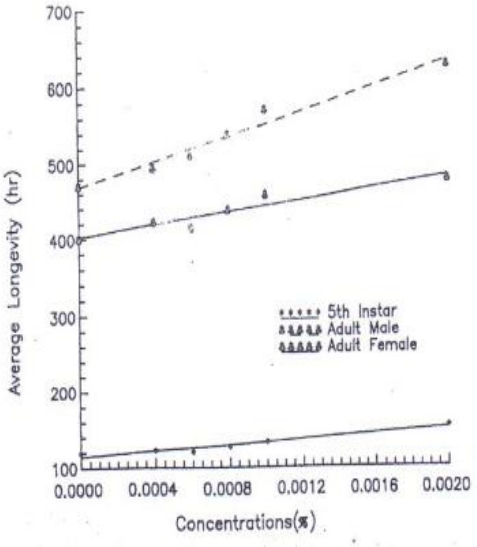

FIGURE 2: Nymphal and adult longevity of *Dysdercus cingulatus* following application of sublethal concentrations of acephate

The total nymphal death within the same instar following the topical application of 0.002, 0.001 and 0.0008% was 27, 18, and 15% more as compared to control (*i.e.* 3%). The lower concentrations (*viz*., 0.0006 and 0.0004%) resulted in insignificant mortality. According to Khowaja *et al.* (1995) the exposure of 10, 08, and 06-ppm temik to the 4th instar nymphs of *Dysdercus cingulatus* caused 52, 43 and 29% total nymphal loss up to adult emergence. The toxicity and economics of ten insecticides against linseed budfly (*Dasyneura lini* Barnes) was assessed by Malik *et al.* (1996) and on the basis of bud infestation, yield and cost benefit ratio, decamethrin 2.8 EC was recorded superior followed by chlorpyrifos 20 EC, phosphamidon 85 SL, endosulfan 35 EC, monocrotophos 40 EC. In field, the treatment of 0.5% chlorpyrifos gave maximum reduction in larval population of *Heliothis armigera* on treated crop followed by fenvalerate, endosulfan and diflubenzuron. The least effective insecticide was azadirachtin (Ravi and Verma, 1997). Fakhri *et al*. (2011) studied the comparative toxicity of conventional and nonconventional insecticides against 4th instar nymphs of *Dysdercus koenigii* and reported that in term of mortality the conventional insecticide (imidacloprid) proved more toxic than the non-conventional insecticide multineem. Likewise the topical application of conventional insecticide *i.e.* monocrotophos on 4th instar nymphs of *D. koenigii* showed higher mortality than nonconventional neemjeevan. Fakhri *et al*. (2011). However the toxicity of two conventional insecticides *i.e.* quinalphos and oxydemeton-o-methyl against 4th nymphal instar of *Dysdercus koenigii* remain almost same Fakhri et al (2012). The longevity of 5th instar nymphs that survived the treatment increased linearly (Y = 114.83 + 17896.55 X, r = 0.9614, P < 0.001) with increasing concentrations of acephate (Fig.2). Likewise, the average life span of adult males and females that emerged from the 5th instar treated nymphs also increased linearly (Y = 469.20 + 81500.00 X, r = 0.9826 and Y = 402.12 + 39706.89 X, r = 0.9441, P < 0.001 respectively), and exhibited positive correlation (Fig.2). The average nymphal duration of 5th instar treated

nymphs following the topical application of 0.002 and 0.001% of acephate was enhanced by 33.3 and 13.5 hrs respectively as compared to control. Similarly, the average survival of adult males and adult females that emerged from the treated 5th instar nymphs also increased by 155.3, 98.9 and 76.1, 56.1 hrs with respect to 0.002 and 0.001% acephate as compared to control (Fig.4).

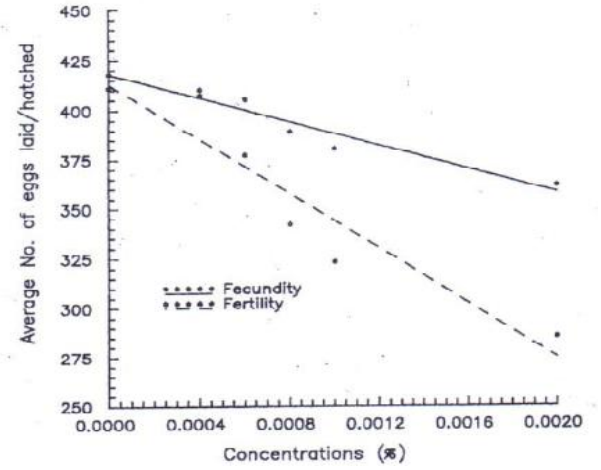

FIGURE 3: Fecundity and fertility of *Dysdercus cingulatus* following application of sublethal concentrations of acephate

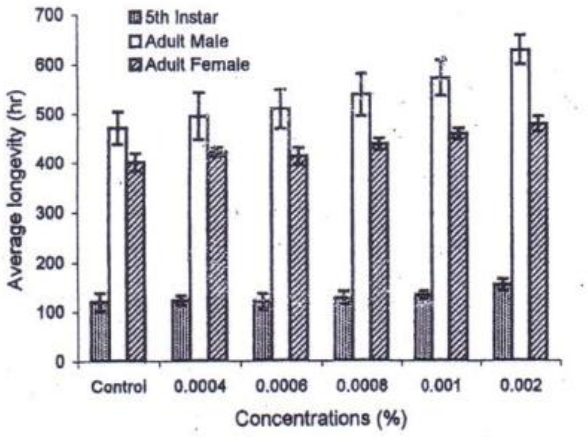

FIGURE 4: Nymphal and adult longivity of *Dysdercus cingulatus* following application of sublethal concentrations of acephate

The nymphal-adult moulting of the treated insects was also affected adversely and 9 out of 20 survived nymphs could not cast off their exuvae and succumbed to incomplete moulting. According to Khowaja *et al.,* 1992, the topical application of 0.003% monocrotophos on 4th instar nymphs of *Dysdercus cingulatus* caused an increase of 41.86 and 50.9% in longevity of 4th as well as 5th instar nymphs respectively compared to control. The longevity of male *Blatella germanica* following the exposure of L_D-50 dose of cyfluthrin and hydramethylnon reduced by 52 and 81% respectively compared to control (Abd-Elghafar & Appel, 1992). The larval duration of 6th instar *Spodoptera litura* when exposed to 1000-ppm cythion (malathion) was enhanced by 72.02% as compared to control (Khowaja *et al*., 1993). Likewise, the nymphal duration of 5th instar nymphs of *D. cingulatus* following the topical application of 30 ppm monocrotophos enhanced by 43.1%, whereas, the longevity of the adult males and females emerged from the treated 5th instar nymphs increased by 31.51 and 26.5% respectively as compared to control (Khowaja *et al*., 1994). The topical application of 80 ppm cythion on 4th instar nymphs of *D. koenigii* increased 4th instar nymphal longevity by 64.8 hr (*i.e.* 90%), whereas, the longevity of 5th instar nymphs, adult males and females which emerged from the 4th instar treated nymphs was enhanced by 43.2 hr (*i.e.* 34%), 189.7 hr (*i.e.* 40.8%) and 177.6 hr (*i.e.* 42.5%) respectively as compared to control (Khowaja *et al*., 2001). The longevity of the nymphs which survived by the treatment of both quinalphos and oxydemeton-o-methyl insecticides increased significantly as compared to control Fakhri *et al.* (2012). On the other hand the average egg production of the females that emerged from the survived treated nymphs showed negative linear correlation with increasing concentration ($Y = 418.04 - 30258.62\ X$, $r = -0.9689$, $P < 0.001$, Fig. 3). Following the topical application of 0.002 and 0.001% acephate/5th instar nymphs, the fall in fecundity of the affected females was 13.64 and 9.09% respectively as compared to control (Fig. 5). The results of Chattoraj and Bhise (1980) also indicated that fecundity of adult *Spodoptera litura*, emerged from the treated larvae exposed to sublethal dose of 3µg dieldrin, was reduced significantly as compared to control. In laboratory test, the exposure of sublethal concentrations (Lc-30 and Lc-50, 0.0088, 0.0182% respectively) of parathion (ethyl parathion) to the larvae of *Spodoptera litura* resulted in reduction in oviposition and egg hatching rate of the adults (Patil and Khanvilkar, 1977). Likewise in the present investigation, the hatching of the eggs laid by the affected females also decreased linearly with increasing concentrations of acephate ($Y = 412.81 - 69137\ X$, $r = -0.9465$, $P < 0.001$, Fig. 3) except in case of 0.0004% acephate. The viability of the eggs, following the application of 0.002 and 0.001% acephate, was slipped by 19.38 and 13.33% respectively as compared to control (Fig. 5). However, the topical application of the lowest selected concentration (0.0004%) of acephate slightly enhanced the hatchability by 0.94% as compared to control (98.33%). The results of Khowaja *et al.,* 1992 indicate that the topical application of sublethal concentration of monocrotophos (0.003%) on 4th instar nymphs of *D. cingulatus* resulted in 21.86 and 29.89% reduction in fecundity and fertility of the surviving females. Similarly, the topical application of 30 ppm monocrotophos on 5th instar nymphs of *D. cingulatus* caused 18.1 and 22.4% reduction in the total egg production and hatching of the eggs (Khowaja *et al*., 1994). The topical application of cythion (malathion) on 6th instar larvae of *Spodoptera litura* and on 4th instar nymphs of *D. koenigii* (Khowaja *et al*., 1993 & 2001), quinalphos and oxydemeton-o-methyl on 4th instar nymphs of *D. koenigii* (Fakhri *et al.,* 2012) caused significant reduction in fecundity and fertility of the adults emerged from the survived treated nymphs. Among the emerged adults, 14.3% had malformed wings and legs. The ingestion of sub-lethal doses of arsenates by 4 species of

adult diptera *viz*., *Rhagoletis pomonella* (Walsh), *Drosophila melanogaster* Meigen, *D. hydei* Sturtevant, and *Musca domestica* was found to suppress egg production through a suppressing effect on ovarian development (Pickett and Patterson, 1963). The ovaries of one-day-old affected females had no changes in their anatomical and histological structures when compared with the ovaries of the control females of the corresponding age. However, the ovaries of the 5-day-old females that survived the treatment of 0.002 and 0.001% acephate developed some abnormalities. In both the cases, the development of the ovarioles was inhibited significantly and the size of the ovarioles was reduced drastically. Furthermore, the number of mature oocytes in each ovariole was generally reduced remarkably and even in some severely affected ovaries, the ovarioles had only 2-3 mature oocytes. In some other ovarioles degeneration was initiated in the oocytes and there was a darkly stained cellular mass at the lower end of the ovariole. The study of Siddappaji *et al.* (1979) revealed that methamidophos, quinolphos and trichlorfon at 0.025% concentration possess excellent ovicidal property and showed 100% inhibition. The treatment of *Musca domestica* with tepa, thiotepa and apholate caused condensation and pycnosis of nuclei, vacuolization of cytoplasm and general atrophy of follicular epithelium of the ovaries (Morgan and LaBreeque, 1964, Landa and Rezabova, 1965, Combiesco *et al.*, 1967). Similarly in the present investigation the cellular structure of the ovarioles of those females that survived the treatment of 0.002 and 0.001% acephate showed some anomalies. The cellular organization of the apical portion (germarium) of ovariole lost its originality and the trophocytes of the anterior region of the germarium showed clumping of chromatin in their nuclei. In some ovarioles the trophic core as well as the nutritive cords were not intact and became fragile. The density of the pre-follicular cells was also very thin and even some oocytes were retained in the germarium. Most of the space of the germarium in the posterior region was occupied by such oocytes and they pushed the trophocytes along with the clumped chromatin towards the anterior part of the germarium. Hsieh and Pienkowski (1973) also noticed similar type of changes where the upper zone of the germarium of *Trogoderma granarium* was mostly damaged by metepa and hempa and the trophocytes were proliferated. The development and maturation of the oocytes in the vitellarium was not uniform because the ooplasm of some oocytes was not homogeneously granular and even the deposition of the yolk was very less. Some young oocytes present in the germarium as well as vitellarium had few small size vacuoles, whereas, in some severely affected ovaries, the oocytes in the vitellarium showed different stages of degeneration and resorption. Matolin *et al.* (1978) also observed that the topical application of metepa to adult colorado beetle *Leptinotarsa decemlineata* interfered with the formation of oocytes, damaging their structure and caused proliferation of follicular epithelium. The histological structure of the ovaries of *Mylabris pustulata* when treated with SAN-322 and DDVP showed vacuolation within yolk, distorted shape of oocyte nucleus and necrosis of follicular epithelium (Mulmule *et al.*, 1988). The ovaries of the 10 days old females that emerged following topical application of 0.002 and 0.001% showed almost similar damage, however, the degree of damage was less as compared to 5 days old females. After 20 days the ovaries of the emerged females from the nymphs following the application of the aforesaid concentrations did not show much difference in the anatomical and histological structures as compared to those of 10 days old affected females. However, the topical application of the lower concentrations of acephate on the nymphs did not noticeably affect the anatomical and histological structure of the ovaries of 1,5,10 and 20 days old affected females.

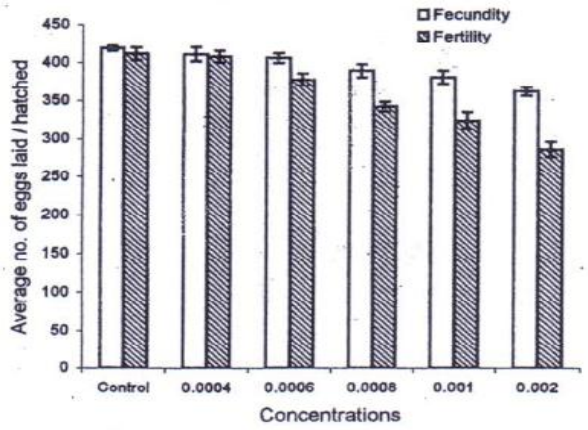

FIGURE 5: Fecundity and fertility of *Dysdercus cingulatus* following application of sublethal concentrations of acephate

REFERENCES

Abd-Elghafar, S.F. and Appel, A.G. (1992) Sublethal effects of insecticides on adult longevity and fecundity of German cockroachs (Dictyoptera: Blattelidae). *J. Econ. Entomol*., **85(5)**: 1809-1819.

Chattoraj, A.N. and Bhise, A. (1980) Latent effect of DDT and dieldrin on fecundity of *Spodoptera litura*

following sublethal larval treatment. *Zeitschrift fur Angew and te zoologie*, **67(2)**: 239-247.

Combiesco, I., Enesco, A. & Ticu, V. **(**1967) Etude de l'action des chemosterilasantes thiotepa et apholate sur le development des ovaries del'espece *Musca domestica* L. Note. II. Archs. roum. path. exp. Microbiol., **26**: 215-228.

Culling, C.F.A. (1974) In: *Hand book of histopathological and Histochemical techniques,* 3rd edn., pp 214.

Finney, D.J. (1985) The median lethal dose and its estimation. *Archives of Toxicology*., **56:** 215-218.

Fakhri, M.S.A., Ansari, M.S., Khowaja, J. and Ali, H. (2012) Comparative toxicity of quinolphos and oxydemeton-o-methyl on different life stages of *Dysdercus koenigii* (Fabr.) *Ann. Pl. Protec. Sci.*, **20(1)**:51-54.

Fakhri, M.S.A.. Ansari, M.S.. Khowaja, J. and Ali, H. (2011) Comparative assessment of conventional and non-conventional insecticides on the biology of *Dysdercus koenigii* Fabr. *J .ent. Res*., **35(3)**: 209-214.

Fakhri, M.S.A., Ansari, M.S., Khowaja, J. and Ali, H. (2011) Comparative efficacy of neemjeevan and monocrotophos against different life stages of *Dysdercus koenigii* Fabr. *Annl. Pl. Protec. Sci.*, **19(1)**:97-100.

Hsieh, Feng-kuo and Pienkowski, R.L. (1973) Alfalfa weevil: Influence of apholate on reproduction in the laboratory. *J. Econ. Entomol*., **66:** 1023-1028.

Khowaja, J. and Ayesha, Q. (2005) Lethality of cythion (malathion) on the 5th instar nymphs of *Dysdercus koenigii* (Hemiptera:Pyrrhocoridae). *J. Exp. Zool. India.* Vol.8 (2), 285-294.

Khowaja, J., Khan, M. A., Qamar, A. and Khan, B.A. (1992) Changes in fecundity, fertility, longevity and weight of *Dysdercus cingulatus* (Hemiptera: Pyrrhocoridae) following topical application of monocrotophos. *Proc. 79th Ind. Sc. Cong*. Part III: 76-77.

Khowaja, J., Khan, M. A., Qamar, A. and Khan, B. A. (1993) Cythion (an organophosphate) as inhibitor of growth and reproduction of *Spodoptera litura* Fabr. (Lepidoptera: Noctuidae). *Bioscience Research Bulletin*, **9(1-2)**: 27-32.

Khowaja, J., Khan, M.A., Qamar, A. and Khan B.A. (1994) Biological effects of sublethal concentrations of monocrotophos on *Dysdercus cingulatus* Fabr. (Hemiptera: Pyrrhocoridae). *J. ent. Res*., **18(1)**: 37-44.

Khowaja, J., Khan, M. A., Qamar, A. and Khan, B. A. **(**1995) Toxicity of temik (aldicarb) to the red cotton bug *Dysdercus cingulatus* (Hemiptera: Pyrrhocoridae). *Ad. Bios*., **14(II)** : 49-56.

Khowaja, J., Qamar, A., Murad, H. and Murad, N. (2001) Inhibitory effect of cythion (malathion) on the biology of red cotton bug, *Dysdercus koenigii* F. (Hemiptera: Pyrrhocoridae). *J. ent. Res*., **25(2)**: 113-120.

Landa, V. and Rezabova, B. (1965) The effect of chemosterilants on the development of reproductive organs in insects. *12th Int. Congr. Ent*., 516-517.

Malik, Y.P., Singh, B., Pandey, N.D. and Singh, S.V. (1996) Bioefficacy of insecticides against linseed budfly. *Indian J. Ent.,* **58(2)**: 115-120.

Matolin, S., Soldan, T. and Landa, V. (1978) Effects of chemosterilants on the reproductive organs of *Leptinotarsa decemlineata. Acta entomologica bohemoslovaca*, **75**: 102-108.

Morgan, P. B. and LaBrecque, G.C. (1964) Effect of tepa and metepa on ovarian development of houses flies. *J.Econ.Entomol.*, **57**: 896-899.,

Mahmoud, M., Quistad, G.B. and Casida, J.E. (1997) Acephate Insecticide Toxicity: Safety Conferred by Inhibition of the Bioactivating Carboxyamidase by the Metabolite Methamidophos. *Chem. Res. Toxicol.*, **10**, 64-69

Mulmule, S., Thakur, V.K. and Kaur, J. (1988) Histopathological effects of some insecticides on the ovaries of meloid beetle, *Mylabris pustulata*.*Entomon*, **13(1)**: 61-68.

Pantin, C.F.A. (1959) In *Notes on microscopical technique for zoologists*., Cambridge University Press, pp 77.

Patil, A.K. and Khanvilkar, V.G. (1977) Effect of sublethal concentrations of parathion on the biology of *Spodoptera litura* (Fab.). *Ind. J. Plant Protec*.(1977, Publ.1979), **5(2)**: 172-177.

Pickett, A.D. and Patterson, N.A. (1963) Arsenates effects on fecundity in some Diptera. *Science*, 196(3566): 493-494.

Ravi, G. and Verma, S. (1997) Persistence and dessipation of insecticides against *Heliothis armigera* on chickpea. *Indian J. Ent.,* **59(1)**: 62-68.

Siddappaji, C., Prabhu, H. S. and Desai, G. S. **(**1979) Ovicidal effect of some insecticides on the eggs of citrus butterfly. *Mysor J. Agric. Sci.,* **11(4)**: 554-558

Sokal, R.R. and Rohlf, F.J. 1981) In *Biometry*. W.H. Freeman & Co., New York**.**

Snedecor, G.W. and Cochran, W.G. (1967) In *Statistical Methods*. sixth edn. Iowa State University Press, Ames., Iowa.