Volume 14, Issue 2
December 2019

ISSN No.:2348-4667

Anthropological Bulletin

a peer reviewed international journal

AB

Department of Anthropology
University of Lucknow, Lucknow, India

ISSN: 2348-4667
Anthropological Bulletin
14 (2), 5-23, 2019

Laboratory evaluation of the toxicity of Acephate for the management of jute hairy caterpillar, *Diacrisia obliqua* walk. (Lepidoptera: Arctiidae).

Khowaja Jamal[1] and Sehba Masood[2,*]

ABSTRACT

Laboratory trials were conducted to assess the insecticidal activities of sublethal concentrations of acephate on mortality, longevity, fecundity, fertility, structural and cellular organization of the reproductive system of Diacrisia (Spilosoma or Spilarctia) obliqua Walker. The toxicity of acephate insecticide against 24 hrs old 5th and 6th instars larvae was dose-dependent and increase with increasing concentrations. Observations on developmental response showed that acephate prolonged larval and pupal period, reduced percent pupation and supress reproductive potential. In addition, acephate also induced morphological abnormalities on survived larvae, pupae and adults.Beside these anatomical and histological studies of the male and female reproductive system of the adults emerged from the survived treated larvae showed dose-dependent damage. The size of the ovaries of the affected females was smaller as compared to unaffected (control-I) females. The germarium and the vitellarium were clearly distinguishable. The common oviduct became slightly broader as compared to that of control females. The bursa copulatrix became comparatively small and less sclerotized. The ovarioles of the affected females showed cellular damage. The nature of the cellular damage was almost identical but the degree of damages was dose based. It is clear that acephate which possesses insecticidal, growth and reproductive retardant and may be useful in the management of jute hairy caterpillar.

KEY WORDS: *Diacrisia obliqua*, acephate, toxicity, mortality, longevity, fecundity, fertility, anatomy and histology

Introduction

Diacrisia obliqua Walker (Syn: *Spilosoma obliqua*, *Spilarctia obliqua*) (Lepidoptera: Arctiidae), commonly known as Bihar hairy caterpillar in Asia, is a polyphagous pest infesting about 126 crops (Singh and Varathrajan, 1999) including forest shrubs and trees (Yadav et al., 2001), various agricultural crops (pulses, oil seeds, cereals), fibre crops, vegetables, mulberry, medicinal, aromatic and other economically important plants (Singh and Singh, 1992; Gupta and Bhattacharya, 2008). It is widely distributed in India, China,

[1] Department of Zoology, Aligarh Muslim University, India, drkhowajajamal@gmail.com
[2] Department of Computer Science, Aligarh Muslim University, India, sehba.masood.cs@amu.ac.in
[*] Corresponding author

Pakistan, Bangladesh, Nepal, Myanmar, and Sri Lanka (Bhattacharya and Rathore 1977; Nagia et al. 1990; Bhadauria et al. 2001; Goel et al. 2004; CPC, 2004).In India, it is a serious pest in West Bengal, Bihar, Madhya Pradesh, Uttar Pradesh, Punjab, Manipur, and other states (Kalleshwaraswamy and Basavarajappa 2005). The adult single female moth lays eggs in batches and after hatching first to third instar larvae feeds gregariously while fourth to sixth instar larvae feeds solitarily that cause significant reduction in yield (Hussain and Begum 1995; Gupta and Bhattacharya, 2008). Timely management of this pest is very important as delay may even lead to complete defoliation ofcrop if remains unchecked in the field. The chemical control of advance larval stage is difficult because of dense hairiness and moreover, some time certain levels of behavioural resistance to different class of insecticides has also been reported (Perry et al. 1998; Dhingra et al, 2007; Mandal et al. 2013). To overcome this, rotations of insecticides with different modes of action have been the strategies to avoid resistance development (Roush and Miller, 1989; Tabashnik, 1989; Bharathi, et al.,2010), hence continuous assessment of the toxicity of various insecticides is essential as an alternative tool for the successful control of this pest. Perhaps, all categories of insecticides may decline the production of pest offspring either through affecting feeding behavior, larvae mortality, developmental time, pupal weight, sex ratio, time to adult emergence, fecundity, and development of the female ovipositor and egg hatch (Moreau and Bauce 2003; Seth et al. 2004; Galvan et al. 2005; Borchert et al. 2005; Eizaguirre et al. 2005; Abbott et al. 2008).The reproductive function especially that of males is a potential target for the toxic effects caused by exposure to pesticides (Bretveld et al., 2007). Results of Farag et al. (2000, 2007, 2010), Ismail and Al-Taher (2011), Joshi et al. (2003) revealed that exposure of different organophosphate pesticides to rodents resulted reduction in fertility, changes in sperm count and motility, changes in reproductive organs, inhibition in brain and skeletal muscle acetylcholinesterase activity and degeneration of muscle fibers. Similar results were also observed in humans' studies where prolonged exposure to pesticides caused low semen sperm counts beside hormonal changes which ultimately resulted in infertility(Oliva et al., 2001, Perry et al., 2007). Therefore, the present experiment was undertaken to investigate the acute and subacute toxicity of acephate against 5th and 6th instar larvae on mortality, longevity, fecundity, fertility as well as anatomical and histological structure of male and female reproductive system of*Diacrisia obliqua* under laboratory condition.

Acephate [*O*, *S*-dimethyl-acetyl-phosphoramidothioate, molecular weight (MW) = 183.17] is a class II "moderately hazardous" (World Health Organization,2009) systemic organophosphate pesticide that has been widely used to control insect pests in agricultural fields as well as parasites on mammalians for decades. It is highly soluble in waterand can easily absorb by soil thus easily absorbed by plants and accumulated in edible parts of plants (Mohapatra et al., 2011; Syed et al., 2014). Acephate and its metabolitemethamidophos are two of the most common and efficient OPs that are used for pest control in agriculture (Maia et al., 2011; Kumar et al., 2015; Pan et al., 2015).

MATERIALS AND METHODS

Insects culture

Adults of *Diacrisia obliqua* Walker were collected on light during the month of September and Octoberand were kept in glass rearing jars (20x15 cms),covered with a piece of muslin cloth which was tightly fixed by means of a rubber band and maintained at 27 ± 2°C temperature and 70-80% relative humidity in BOD incubator. The adults were offered 5% glucose solution soaked in sterilized cotton wool as food. The females laid eggs on folded paper strips 2"x20" kept in the jars.Following egg laying adults were transferred to other rearing jars for further egg laying. After hatching the larvae were provided with fresh castor (*Ricinus communis*)leaves as food. These larvae pupated at the surface of the jars or at the under surface of the leaves. The emergence of adults took place after a week following pupation. Then the newly emerged adults were transferred in pairs to separate rearing jars for further generations. For treatment newly moulted 5th and 6th instar larvae were sorted out for experimental purpose.

Laboratory Bioassay for Acephate

The topical application method was used for bioassay with acephate (O, S-dimethyl acetyl phosphoramidothioate) to determine the mortality, longevity, fecundity, fertility, anatomy and histology of the reproductive system of *S. obliqua*. In a preliminary experiment, LC-50 value of acephate for 5th instar larvae was obtained. On the basis of LC-50 value, five serial dilutions (0.1, 0.08, 0.06, 0.04 and 0.02%) of acephate were prepared in acetone (Analytical grade). One hundred (24 hrs old) 5th and 6th instar larvae in 4 replicates were topically treated with 2 µl of each concentration with the help of a 2 ml tuberculin syringe fitted to a manually operated micro-applicator. For control (designated as control-I) only 2 µl acetone (Analytical grade) was applied on the same number of larvae obtained from the same stock. To compare the effect of solvent (acetone), same number of larvae were treated with 2 µl distilled water (designated as control-II) were maintained at the same controlled conditions. Daily mortality of the treated insects as well as the emergence of the adults from the survived nymphs was recorded to observe longevity.

For the observations on egg production and egg viability, the adult females emerged from the nymphs treated with each concentration of acephate and acetone (control) were paired with males of the corresponding age obtained from untreated stock. 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.

Anatomical and Histological Preparation

Active and healthyfemales which emerged from the survived treated and untreated nymphs were paired with their corresponding males. From such stock, 10 females and 10 males were dissected out simultaneously, at the intervals of 01, 05, 10 and 20 days following emergence, to study the changes both in anatomical as well as histological structure of the reproductive system. For anatomical study, the dissected material was prepared according to Pantin (1959). For histological observations, the testes and ovaries wereremoved in the physiological saline and 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^{o}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 (Khowaja, J.2015). The staining was achieved by YSI-106 Yorko automatic staining machine having 12 stations using Culling (1974) method.

Statistical Analysis

The larval mortality was observed after 24, 48 h, and 72 h and the data were corrected by adjusting mortality in controls using Abbott's formula (Abbott's, 1925).

$$\text{Corrected mortality} = \frac{\text{Treated mortality- Control}}{\text{100-Control mortality}} \times 100$$

The moribund larvae were also counted as dead. Finally, the mortality data were analysed by probit analysis (Finney 1985) to calculate Lc-50 value using SPSS statistical package programme.

Results and Discussion

The mortality of the test insects was found to increase with an increase in concentrations of acephate. The topical application of serially diluted 0.1, 0.08, 0.06, 0.04 and 0.02% acephate on 5th instar (24 hrs old) larvae of *Diacrisia obliqua* caused 35, 24,15,09, and 04% initial mortality (Fig.01) respectively within 24 hrs ($Y= -5.8571x + 35.857$, $R^2 = 0.9221$, $P< 0.001$; Fig.5), whereas the total larval mortality up to adult emergence was 53, 40, 26, 11, and 07% (Fig. 02) respectively as compared to 02% in control ($Y= -8.75x + 55.286$. $R^2=0.8979$, $P< 0.001$; Fig. 06). The topical application of the same above-said concentrations on 6th instar larvae produced 20, 19, 06, 04, and 03% initial mortality (Fig. 09) respectively as compared to control ($Y= -3.5x + 21.571$,$R^2 = 0.8133$, $P< 0.001$; Fig. 13), however the total larval mortality up to adult emergence was 34, 27, 17, 06, and 03% (Fig. 10) respectively as compared to 04% in control ($Y= -5.6786x + 35.857$; $R^2 = 0.8793$; $P< 0.001$; Fig. 14). Earlier, Singh and Singh (2000) performed a bioassay studies to determine the relative toxicity of five synthetic pyrethroids (decamethrin [deltamethrin], cypermethrin, lambda-cyhalothrin, fenvalerate, and bifenthrin) and four non-pyrethroids (endosulfan, chlorpyriphos [chlorpyrifos], lindane, and malathion) against the 2nd instar (5±1-day-old) larvae of *Spilarctia obliqua* in the laboratory. Based on the LC_{50} values of the insecticides, the order of toxicity against *Spilarctia obliqua* was decamethrin > cypermethrin > lambda-cyhalothrin > fenvalerate > bifenthrin > endosulfan > chlorpyriphos > lindane > malathion. On the basis of the observations they concluded that pyrethroid insecticides were more toxic to *Spilarctia obliqua* than the other insecticides. Gupta et al., 2004 also studied the efficacy of different synthetic pesticides and reported that endosulfan can effectively control the larva of *S. oblique*. Whereas, further study by Nair et al. (2007) found that indoxacarb was highly toxic than endosulfan against the larva of *S. oblique* in laboratory condition. However, Mishra et al (2017) while evaluating the effectiveness of eleven insecticides against bihar hairy caterpillar, *Spilosoma obliqua* Walker observed that Flubendiamide 480 SC was more toxic than Indoxacarb 14.5 SC and the overall order of effectiveness of these insecticides was Flubendiamide 480 SC > Indoxacarb 14.5 SC > Spinosad 45 SC > Thiodicarb 75 WP > Emmamectin benzoate 5 SG > Rynaxypyre 20 SC > Thiomethoxom 25% WG > Fipronil 5% SL > Imidacloprid 17.8 SL > Chloropyriphos 20 EC. Muthusamy et al (2011) studied the baseline susceptibility of five classes of chemical insecticides namely imidacloprid, cypermethrin, Emamactin, Neem and Flubendiamide on third instar larvae of *S. oblique* and observed that emamectin benzoate was the most potent insecticide (LC_{50}: 2.459 g a.i/ha), followed by cypermethrin (LC_{50}: 41.72 g a.i/ha) and concluded that tThis information can be used for designing IPM programs in groundnut. Kumar et al (2013) tested 5 insecticides against the larvae of *Spilarctia obliqua* and observed that Deltamethrin was most toxic insecticide against the larvae of *Spilarctia obliqua* followed by triazophos, spinosad, chlorpyrifos and fipronil in leaf dip. Selvaraj et al (2015) studied the toxicity of conventional (profenofos 50 EC and λ-cyhalothrin 5 EC) and non-conventional (flubendiamide 480 SC, chlorantraniliprole 18.5 SC, emamectin benzoate 5 SG) insecticides against the third instar larvae of jute hairy caterpillar, *Spilarctia obliqua* under laboratory conditions and reported that the bioefficacy of synergistic binary combinations along with individual insecticides established the superiority of profenophos + λ- -1 cyhalothrin (3:1) with 89.12% reduction in infestation and recorded maximum fibre yield 38.67qha under field condition. Sharma et al (2015) studied the stomach toxicity of Cypermethrin, Lambda cyhalothrin and Chlorpyriphos against 7d old larvae of *Spilarctia obliqua* by leaf dip method. On the basis of LC50 values at different time intervals the order of toxicity was recorded as Cypermethrin > Lambda cyhalothrin > Chlorpyriphos. These findings can be helpful for the selection of suitable insecticides for effective pest management under field condition. According to Khowaja et al. (1995) the exposure of 10, 08, and 06-ppm temik to the 4thinstar nymphs of *Dysdercus cingulatus* caused 52, 43 and 29% total nymphal loss up to adult emergence. 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).

Ali et al (2008) studied the biological activity of eco-friendly insecticide, nimbicidine on the mortality, growth and feeding responses of jute hairy caterpillar, *Spilarctia obliqua* (Walker) and reported that nimbicidine possesses insecticidal, growth retardant and antifeedant properties and may be useful in the management of jute hairy caterpillar. Gupta and Bhattacharya (2008) studied the lethal and sublethal effects of three post-emergence herbicides, 2,4-D ethyl ester (Weedkill 80WP), imazethapyr (Pursuit 10EC) and quizalofop ethyl (Tergasuper 5EC) against the larvae of *Spilarctia obliqua* Walker (Lepidoptera: Arctiidae) and observed that Quizalofop ethyl and imazethapyr caused signifcant reduction of pest survival at almost all levels; however, 2,4-D ethyl ester was nontoxic. Mandal et al (2013) evaluation the toxicity of azadirachtin 1500ppm @3ml/l, endosulfan 35EC @ 300g a.i/ha, triazophos 40 EC @ 250g a.i/ha, thiamethoxam 25 WG @ 40g a.i/ha, ë-cyhalothrin 5 EC @ 40g a.i/ha, indoxacarb 14.5 SC @ 75g a.i/ha and imidacloprid17.8 SL @ 30g a.i/ha for the management of bihar hairy caterpillar (Spilosoma obliqua Walk.) in black gram and reported the order of efficacy: triazophos (90.64%) >ë-cyhalothrin (83.71%) >indoxacarb (78.76%) >endosulfan (69.53%) >imidacloprid (62.31%) > thiamethoxam (57.40%). Azadirachtin was found to be least effective in controlling of hairy caterpillar. Highest cost benefit ratio (1:21.69) was observed in triazophos 40 EC @ 250g a.i/ha. Bandyopadhyay et al (2014) studied the toxicity of the commercial formulation Delfin® containing *Bacillus thuringiensis* var. kurstaki (Btk) serotype 3A, 3B, SA11 (WG) and Margo Econeem™ Plus (EC) based botanical insecticide containing azadirachtin 1% (10,000 ppm) alone and in different sublethal combinations against 3rd instars of *Spilarctia obliqua* Walker (Lepidoptera: Arctiidae) under laboratory conditions and suggested that combination of botanicals with microbes could be strategized for utilization in integrated management. Hussein and Mohammed (2015) studied the toxicity of botanical extracts of Neem, Sarifa, bioagent Beauveria bassiana and conventional Insecticide (Cypermethrin 0.07%) against larvae of Spilosoma obliqua on cabbage leaves in plastic jars under laboratory conditions and observed that Cypermethrin caused 100% population reduction of Spilosoma obliqua (100%) followed by leaf extracts of Neem (90.00%), Sarifa (83.33%) and Beauveria bassiana (73.33%) as compared with Control (0.00%). The Bioefficacy of ethiprole 40+ imidacloprid 40 (Glamore 80WG) against 14d old larvae of *Spilosoma obliqua* (Walker) by leaf dip method revealed a feeding inhibition of 87.60 and 81.86% @0.833 and 0.666% at 24HAF (hours after feeding) as compared to control, respectively. The LC50 values against 9d old larvae at 2.5, 3.0, 3.5, 4.0 and 5.0 days after feeding (DAF) were 1.018, 0.802, 0.606, 0.464 and 0.334%; and against 14d old larvae at 4.0 and 5.0 DAF were 0.628 and 0.563%, respectively. The LT50 values against 9d old larvae were 51.08, 81.90 and 96.85 hr @0.833, 0.666 and 0.499%, respectively. The LC50 values against 9d old larvae in atomization method at 3, 4 and 5 DAE (days after exposure) were 0.466, 0.369 and 0.324%, respectively; and against 5d old larvae it was observed to be 0.293 and 0.130% at 3 and 4 DAE, respectively (Devi and Srivastava, 2018).

The longevity of 5th instar (24 hrs old) treated larvae of *Diacrisia obliqua* increased linearly ($Y = -4.3286x + 108.3$, $R^2 = 0.907$; $P < 0.001$; Fig.07) with increasing concentrations of acephate. Similarly, the survival duration of 6th instar larvae, pupal period, average life span of adult males and females that emerged from the 5th instar treated nymphs also increased linearly ($Y = -2.9679x + 146.94$, $R^2 = 0.7895$; $Y = -9.5429x + 296.8$, $R^2 = 0.7463$; $Y= -10.318x + 426.73$, $R^2 = 0.8302$; $Y= -4.4464x + 216.67$, $R^2= 0.8943$, $P <0.001$ respectively, Fig 07), and exhibited positive correlation. The average larval duration of 5th instar treated larvae and 6th instar survived larvae following the topical application of 0.1, 0.08, 0.06, 0.04, and 0.02% of acephate was enhanced by 25.7, 14.9, 9.1, 4.4,3.2 hrs and 21.6, 11.4, 8.9, 3.9, 6.2 hrs respectively (Fig. 03) as compared to control-I, whereas the average pupal period, average life span of adult males and adult females that emerged from the treated 5th instar larvae also increased by 67.1, 31.1, 18.5, 4.6, 9.4; 68.7, 43.4, 21.6, 14.4, 10.3 and 26.6, 15.2, 9.5, 3.8, 5.7 hrs respectively as compared to control (Fig.03).The longevity of 6th instar (24 hrs old) treated larvae following the topical application of same above said concentration also increased linearly ($Y= -2.425x + 142.43$, $R^2= 0.675$, $P <0.001$, Fig.15) with increasing sublethal concentrations of acephate. Similarly, the pupal duration, adult male and female life span that emerged from the 6th instar treated larvae also increased linearly ($Y=-7.5679x + 290.57$, $R^2 = 0.7392$; $Y= -16.23x + 503.9$, $R^2 = 0.8108$; and $Y= -5.4286x + 268$, $R^2 = 0.9049$, $P <0.001$ respectively, Fig. 15) with increasing sublethal concentrations and exhibit positive correlation.The average survival duration of 6thinstar treated larvae following the topical application of 0.1, 0.08, 0.06. 0.04, and 0.02% of acephate was enhanced by 17.5, 11.4, 02.0, 03.7, 02.5 hrs respectively (Fig. 11) as compared to control-I, whereas the average pupal period, average life span of adult males and adult females that emergedfrom the survived6th instar treated larvae also increased by 53.8, 26.9, 12.2, 05.6, 08.3; 104.9, 72.5, 36.2, 12.0, 16.4 and 30.8, 14.2, 16.0, 04.9, 03.1 hrs respectively as compared to control (Fig.11).

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 4thinstar 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 LD-50 dose of cyfluthrin and hydramethylnon reduced by 52and 81% respectively compared to control (Abd-Elghafar& Appel, 1992). The larval duration of 6thinstar *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 4thinstar 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 insecticidesincreased significantly as compared to control Fakhri et al. (2012).

The average egg production of the females that emerged from the survived treated5thinstar larvae showed negative linear correlation with increasing concentration ($Y = 44.75x + 1589.9$, $R^2= 0.9383$, $P <0.001$, Fig. 08). Following the topical application of higher selected sublethal concentrations viz., 0.1,0.08, and 0.06% acephate/5thinstar larvae, the fall in fecundity of the affected females was 14.22,09.52 and 07.81% respectively (Fig. 04) as compared to control. The hatching of the eggs laid by the affected females also decreased linearly with increasing concentrations of acephate ($Y = 71.571x + 1398.4$, $R^2 = 0.9095$, $P < 0.001$, Fig. 08). The viability of the eggs following the application of 0.1, 0.08 and 0.06% acephate, was slipped by 24.59,14.92 and 11.46% respectively as compared to control (Fig. 04).

Similarly, the average egg laying of the females that emerged from the survived 6th instar treated larvae also showed negative linear correlation with increasing concentration ($Y = 47.036x + 1528.6$, $R^2 = 0.8335$, $P <0.001$, Fig. 16). Following the topical application of higher selected sublethal concentrations viz., 0.1, 0.08, and 0.06% acephate/6th instar larvae, the fall in fecundity of the affected females was 17.98, 09.05 and 08.01% respectively (Fig. 12) as compared to control. The average hatching of the eggs laid by the affected females also decreased linearly with increasing concentrations of acephate ($Y = 79.929x + 1291.1$, $R^2 = 0.9117$, $P < 0.001$, Fig. 08). The viability of the eggs following the application of 0.1, 0.08 and 0.06% acephate, was slipped by 28.15., 15.62 and 1.85% respectively as compared to control (Fig. 12).

The results of Chattoraj and Bhise (1980) also indicated that fecundity of adult *Spodoptera litura*, emerged from the treated larvae exposed to sub lethal dose of 3µg dieldrin, was reduced significantly as compared to control. In laboratory test, the exposure of sub lethal concentrations (Lc-30 and Lc-50,0.0088, 0.0182% respectively) of parathion (ethylparathion) to the larvae of *Spodoptera litura* resulted in reduction in oviposition and egg hatching rate of the adults (Patil and Khanvilkar, 1977).

The results of Khowaja et al., 1992 indicate that the topical application of sublethal concentrations 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 5thinstar 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) on6th 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 the two days old females which emerged from the survived 5th instar as well as 6th instar treated larvae following the topical application of 0.10 and 0.08% concentrations of acephate showed some anatomical deformities. The size of the ovaries of the affected females was smaller as compared to unaffected (control-I) females however the germarium and the vitellarium were clearly distinguishable. Although, the length of each ovariole was normal, the development of oocytes was slow in the vitellarial portion and there was loose arrangement of chorionated eggs at the base of some ovarioles. In some ovarioles more than one oocytes were present in a single egg chamber resulting in the formation of compound egg chamber. In addition, there was formation of an ovariole loop and the common oviduct became slightly broader as compared to that of control females. The bursa copulatrix became comparatively small and less sclerotized. Although, the shape of the accessory glands was unchanged, its size was reduced proportionately with respect to the size of the ovary as compared to that of the control. However, the females that emerged from the survived 5th instar larvae treated with 0.06, 0.04 and 0.02% acephate did not show any remarkable damage in the anatomical structure of their ovaries. The shape and size of the ovaries of the affected females was almost similar to that of the control-I.

The histological picture of the ovarioles of the affected females following the topical application of 0.10, 0.08 and 0.06% acephate on the 5th and 6th instar larvae showed cellular damage where the nature of the changes was almost identical with respect to each treatment but the degree of damages varied with concentrations. The germinal part of the ovariole was intact having normal primordial germ cells. Below the germarium, there was a long immature vitellarial portion which contained more immature oocytes as compared to control. The linear arrangement of both immature and mature eggs in certain ovarioles was not uniform and the density of ooplasm was very thin in the oocytes. The development of these oocytes was rather slow and contained less ooplasm as compared to the control. Below this portion, the ovarioles had the chorionated eggs. The accumulation of yolk protein spheres at the egg cortex was not normal in the eggs of the same region of the ovarioles as evident by differential staining property. In some ovarioles the ooplasm of the chorionated eggs developed differential growth pattern including the accumulation of the yolk protein spheres as compared to normal pattern of the controls. The staining property of these oocytes showed their young condition though their accompanying trophocytes were almost exhausted. The follicle cells of immature oocytes in few cases had loose arrangement leaving some space between them. The trophocytes in some ovarioles became short and loosely arranged. The ovarioles of the affected females by lower concentrations (0.06, 0.04 and 0.02%) of acephate had either insignificant effect or no damage at all. The histological picture of the ovaries of these females was almost identical with that of the unaffected (control-I) females.

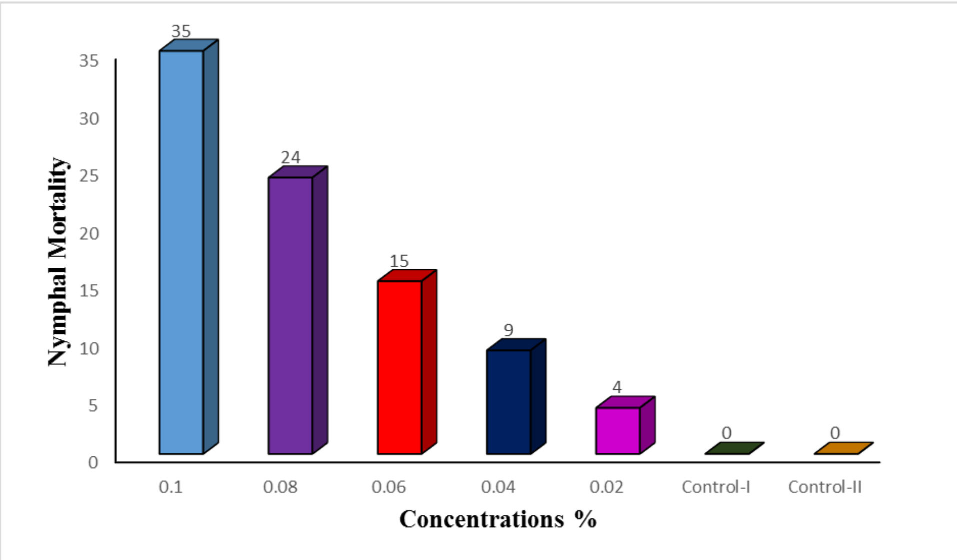

Fig.01. Initial larval mortality within 24 hrs (Knockdown effect) following topical application of sublethal concentrations of Acephate on 5th instar larvae of *Diacrisia obliqua*

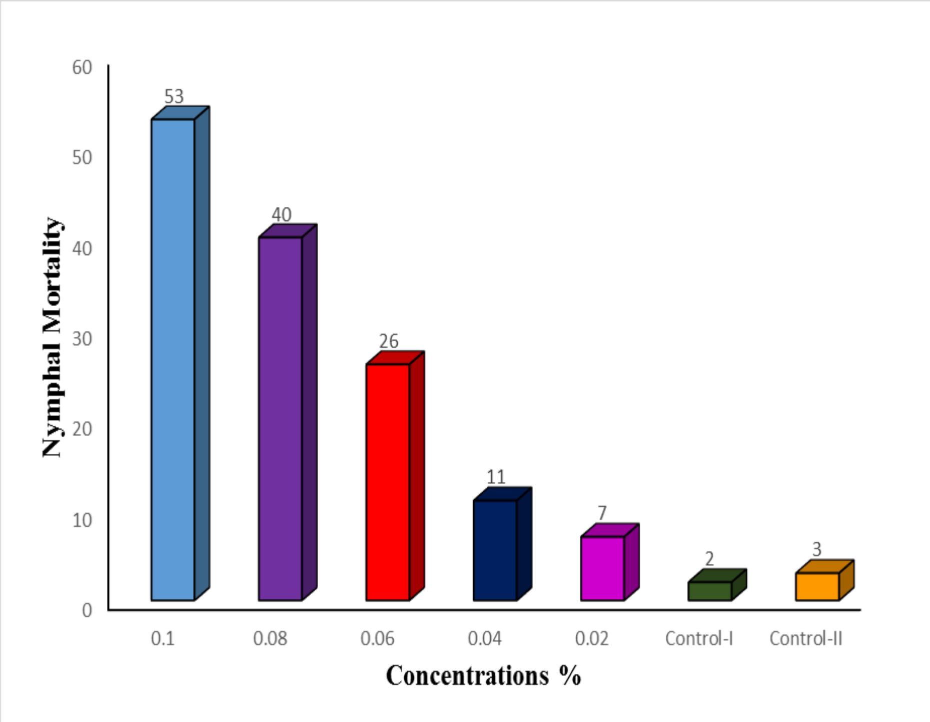

Fig.02. Total larval mortality up to adult emergence (Residual effect) following topical application of sublethal concentrations of Acephate on 5th instar larvae of *Diacrisia obliqua*

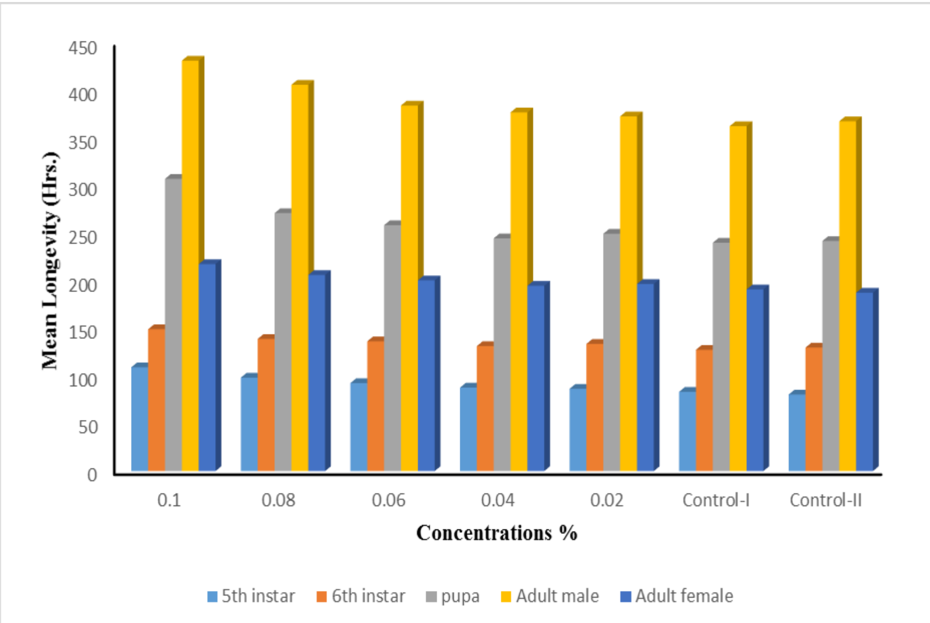

Fig. 03. Larval, pupal and adult longevity following topical application of sublethal concentrations of Acephate on 5th instar larvae of *Diacrisia obliqua*

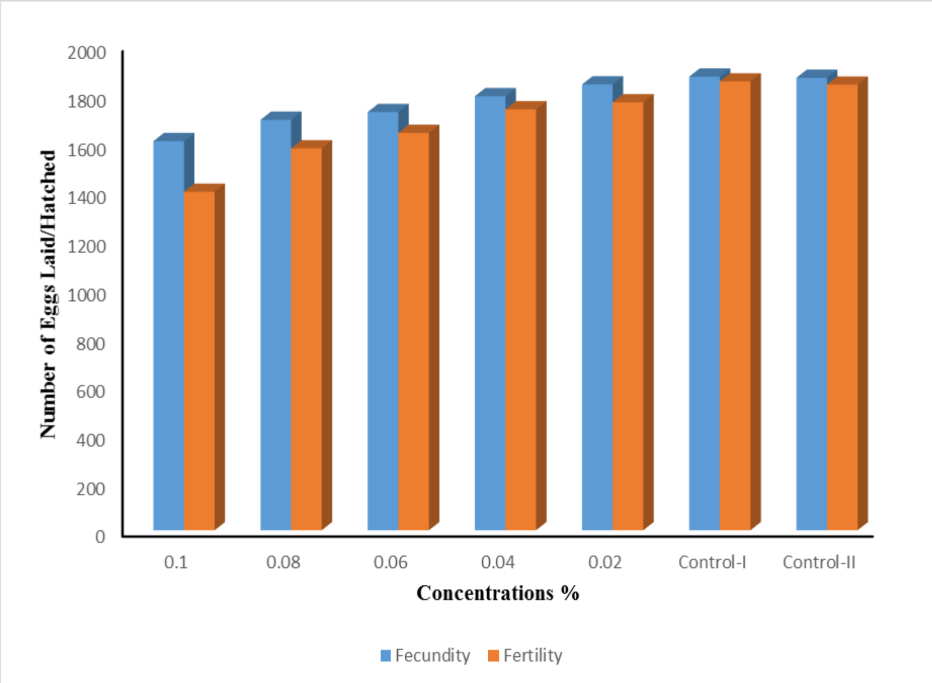

Fig. 04. Fecundity and fertility of the adult females following topical application of sublethal concentrations of Acephate on 5th instar larvae of *Diacrisia obliqua*

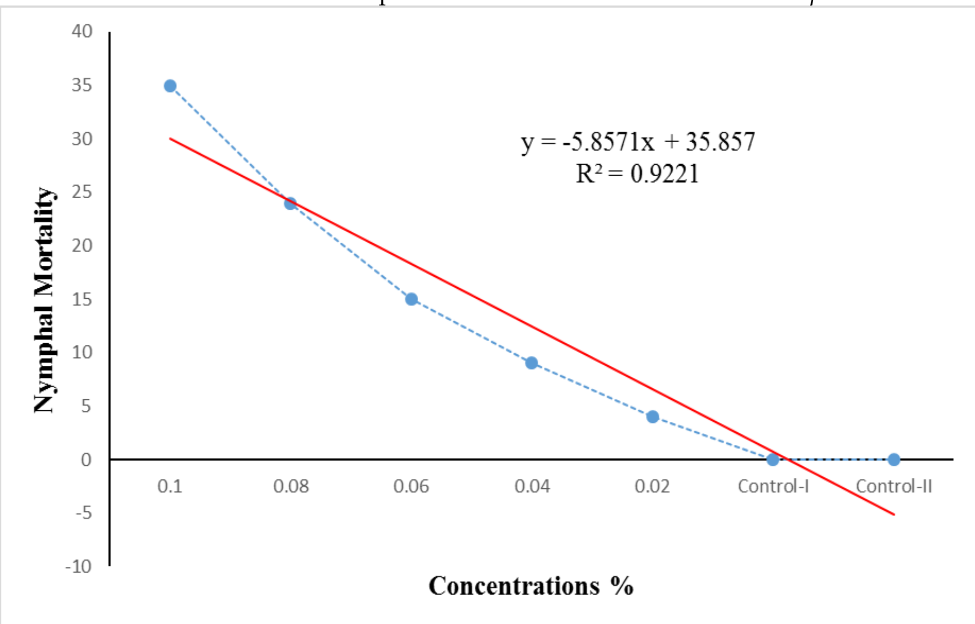

Fig.05. Initial larval mortality within 24 hrs (Knockdown effect) following topical application of sublethal concentrations of Acephate on 5th instar larvae of *Diacrisia obliqua*

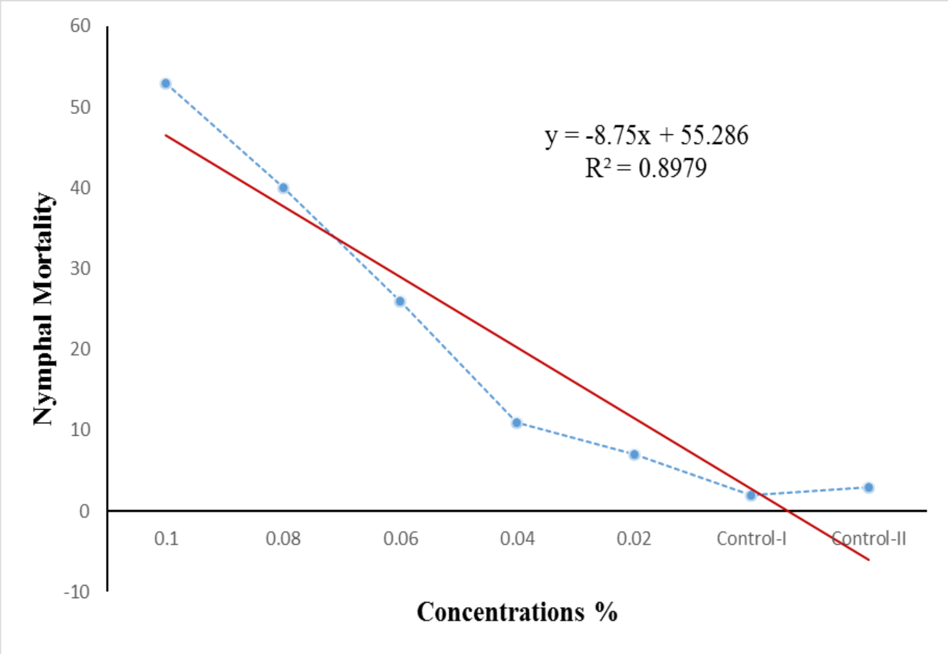

Fig. 06. Total larval mortality up to adult emergence (Residual effect) following topical application of sublethal concentrations of Acephate on 5th instar larvae of *Diacrisia obliqua*

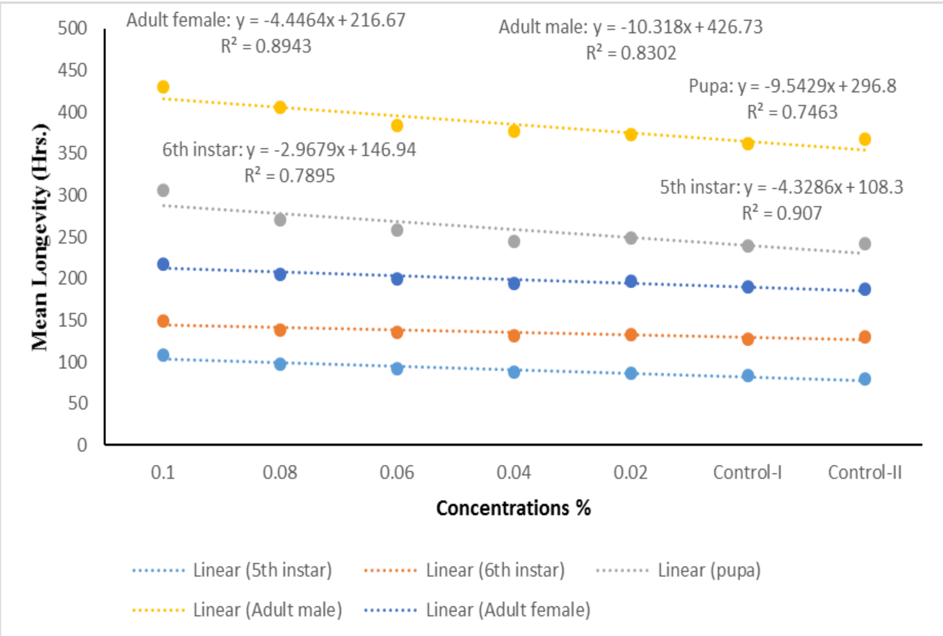

Fig. 07. Larval, pupal and adult longevity following topical application of sublethal concentrations of Acephate on 5th instar larvae of *Diacrisia obliqua*.

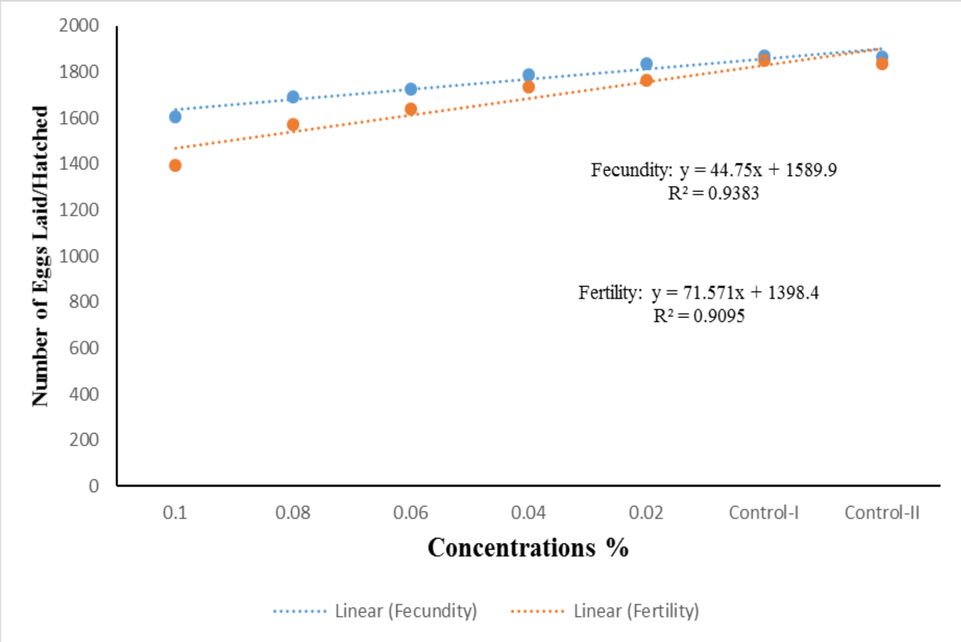

Fig. 08. Fecundity and fertility of the adult females following topical application of sublethal concentrations of Acephate on 5th instar larvae of *Diacrisia obliqua*

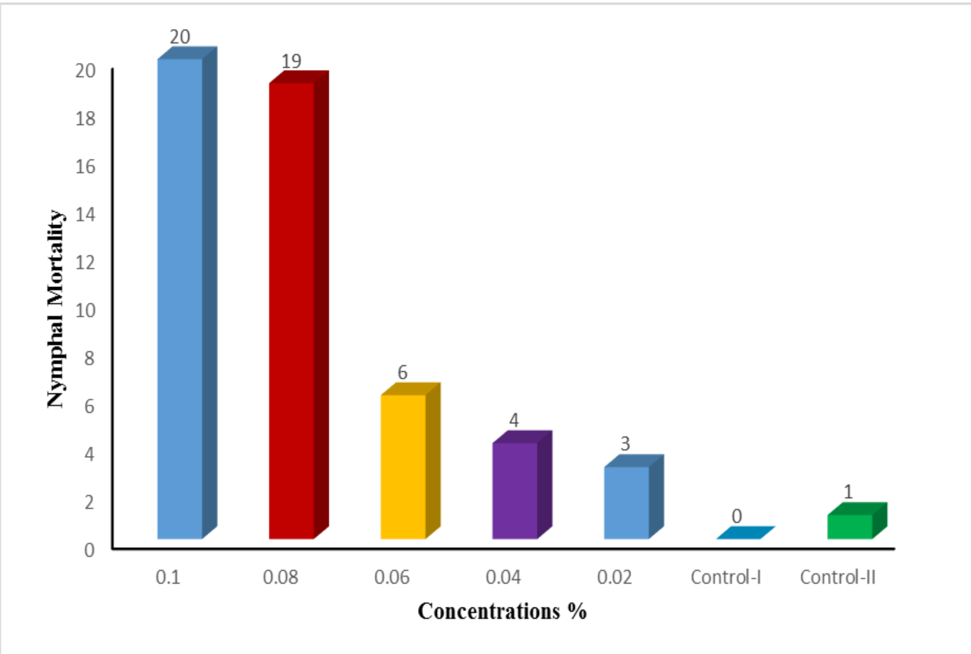

Fig.09. Initial larval mortality within 24 hrs (Knockdown effect) following topical application of sublethal concentrations of Acephate on 6th instar larvae of *Diacrisia obliqua*

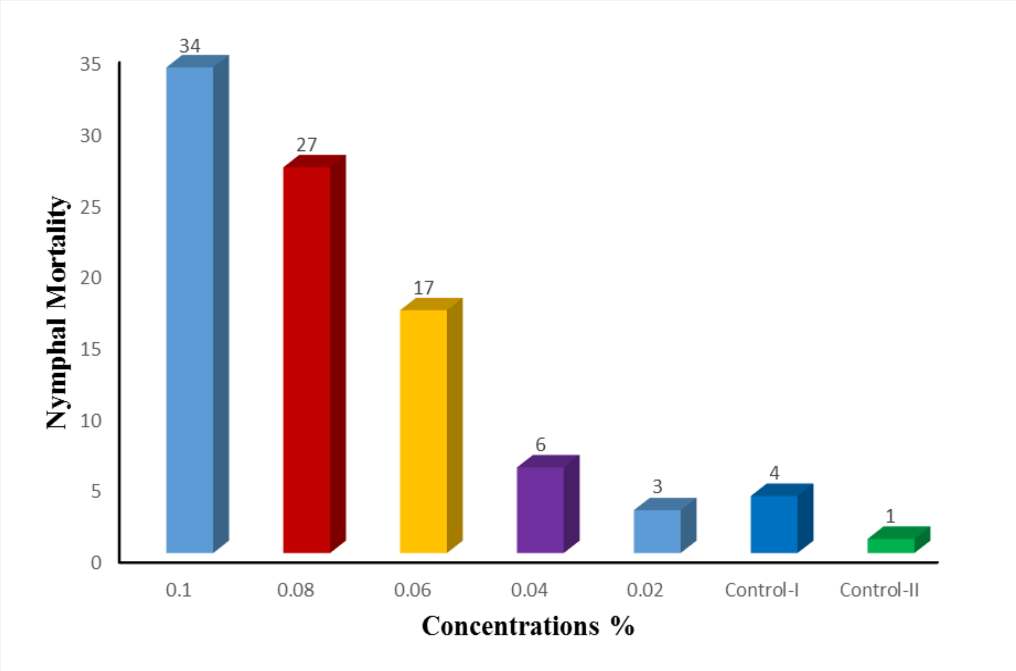

Fig.10. Total larval mortality up to adult emergence (Residual effect) following topical application of sublethal concentrations of Acephate on 6th instar larvae of *Diacrisia oblique*

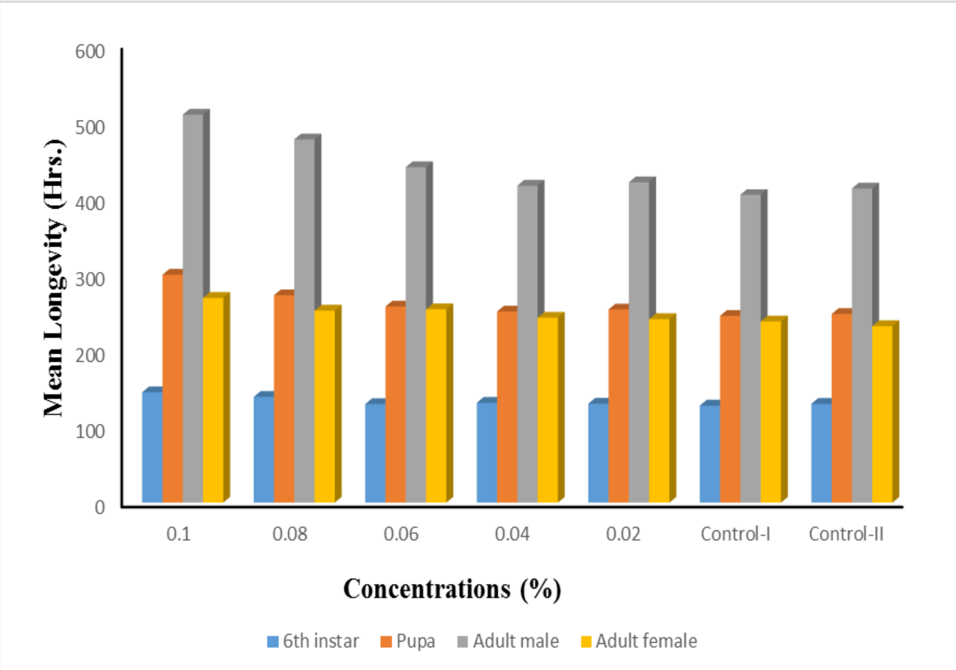

Fig. 11. Larval, pupal and adult longevity following topical application of sublethal concentrations of Acephate on 6th instar larvae of *Diacrisia obliqua*

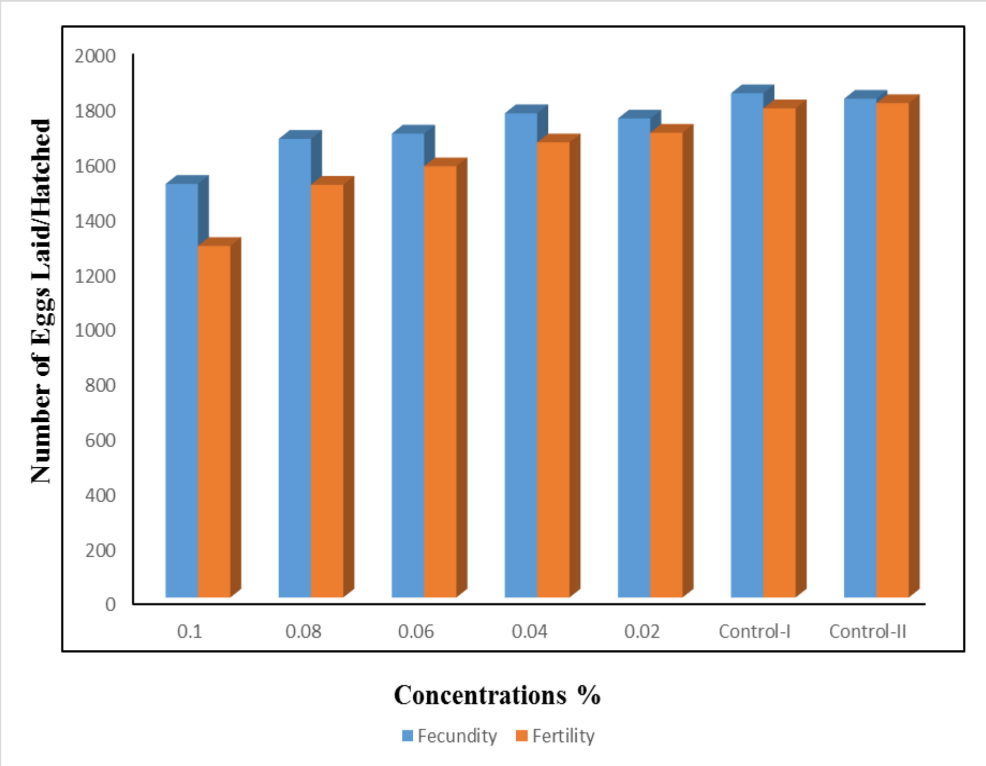

Fig. 12. Fecundity and fertility of the adult females following topical application of sublethal concentrations of Acephate on 6th instar larvae of *Diacrisia obliqua*

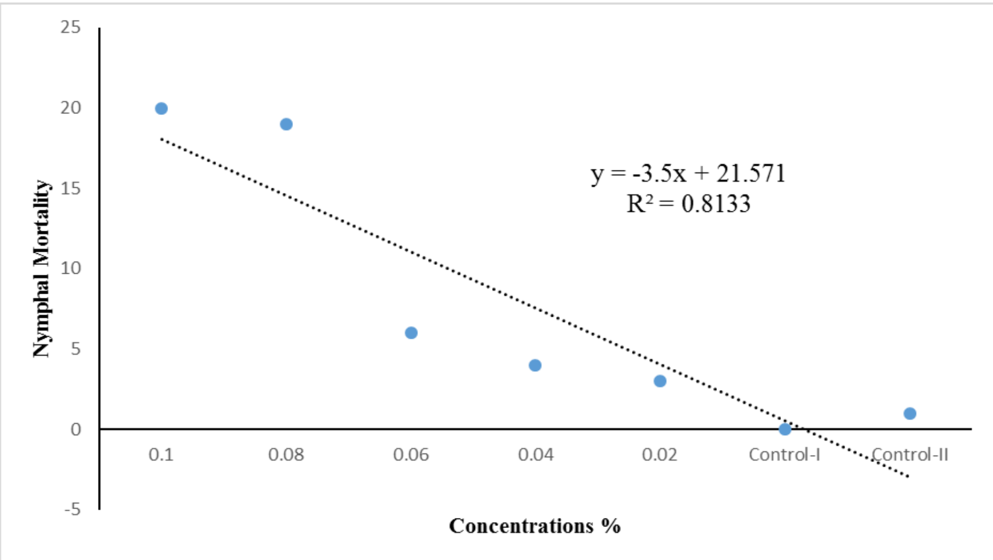

Fig.13. Initial larval mortality within 24 hrs (Knockdown effect) following topical application of sublethal concentrations of Acephate on 6th instar larvae of *Diacrisia obliqua*

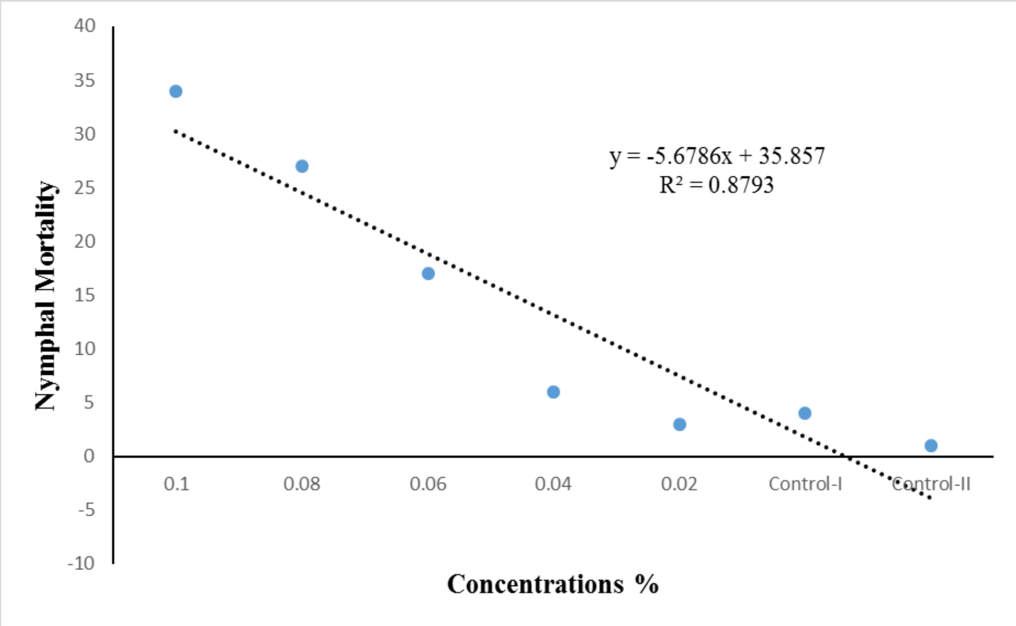

Fig.14. Total larval mortality up to adult emergence (Residual effect) following topical application of sublethal concentrations of Acephate on 6th instar larvae of *Diacrisia obliqua*

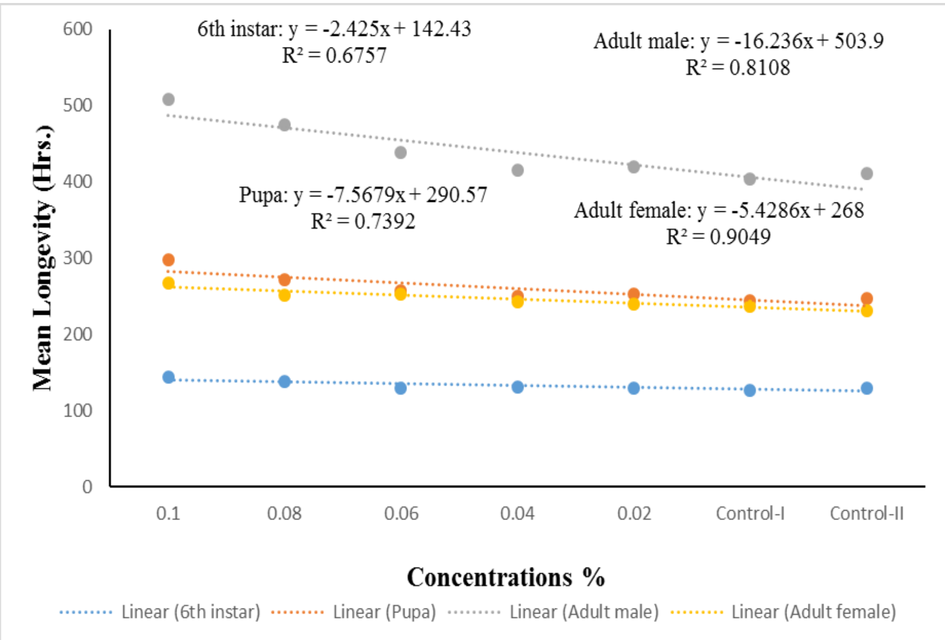

Fig.15. Larval, pupal and adult longevity following topical application of sublethal concentrations of Acephate on 6th instar larvae of *Diacrisia obliqua*.

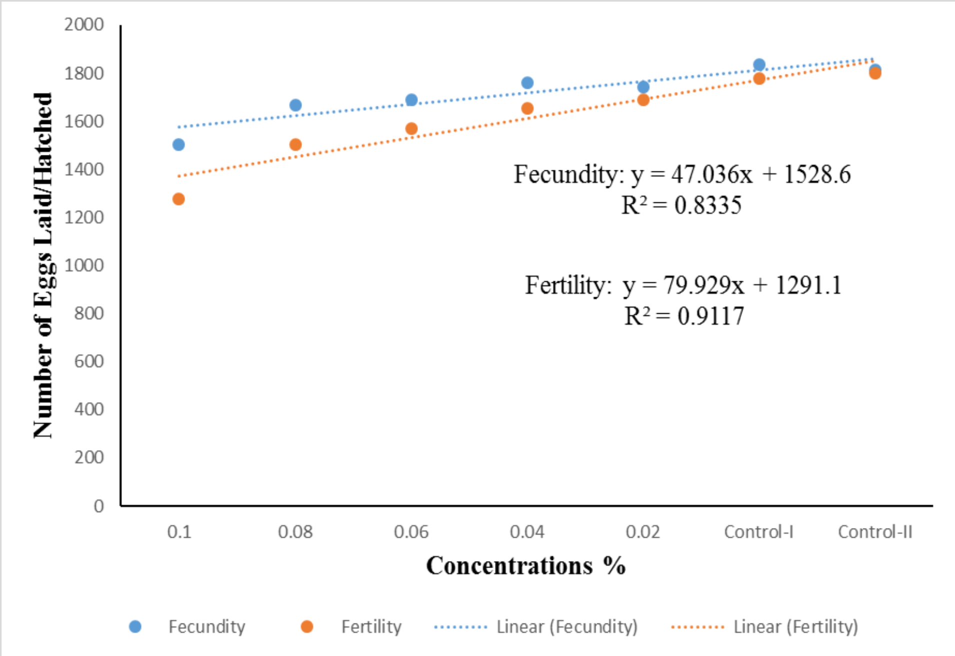

Fig. 16. Fecundity and fertility of the adult females following topical application of sublethal concentrations of Acephate on 6th instar larvae of *Diacrisia obliqua*

REFERENCES

Abbott, V.A.; Nadeau, J.L.; Higo, A.H.; Winston, M.L. (2008) Lethal and sublethal effects of imidacloprid on Osmia lignaria and clothianidin on Megachilerotundata (Hymenoptera: Megachilidae). J Econ Entomol 101:784–796.

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide, J. Econ. Ent. 18: 265-267.

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.

Ali, M. M.; Haque, M. A.; Ahmad, M. (2008). Biological activity of nimbicidine against jute hairy caterpillar, *Spilarctia obliqua* (Walker). J. Agrofor. Environ. 2(2): 107-111.

Bandyopadhyay, S.; Gotyal, B. S.; Satpathy, S.; Selvaraj, K.; Tripathi, A.N.; Ali, N. (2014). Synergistic effect of Azadirachtin and *Bacillus thuringiensis* against Bihar Hairy Caterpillar, *Spilarctia obliqua* Walker. Biopestic. Int. 10(1): 71-76.

Bhadauria, N. K. S.; Bhadauria, N. S.; Jakhmola, S. S. (2001). Larval development and survival of Bihar hairy caterpillar, *Spilosoma obliqua* (Walk.) on different host plants. Indian J. Entomol 63:475–477

Bharathi, K.; Kuttalam, S.; Monoharan, T.; Ravi, M.; Ramasubramanian, T. (2010) Developmentand use of species specific insecticide mixtures:a noval concept for the management of insecticide resistance in Helicoverpa armigera.Indian J. Agric. Sci., 63, 555–558.

Bhattacharya, A. K.; Rathore, Y. S. (1977). Survey and study of the bionomics of major soybean insects and their chemical control. Pantnagar, Nainital, India, G.B. Pant University of Agriculture and Technology, Pantnagar, p 164

Bretveld, R.; Brouwers, M.; Ebisch, I.; Roeleveld, N. (2007). Influence of pesticides on male fertility. Scandinavian Journal of Work, Environment & Health, Vol. 33 (1): 13-28.

C.P.C. (2004) Crop Protection Compendium. CAB International, Wallingford, UK.

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

Devi, N. I.; Srivastava, R. P. (2018). Bioefficacy of Ethiprole 40 + Imidacloprid 40 (glamore 80WG) against bihar hairy caterpillar, *Spilarctia obliqua* (Walker). Indian Journal of Entomology, 80(2): 197-202. DOI No.: 10.5958/0974-8172.2018.00035.4

Dhingra, S.; Bhandari, J. K. S.; Shankarganesh, K. (2007) Relative resistance of Bihar hairy caterpillar to insecticide mixtures. J. Entomol. Res., 31, 209–212.

Eizaguirre, M.; Tort, S.; Lo´pez, C.; Albajes, R. (2005) Effects of sublethal concentrations of *Bacillus thuringiensis* on larval development of Sesamianonagrioides. J Econ Entomol 98:464–470.

Fakhri, M. S. A.; Ansari, M. S.; Khowaja, J; 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. (2012).Comparative toxicity of Quinalphos and Oxydemeton-O-Methyl on life stages of *Dysdercus koenigii* (Fabr.) *Ann. Pl. Protec. Sci. 20(1): 51-54.*

Farag, A. T.; Eweidah, M. H.; El-Okazy, A. M. (2000).Reproductive toxicology of acephate in male mice. Reproductive Toxicology, 14, (5): 457-462.

Farag, A. T.; El-Aswad, A. F.; Shaaban, N. A. (2007). Assessment of reproductive toxicity of orally administered technical dimethoate in male mice. Reproductive Toxicology, 23(2): 232-238.

Farag, A.T.; Radwan, A.H.; Sorour, F.; El Okazy, A.; El-Sayed El-Agamy El-Sebae, A.E. (2010). Chlorpyrifos induced reproductive toxicity in male mice. Reproductive Toxicology, 29(1): 80-85.

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

Galvan, T.L.; Koch, R.L.; Hutchison, W.D. (2005) Effects of spidosad and indoxacarb on survival, development, and reproduction of the multicolored Asian lady beetle (Coleoptera: Coccinellidae). Biol Control 34:108–114.

Goel, S.C.; Kumar, S.; Bhardwaj, D.K. (2004) Salient features and bibliography of Bihar hairy caterpillar, *Spilosoma obliqua* Wlk. (Lepidoptera: Arctiidae). U. P. J. Zool. 24:1–31

Gupta, A. K.; Kaushik, U. K.; Dixit, S. A. (2004). Studies on chemical control of *Spilosoma obliqua* (Walker) under laboratory condition. Journal of Maharashtra Agricultural Universities. 29:228-29

Gupta, G.; Bhattacharya, A. K. (2008). Assessing toxicity of post-emergence herbicides to the *Spilarctia obliqua* Walker (Lepidoptera: Arctiidae). J Pest Sci. 81:9–15

Hussain, M.; Begum, M. (1995). Food preference of jute hairy caterpillar *Spilosoma obliqua* (Walk.) on some varieties of jute. Bangladesh J Entomology, 5(1,2):57-59.

Hussein A. S.; Mohammed, S. A. (2015). Effect of botanical extracts, biological and chemical control against *Spilosoma oblique* on cabbage (Brassica oleracea). Journal of Entomology and Zoology Studies; 3 (1): 43-46.

Ismail, M.; AL-Taher, A.Y. (2011). Effect of propetamphos on the male rats reproductive system. Environmental Toxicology and Pharmacology, 31 (2): 333-338.

Joshi, S.C.; Mathur, R.; Gajraj, A.; Sharma, T. (2003). Influence of methyl parathion on reproductive parameters in male rats. Environmental Toxicology and Pharmacology, 14 (3): 91-98.

Kalleshwaraswamy, C. M.; Basavarajappa, M. P. (2005). Outbreak of *Spilosoma obliqua* Walker on sunflower in Bijapur district of Karnataka. Insect Environment 11:143.

Khan, B. A.; Khan, M. A.; Khowaja, J. and Qamar, A. (1992). Muristerone (a phytoecdysone) as growth inhibitor to *Utetheisa pulchella* (Arctidae: Lepidoptera). *Agric. Biol. Res*. 8(2): 77-81.

Khowaja, J. (2015). Toxic impact of sublethal concentrations of acephate (an organophosphate) on mortality, longevity, fecundity, fertility, and reproductive system of *Dysdercus cingulatus* (Hemiptera: Pyrrhocoridae). Global Journal of Bio-science & Biotechnology (GJBB), Vol. 4(1): 1-5. (ISSN 2250-3560 Print; ISSN 2250- 3579 Online)

Khowaja, J.; Ayesha, Q; 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

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

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

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

Kumar, A.; Bhatnagar, S.; Sharma, R. K. (2013). Toxicity of some commonly used insecticides against *Spilarctia obliqua* (walker). Indian Forester, 139 (3) : 260-263, 2013.

Kumar, V.; Upadhyay, N.; Kumara, V.; Sharma, S. (2015). A review on sample preparation and chromatographic determination of acephate and methamidophos in different samples. Arab. J. Chem. 8, 624–631. doi: 10.1016/j.arabjc.2014.12.007.

Maia, L. O.; Júnior, W. D.; Carvalho, L. S.; Jesus, L. R.; Paiva, G. D.; Araujo, P. (2011). Association of methamidophos and sleep loss on reproductive toxicityof male mice. Environ. Toxicol. Phar. 32, 155–161. doi: 10.1016/j.etap.2011.04.007.

Mandal, D.; Bhowmik, P.; Baral, K. (2013). Evaluation of insecticides for the management of Bihar hairy caterpillar, *Spilosoma obliqua* walk. (Lepidoptera: Arctiidae) in black gram (Vigna mungol.) journal of life sciences ,8(2): 429-431.

Mandal, D.; Bhowmik, P.; Baral, K. (2013). Evaluation of insecticides for the management of bihar hairy caterpillar, *Spilosoma obliqua* walk. (Lepidoptera: Arctiidae) in black gram (Vigna mungo L.). The Biascan, 8(2): 429-431.

Mishra, S. K.; Gupta, V.; Saraf, R. K. (2017). Evaluating the efficacy of novel insecticides against Bihar hairy caterpillar, *Spilosoma obliqua* walker (Lepidoptera: Arctiidae) in soybean crop. International Journal of Plant Protection Volume 10,(2): 241-246

Mohapatra, S.; Ahuja, A. K.; Deepa, M.; Sharma, D. (2011).Residues of acephate and its metabolite methamidophos in/on mangofruit (Mangiferaindica L.). B. Environ. Contam. Tox. 86, 101–104.doi: 10.1007/s00128-010-0154-2

Moreau, C.; Bauce, E. (2003) Lethal and sublethal effects of single and double applications of Bacillus thuringiensis variety kurstaki on spruce budworm (Lepidotpera: Tortricidae) larvae. J Econ Entomol 96:280–286

Muthusamy, R.; Karthi, S.; Shivkumar, M. S. (2011). Baseline susceptibility of five classes of insecticides on Bihar hairy caterpillar *Spilosoma obliqua* (Walk.) (Lepidoptera: Arctiidae). Resistant Pest Management Newsletter, Vol.21 (1): 11- 13.

Nagia, D.K., Kumar, S., Saini, M.L. (1990) Laboratory evaluation of some insecticides against Bihar hairy caterpillar, *Spilosoma obliqua* (Walker) (Arctiidae: Lepidoptera) on castor (Ricinus communis L.). Pl Protec Bull Faridabad 42:13–16

Nair, N.; Sekh, K.; Debnath, M.; Chakraborty, S.; Somchoudhury, A.K. (2007). Relative toxicity of some chemicals to bihar hairy caterpillar, *Spilarctia obliqua* Walker (Arctiidae, Lepidoptera). Journal of Crop Weed. 3(1):1-2

Oliva, A.; Spira, A.; Multigner, L. (2001). Contribution of environmental factors to the risk of male infertility. *Human Reproduction*, 16(8): 1768–1776.

Pan, R.; Chen, H.; Wang, C.; Wang, Q.; Jiang, Y. (2015). Enantio selective dissipation of acephate and its metabolite, methamidophos, during teacultivation, manufacturing, and infusion. J. Agric. Food Chem. 63, 1300–1308.doi: 10.1021/jf504916b.

Perry, A. S.; Samamoto, I.; Ishaaya, I.; Perry, R.Y. (1998) Insecticides in Agriculture and Environment, Springer, Berlin.

Perry, M.J.; Venners, S.A.; Barr, D.B.; Xu, X. (2007). Environmental pyrethroid and organophosphorus insecticide exposures and sperm concentration. Reproductive Toxicology, 23(1): 113-118.

Roush, R.T.; Miller, G.L. (1986) Consideration fordesign of insecticide resistance monitoringprogrammes. J. Econ. Entomol., 79, 293–298.

Selvaraj, K.; Babu, V.R.; Gotyal, B.S.; Satpathy, S. (2015). Toxicity and bioefficacy of individual and combination of diversified insecticides against jute hairy caterpillar, *Spilarctia oblique*. Journal of Environmental Biology, Vol. 36, 1409-1414.

Seth, R.K.; Kaur, J.J.; Rao, D.K.; Reynoldse, S.E. (2004) Effects of larval exposure to sublethal concentrations of the ecdysteroid agonists RH-5849 and tebufenozide (RH- 5992) on male reproductive physiology in Spodoptera litura. J Insect Physiol 50:505– 517.

Sharma, P.; Geetanjly; Rai, V. L. (2015).Toxicity of some insecticides against seven day old larvae of Bihar hairy caterpillar, *Spilarctia obliqua* Walker. Journal of Applied and Natural Science 7 (2) : 960 – 963.

Singh, D. S.; Singh, J. P. (2000). Status of pyrethroid and non-pyrethroid insecticides to the larvae of bihar hairy caterpillar, *Spilarctia obliqua*. Indian Journal of Entomology V 62 (2): 141-145.

Singh, I.; Singh, G. (1992). Assessment of foliage loss caused by different larval instars of Bihar hairy caterpillar, *Spilosoma obliqua* Walker on sunflower. Journal of Insect Science. 1992; 6(2):185-186.

Singh, Y.R.; Varatharajan, R. (1999). Host range of Bihar hairy caterpillar, *Spilosoma obliqua* (Walker) (Arctiidae: Lepidoptera). Hexapoda., 11, 65-74.

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

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

Syed, J. H.; Alamdar, A.; Mohammad, A.; Ahad, K.; Shabir, Z.; Ahmed, H. (2014). Pesticide residues in fruits and vegetables from Pakistan: a review ofthe occurrence and associated human health risks. Environ. Sci. Pollut. Res. 21,13367–13393. doi: 10.1007/s11356-014-3117-z.

Tabashnik, B.E. (1989) Managing resistance withmultiple pesticide tactics: theory, evidence, and recommendations. J. Econ. Entomol., 82, 1263–1269.

World Health Organization [WHO], (2009). The WHO Recommended Classification of Pesticides by Hazard and Guidelines to Classification. Geneva:WHO.

Yadav, J. S.; Valli, M. Y.; Prasad, A. R. (2001). Isolation, identification, synthesis, and bioefficacy of female *Diacrisia obliqua* (Arctiidae) sex pheromone blend. An Indian agricultural pest. Pure Applied Chemistry, 73 (7): 1157–1162.

****