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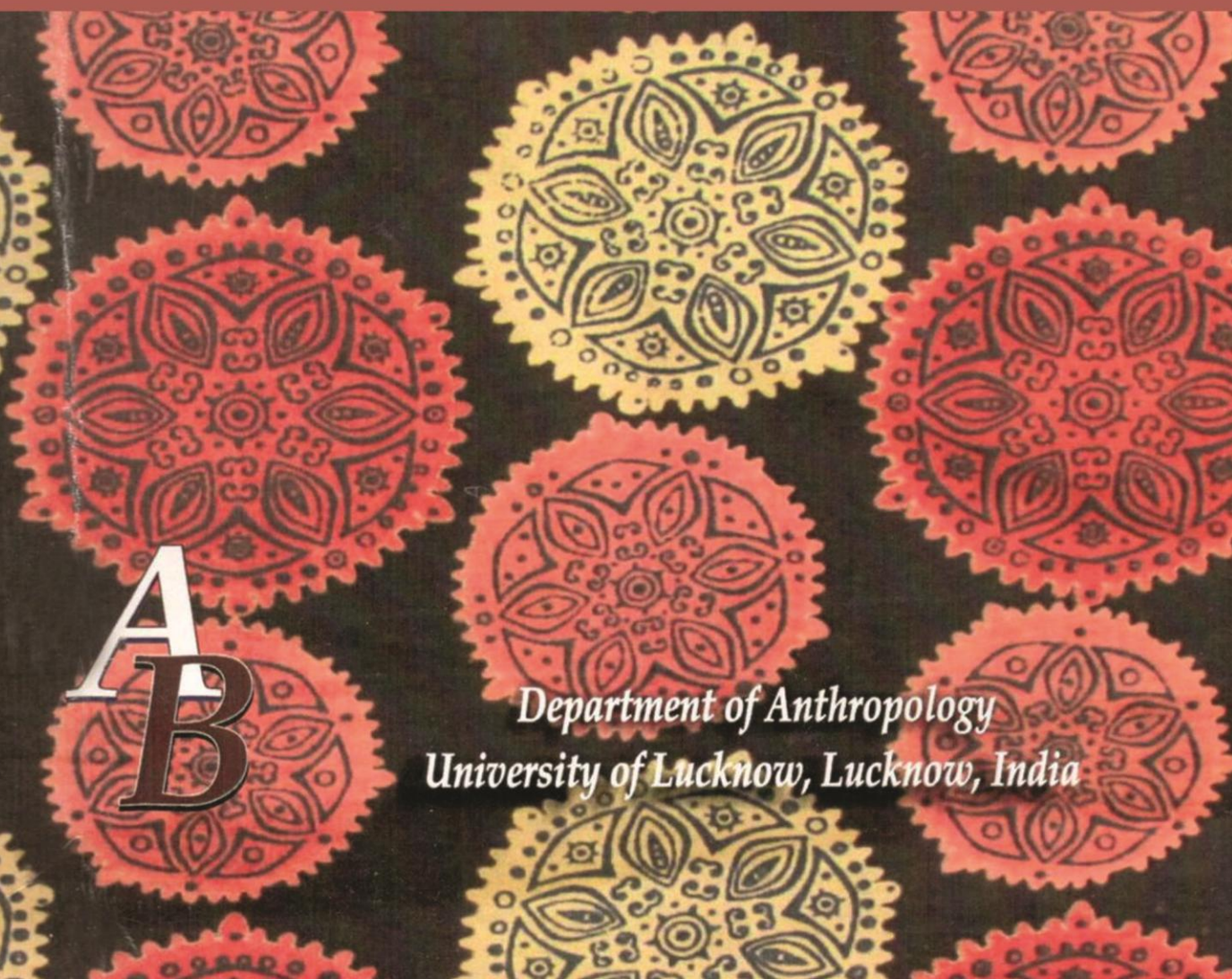

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Insecticidal activity of Acephate for the management of cotton pests *Dysdercus cingulatus* under laboratory condition.

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ABSTARCT

Cotton plays a very important role in the agricultural and industrial economy of India where around 40 million to 50 million people are employed in cotton processing and trade. The red cotton stainer, Dysdercus cingulatus, is a serious polyphagous pest, causes heavy loss to cotton crops which badly affects the economy of poor farmers. The present paper describesthe toxic effect of sublethal concentrations of acephate (an organophosphate insecticide) on individual 4th instar nymphs of Dysdercus cingulatus. The toxicity of topically applied 0.0004, 0.0006, 0.0008, 0.001 and 0.002% acephate resulted in concentration-based effects. The total nymphal mortality as well as nymphal and adult longevity were directly proportional to the concentrations, increase linearly and showed positive correlation. On the contrary, the fecundity and fertility of the eggs laid by the affected females were inversely proportional to the concentrations and showed negative correlation. The structural architecture and cellular organization of the ovaries of one day old affected females remain unchanged, however, the size of the ovaries of 5 day and 10-day old females that emerged from 0.001 and 0.002% acephate treated nymphs reduced significantly. Furthermore, the number of oocytes in the vitellarium dropped to 4-7 as compared to 8-10 in normal females. The cellular organisation of the germarium of the severely affected ovarioles lost its characteristics and some oocytes in the vitellarium showed degeneration and resorption.

Key words: *Dysdercus cingulatus*, acephate, mortality, fecundity, fertility, longevity, anatomy, histology, management of cotton pests.

INTRODUCTION

To fight hunger throughout the world, the food production has always been a challenge facing mankind. A main keystone in this challenge is the competition from insects which on an average destroy 20-30% crop production annually and even at some instances they provoke a total loss. One of the most important global problems is protecting the crop from insects because they are the most successful group of animals and can be found in any ecosystem. To control these pests' large number of plant protection products which mainly composed of xenobiotics comprises approximately 45% herbicides, 25% insecticides and 25% fungicides (UIPP 2011). Despite many methods available, application of insecticides remains the basic management tactics to suppress the pest population in almost all cropping systems

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around the world (Yang et al., 2005) that have saved millions of humans since the date of their synthesis and use. They have played an important role that brought revolution in the field of agriculture on control of insect pests of crops. Among insecticides, neurotoxics are the main active substance which educe various effects in the organisms through their interaction with several molecular targets that can cause lethal and adverse sublethal effects (Sattelle and Yamamoto 1988, Soderland and Bloomquist 1989). This can be embodied by the neurotoxic organophosphates, carbamates, pyrethroids, neonicotinoid and phenylpyrazole compounds which can trigger not only more or less severe neural effects but also reprotoxicity through mechanisms independent of their neural action (Yousef 2010; Zhang et al., 2010a, b; Joshi et al., 2011; Zhao et al., 2011). Thus, although all effects could be regarded as molecular, the molecular interaction between toxics and targets may result in "macroscopic" impacts that can be observed at cell, tissue, organ, system, individual and population levels, depending on their noxious action. Because of their broad spectrum, more specificity and high efficiency, the organophosphate (OPs) compounds are one of the most widely used insecticides. Among organophosphates, acephate and methamidophos are two most common and efficient insecticides which are used for pest control in agriculture (Maia et al., 2011; Kumar et al., 2015; Pan et al., 2015). Acephate [O,S-dimethyl-acetyl-phosphoramido-thioate] is an important systemic insecticide that has been widely used to control insect pests in agricultural fields for decades (Mahajan et al., 1997) because of its low mammalian toxicity. It is a class II "moderately hazardous" insecticide, however, methamidophos is classified as class IV "highly toxic" insecticide (World Health Organization [WHO], 2009). Despite their importance, insecticides also have negative impact like toxic residues in food, long term persistence in environment, resurgence, development of resistance as well as effect on non-target organisms. So, scientist in industry and academia are continuously trying to produce newer and newer compounds to manage the pest population. Hence, it was considered desirable to evaluate the sublethal effects of various concentrations of acephate upon several important biological traits of *Dysdercus cingulatus* which also known as the red cotton bug. It is called by various local names in different regions of India like Chainpa in Punjab, Kappa poka in Orissa, Lal chingum in Uttar Pradesh and Lal chusiya in Gujarat (Verma and Patel, 2012). It is important polyphagous heteropteran cosmopolitan pest of malvaceae and bombaceae. It is a well-known destructive pest of cotton in India and many parts of the world (Karihaloo and Kumar, 2009) which badly affects the economy of poor farmers. The main damage is done due the penetration of mouth parts into the developing cotton bolls often followed by the transmission of fungi *Nematospora gossypii* (Frazer, 1944), resulting to pods enviable beside staining the lint to the typical yellow colour thereby reducing the commercial value. Both of its stage (nymphal and adults) feed on developing and mature seeds. Few years ago, it was placed under minor pest category but recently it has shifted to major pest category due to its increasing population.

MATERIALS AND METHODS

A stock culture of *Dysdercus cingulatus* was maintained in sterilized glass jars at 29 ± 2°C temperature, 70-80% relative humidity and 12:12 hours light and dark (L:D) photo periodin BOD cabinet according to Khowaja and Qamar(2002). Overnight fresh soaked health cotton seeds were given daily as food. A stock solution of 0.1% was prepared in analytical grade acetone and five different sublethal concentrations viz., 0.002, 0.001, 0.0008, 0.0006, and 0.0004% of acephate were prepared on the basis of LC-50 value.1µl of each concentration of acephate was applied topically on one hundred newly moulted (one-day-old) 4th instar nymphs. Two sets of controls, one set containing one hundred nymphs of the same age and stage were treated individually with 1µl acetone (solvent) to serve as treated control while the other had untreated one hundred nymphs of the corresponding age and served as untreated control, were also run parallel with each series of experiment. Observations were recorded on different parameters(viz., mortality, malformation, fecundity, fertility, longevity,

anatomical and histological deformities of the gonads) and the results were compared with that of acetone treated control insects. Four replicates were taken in each treatment. The females emerged from the treated nymphs were mated with the males of the corresponding age obtained from untreated stock for observations on fecundity and fertility. The ovaries of the females were dissected out in physiological saline. For anatomical study, the dismembered material was prepared according to Pantin (1959). For histological study, the ovaries were kept in Bouin's fixative for 12 hours, washed in tap water and distilled water, dehydrated in ascending grades of alcohol (30, 50, 70, 90, 96 and 100%), kept in xylene solution for 10 minutes, incubated in xylene and paraffin (1:1) solution for 15 minutes at 60^{o}C and finally placed in pure wax in paper boats for block making. Serial sections of 5 micron thick were cut on rotary microtome. The ribbons were placed on a glass slide that were pre-lubricated with albumin and glycerine solutionand then were warmed gently using electric stretching board to remove the crease. To remove the wax the slides were dipped in xylene, rehydrated by passing through the descending grades of alcohol, kept in distilled water for 10 minutes and finally 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. After staining the tissue were again dehydrated, cleared in xylene and finally mounted in DPX and observed under microscope.

RESULTS AND DISCUSSION

Increase in mortality was observed with increase in the concentrations, which is indication of a significant and positive correlation between the percentage mortality caused by the insecticide and the concentration strength (Y= 30464x -0.8454, r= 0.9408, P<0.001, Fig. 01), where y = mortality and x = concentration strength. The knockdown effect of the higher selected sublethal concentrations i.e.,0.0008, 0.001 and 0.002% acephate was very high and caused respectively 19%, 31%, and 43% mortalitywithin the same instar, whereas, 05%, 04%, and 07% nymphs could not cast off their exuviae and died during the process of moulting respectively. The adults which emerged from the survive nymphs, 4.9%, 7.2% and 11.6% had malformed wings respectively. The total mortality up to adult emergence was 25%, 41% and 56% respectively, however,the topical application of lower sublethal concentrations i.e., 0.0004 and 0.0006% did not cause any significant mortality either within the same instar or later up to adult emergence (Fig. 02). Khowaja et al., (2001) studied the toxic effect five sublethal concentrations of cythion (10, 20, 40, 60 and 80 ppm) on 4th instar nymphs of *Dysdercus koenigii* and observed that the nymphal loss and survival duration of the treated insects were directly proportional and increased linearly with increase in concentrations. Khowaja and Qamar (2005) reported that topical of 60 and 80 ppm cythion (an organophosphate) to one day old 5th instar nymphs of *Dysdercus koenigii* resulted in 29 and 40% total nymphal mortality and the adults which emerged from the survived treated nymphs showed 3 and 7% malformation in their wings. Fakhri et al., (2011a and 2011b) studied the comparative efficacy of conventional (monocrotophos and imidacloprid) and non-conventional (neemjeevan and multineem) insecticides on 4th instar nymphs of *Dysdercus koenigii* and observed that conventional insecticides were more toxic than the non-conventional insecticides in term of nymphal mortality.Murtza et al., (2013) studied the toxic effect of two pyrethroids viz., Lacer (Cypermethrin a.i. 20% W/W) and Fenaro (Fenvaleratea.i. 20%) on 5th instar nymphs of *Dysdercus koenigii* and observed that the mortality, malformation and longevity of the treated nymphs increased linearly and showed positive correlation.The topical application of 0.001, 0.002, 0.004% quinalphos and oxydemeton-o-methyl on 4th instar nymphs of *Dysdercus koenigii* caused 21.6, 31.6, 54.9% and 21.6, 34.9, 54.9% nymphal mortality respectively (Fakhri et al., 2012). The topical application of 06, 08 and 10 ppm temik (a.i. Aldicarb) on 4th instar nymphs of *D. cingulatus*caused29, 43 and 52%mortality up to adult emergence as compared to 2% in control, further the adults which emerged from the survived nymphs showed 7.13, 6.29 and 4.79% malformation in

their wings.When the same concentrations were applied on 5th instar, the respective nymphal death was 21, 29 and 38% and 9.63, 8.17 and 6.92% adults were with malformed wings (Khowaja et al., 1995). The topical application of 0.5, 1.0, 2.0, 4.0 and 8.0 ug polypodine-B to the 4th instar nymphs of *Dysdercus cingulatus* caused 10, 19, 30, 39 and 52% total nymphal loss up to adult emergence respectively (Khan et al., 2002).

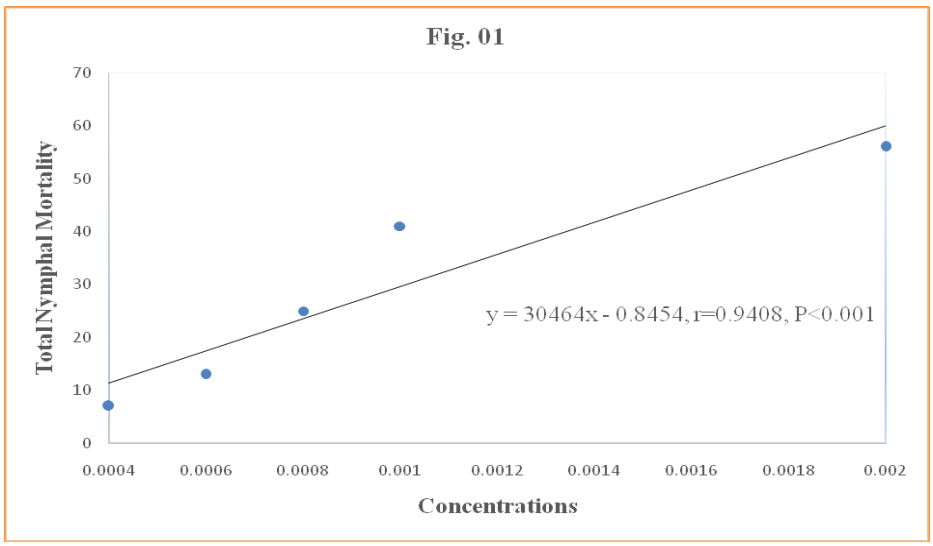

Fig. 01: Total nymphal mortality up to adult emergence following topical application of different sublethal concentrations of acephate on 4th instar nymphs of *Dysdercus cingulatus*

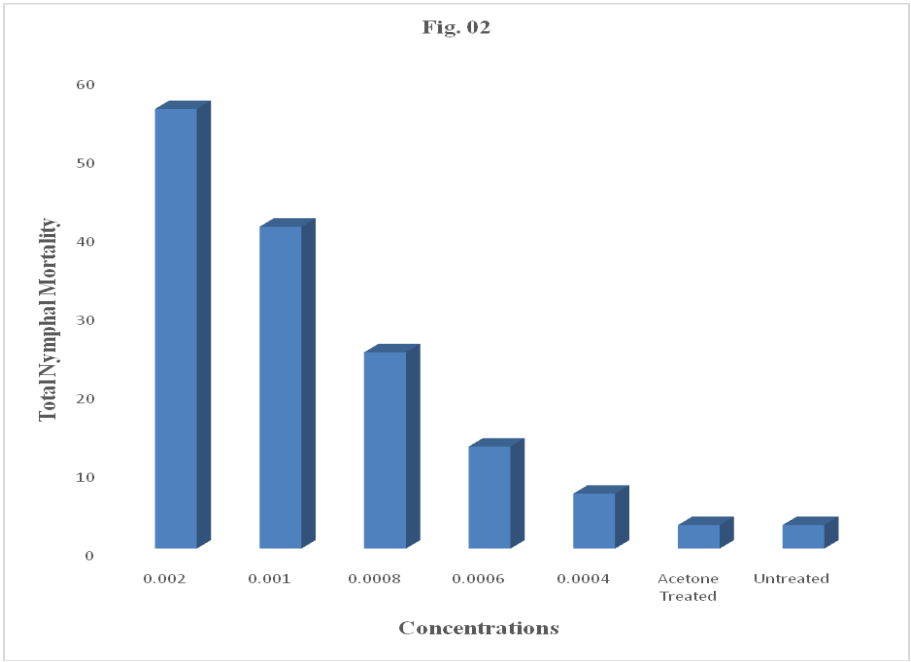

Fig. 02: Total nymphal mortality up to adult emergence following topical application of different sublethal concentrations of acephate on 4th instar nymphs of *Dysdercus cingulatus*.

Same pattern as described above for mortality was also observed for longevity and a positive correlation was found between the survival duration and concentration strength. The longevity of 4th instar treated nymphs (Y= 8632.5x + 71.11, r = 0.9668, P< 0.001, Fig. 03) as well as survived 5th instar nymphs (Y= 13666x + 122.76, r = 0.9337, P<0.001, Fig. 03) also increased linearly and showed a positive correlation with increasing concentrations. Similarly, the longevity of adult females and adult males which emerged from the treated 4th instar nymphs also extended linearly (Y= 33758x + 404.12x, r =0.9734, P<0.001, Fig. 03) and (Y=63325x + 475.29, r = 0.9425, P<0.001, Fig. 03) respectively. The nymphal duration of 4th instar treated nymphs following the topical application of higher sublethal concentrations viz., 0.0008, 0.001 and 0.002%acephate was enhanced significantly by 8.11, 12.82 and 19.4% respectively as compared to control (Fig. 04). The survivability of 5th instar, that emerged from the 4th instar treated nymphs also enhanced by 9.39, 12.87 and 17.90% respectively as compared to control. Likewise, the average survival duration of adult males and females which emerged from the 4th instar treated nymphs after the topical application 0.0008, 0.001 and 0.002%acephatewas enhanced by 10.93, 15.49,20.27% and 2.84, 7.37 and 12.90% respectively as compared to control (Fig. 04).

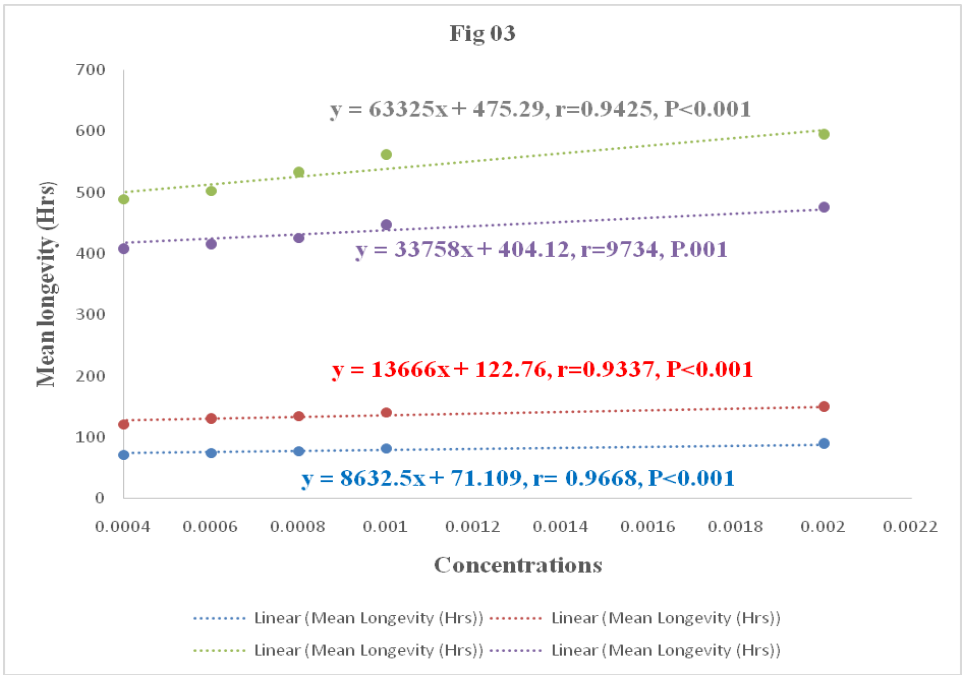

Fig.03: Nymphal and adult longevity following topical application of different sublethal concentrations of acephate on 4th instar nymphs of *Dysdercus cingulatus.*

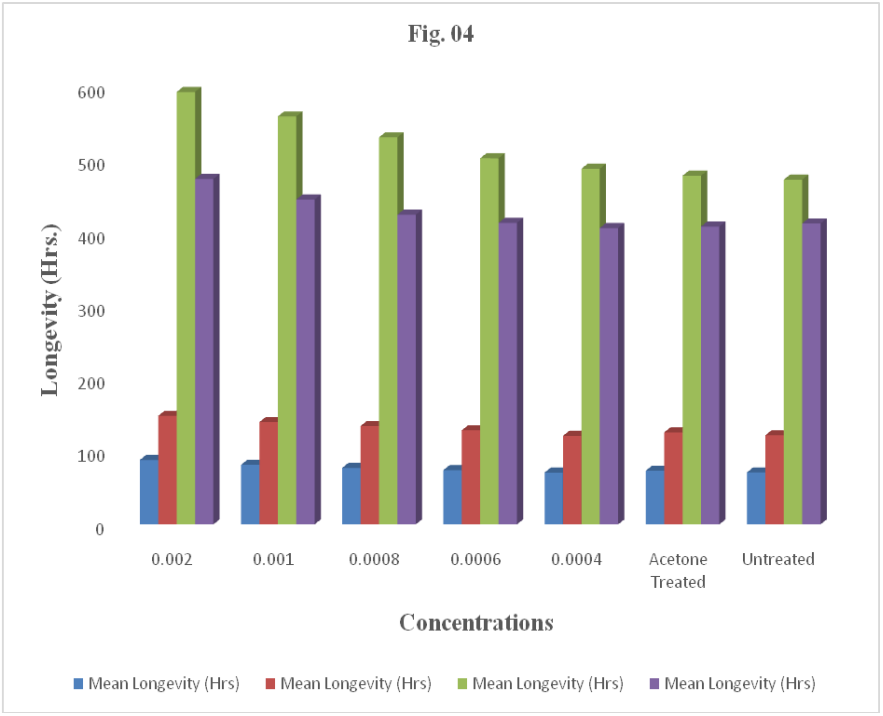

Fig.04: Nymphal and adult longevity following topical application of different sublethal concentrations of acephate on 4th instar nymphs of *Dysdercus cingulatus*.

The prolongation in the survival duration following the exposure to insecticides have been also reported by earlier workers (Ouye and Knutson, 1957; Katiyar and Lemonde, 1972; Parker et al., 1976; Ball and Su, 1979). The topical application of 0.25, 0.5 and 1% cythion on 6th instar larvae of *Spodoptera litura* caused 44.1, 42.7 and 35.7% enhancement in the larval stage, whereas, the pupal duration was increased by 26.2, 16.7 and 10.0% respectively. However, the average life span of adult males and females was reduced slightly as compared to control (Khowaja et al., 1993). The survival duration of 5th instar nymph *Dysdercus cingulatus* after the exposure of 30 ppm monocrotophos was extended by 43.14% whereas, the longevity of adult males and females was enhanced by 31.48 and 26.3% respectively as compared to control (Khowaja et al., 1994). The exposure of insecticides to insect inhibited the hydroxylation process of some steroids which slow down the production of moulting hormone, thereby prolonging the nymphal duration (Conneyet al., 1966). Gupta et al., (1997) studied the effect of RD-9 Repelin on growth and development of *Dysdercus koenigii* and found that the higher concentrations (i.e., 1, 2 and 3%) prolonged the nymphal period of the bug, as such the number of generations were reduced to almost half of the normal populations. It also affected the emergence of adults and those emerged could not lay eggs. However, the lower concentrations of RD-9 Repelin affected the growth and development of the test insects in the subsequent generations. Consequently, the hatching was nil in the third generation in the treated insects.

The average no. of eggs laid by the females which emerged from the survived 4th instar treated nymphs decreased linearly ($Y= -45799x+ 418.57$, $r = -0.9471$, $P<0.001$, Fig. 05), showing a negative correlation with increasing concentrations of acephate. The average egg laying of the females which survived the higher concentrationsviz., 0.0008, 0.001 and 0.002%

acephate was dropped by 9.98, 14.73 and 20.90% respectively as compared to control(Fig. 06). Further, the hatching of the eggs laid by the affected females also decreased linearly (Y= -89149x + 407.78, r = -0.9337, P<0.001, Fig. 05) and yielded a negative correlation with increasing concentrations of acephate. The average hatching of the eggs laid by the affected females after the topical application of the aforesaid sublethal concentrations (i.e.,0.0008, 0.001 and 0.002% acephate) reduced significantly by 12.24, 19.11 and 26.08% respectively as compared to control (Fig 06).

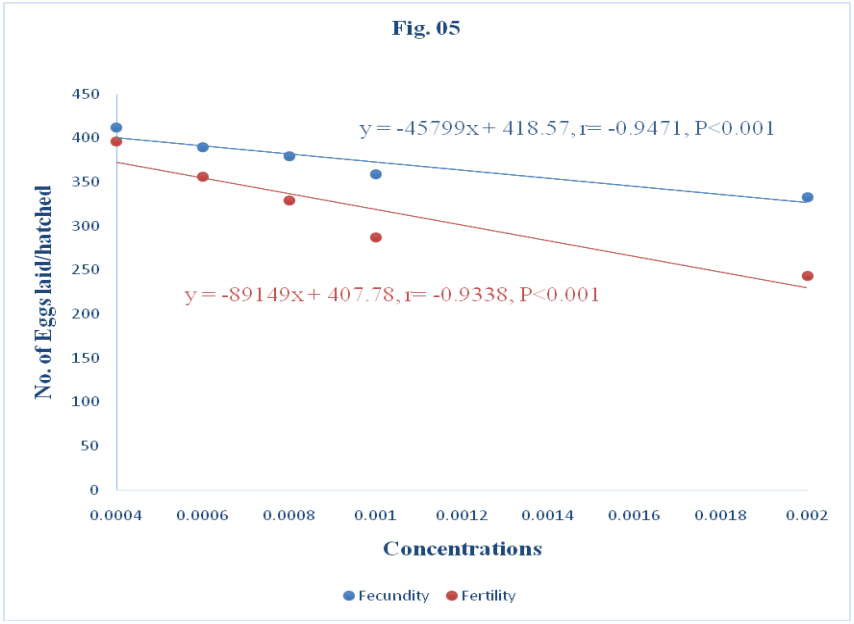

Fig. 05: Fecundity and fertility of *Dysdercus cingulatus* following topical application of different sublethal concentrations of acephate on 4th instar.

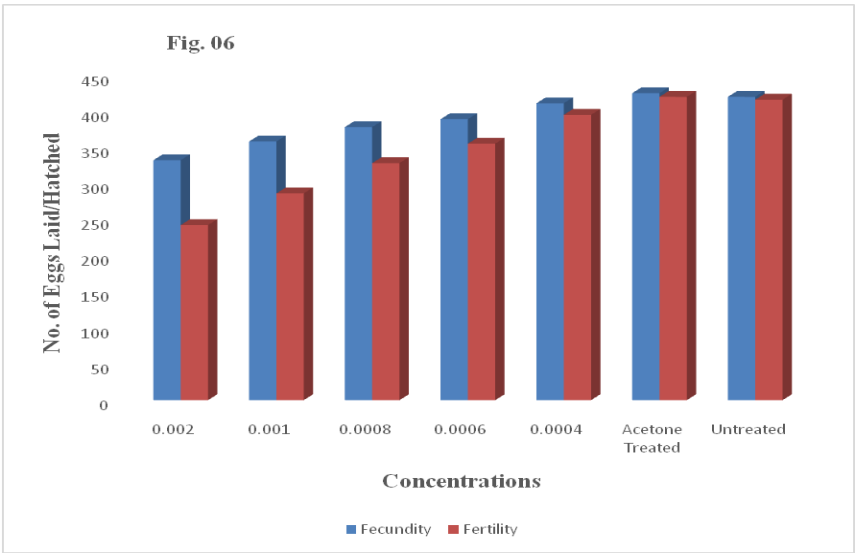

Fig. 06: Fecundity and fertility of *Dysdercus cingulatus* following topical application of different sublethal concentrations of acephate on 4th instar.

These observations are in agreement with the earlier workers (Hamilton and Schal,1990; Abd-Elghafar and Appel, 1992) who observed decreased fecundity as well as fertility of the insects when exposed to insecticides. Khowaja et al., (1994) reported 18.10 and 14.49% reduction in egg production and 22.40 and 13.4% reduction in egg hatching following the topical application of30 and 25 ppm monocrotophos respectively on 5th instar nymphs of *D. cingulatus*. However, when same concentrations of monocrotophos were applied on 4th instar nymphs of *D. cingulatus,*the egg production and egg hatching of the females was reduced by 21.9 and 16.8% and 29.9 and 22.8% respectively (Khowaja and Qamar, 2002). The average egg production and average egg hatching of the females which emerged from the survived 4th instar treated nymphs following the topical application of 6 µg dose/nymphs of 22,25-dideoxyecdysone and 2,22,25-trideoxy-5β-hydroxyecdysone (analogues of β-ecdysone) was dropped by 30.85, 35.28 and 27.55, 29.18% respectively as compared to control (Khowaja et al., 1996).The egg inhibition of egg production as well as egg hatching (Khowaja et al., 1998) was also recorded when the 5th instar nymphs of *Dysdercus koenigii* were treated topically with two analogues (22,25-dideoxyecdysone and 2,22,25-trideoxy-5β-hydroxyecdysone) of β-ecdysones. Khowaja et al. (2001) studied the toxic effect five sublethal concentrations of cythion (10, 20, 40, 60 and 80 ppm) on 4th instar nymphs of *Dysdercus koenigii* and observed the fecundity and fertility of the adults which survived the treatment were inversely proportional, decreased linearly and showed negative correlation with concentrations. Murtza et al., (2013) studied the toxic effect of two pyrethroids viz., Lacer (Cypermethrin a.i. 20% W/W) and Fenaro (Fenvaleratea.i. 20%) on 5th instar nymphs of *Dysdercus koenigii* and reported that the fecundity and fertility of the adults emerged from the survived nymphs decreased linearly and showed negative correlation with increasing sublethal concentrations.

The ovaries of one day old affected females had no change 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 and 10-day-oldfemales that survived the treatment of 0.001 and 0.002%acephate developed some abnormalities in their ovarioles. In both the cases, the development of the ovarioles was inhibited significantly and the size of the ovaries was reduced drastically. Furthermore, the number of mature oocytes in each ovariole was generally reduced to 4-6 than 8-10 in case of control females. In severely affected ovaries, the ovarioles had only 2-3 mature oocytes, while in some other ovarioles degeneration was initiated in the oocytes and there was a darkly stained cellular mass at the end of the ovariole.The study of Siddappajiet al., (1979) also reveal that methamidophos, quinolphos and trichlorfon possess excellent ovicidal property and exhibited 100% inhibition at 0.025% concentration. 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;Combiescoet al., 1967). The injection of varying doses of metepa into female *D. cingulatus* F. induced sterility and oocyte degeneration which ended up in the total dissolution of the ovarian follicle when the dose was raised up to 70 µg/female. At time one or two gigantic eggs developed at the expense of the reserves of the other oocytes but none could continue successful growth and eventually all the oocytes degenerated completely (Sukumar, 1985). Similarly in the present investigation the cellular structure of the ovarioles of the females that survived the treatment of 60 and 80 ppm cythion showed some anomalies. The cellular organization of the apical portion (germarium) of ovariole lost its originality and the trophocytes of the anterior region 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 some oocytes were even 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. Likewise, Matolinet 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 *Mylabrispustulata* when treated with SAN-322 and DDVP showed vacuolation within yolk, distorted shape of oocyte nucleus and necrosis of follicular epithelium (Mulmuleet al., 1988). The ovaries of the 10 days old females that emerged following topical application of 80 and 60 ppm cythion 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 cythion 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.

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