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Influence of Neem Oil on the Morphogenesis of Uzifly Exorista Bombysis

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ABSTRACT:- The diverse biological activity of neem include insect growth regulatory effects, such as moulting disturbances, prevention of pupation and inhibition of adult emergence or malformation production of abnormal pupae and production of sterility in adults (Morduce and Blackwell, 1993). The tetranortriterpinoids, meliantriol, salannin and azadirachtin occurring mainly in seeds act as antifeedants, disruptants of insect growth and development (Jacobson, 1989). Considering the importance of botanicals in the silkworm pest management as an eco-friendly approach, neem oil has been tried to control Uzifly in the present study. Sixty maggots and zero hour pupae of Uzifly were treated topically with 0.5, 1, and 1.5 µl of neem oil /1 µl of acetone with the help of Hamilton microsyrange. The treated resultants were observed for morphological deformities. Controls and Carrier controls were maintained to compare the results and the experiments were replicated six times. The neem oil treated resultants of third instar Uzifly maggots, a few of them died during moulting, some of them developed into maggot-pupal and pupal-adult intermediates, abnormal pupae, abnormal adults and remaining treated third instar maggots developed into morphologically normal adults exhibiting reduced fecundity when compared to the controls. In the neem oil treated resultants of zero hour pupae of Uzifly, a few of them died during adult eclosion, some of them developed into pupal-adult intermediates, abnormal pupae, abnormal adults and remaining treated zero hour pupae of Uzifly developed into morphologically normal adults and fecundity of these treated resultant females was reduced drastically compared to the controls.

Key word: *Neem oil, Uzifly Exorista bombycis, Maggots, Pupal-adult intermediatesAbnormal Pupa, Abnormal Adults.*

I. INTRODUCTION

The Indian neem tree, *Azadiarachta indica* A. Juss has aroused considerable interest as a means of alternative plant protection during the past two decades. The extracts of fruits and leaves cause inhibition of feeding and oviposition, growth disruption and sterility in insects. It exhibits repellant activity at the higher concentration and phagostimulatory effect at the lower concentrations. Many commercial formulations are used in the diversified agro climatic region of the world. Use of neem derivatives is being advocated for the protection of different species of silkworm (Singh *et al*., 1993, Srivastava and Mishra, 2000).

Botanical pest control agents have been identified as attractive alternatives to synthetic chemical insecticides for pest management. Neem acts as a potent sterilant causing reproductive abnormalities including ovarian regression, abnormal/ arrested oocyte development and vitellogenesis (Sreelatha and Geetha, 2008).

Anjali (2008) observed significant reduction in fecundity, hatchability and survival of eggs in Epilachna dodecastigma exposed to sublethal concentration of neem leaf cake and oil.

Application of Melia azedarach (Borges et al., 2003) on Boophilus microplus; Rumex dentatus Hook.(Polygonaceae),Portulaca oleracea L.(Portulacaceae) and Piper cubebae L.(Piperaceae) (Khalaf, 2005) on Fannia canicularis (Koona and Koona, 2006) on the beetles, Clausena dentata (Rutaceae) on Helicoverpa armigera resulted in reproductive abnormalities like reduced reproductive potential, inhibition of egg production, abnormal vitellogenesis and oocyte maturation and disturbance of ovarian proteins synthesis (Malarvannan et al., 2009).

Azadirachta indica A. Juss (Meliaceae) and *Hydnocarpus wightiana* Bl.(Bixaceae) induced significant reduction in the incidence of *Ocimum rhinoceros* (Chandrika Mohan *et al.*, 2000). *Annona squamosa* L.(Annonaceae) extract caused histomorphological derangements in the ovary of *Oryctes rhinoceros* (Sreelatha and Geetha, 2008).

Mixing of *Eupatorium odoratum* leaves with soil in sweet potato beds before planting, reduces weevil infestation. Inhibitory effects of phytochemicals on insect reproduction might be due to histological and biochemical alterations which lead to physiological impairments. Understanding of the malformations and structural deformities of the ovary could potentially be a foundation for devising strategies for safer pest control measures (Rajamma, 1982).

The diverse biological activity of neem include insect growth regulatory effects, such as moulting disturbances, prevention of pupation and inhibition of adult emergence or

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malformation production of abnormal pupae and production of sterility in adults (Morduce and Blackwell, 1993). The tetranortriterpinoids, meliantriol, salannin and azadirachtin occurring mainly in seeds act as antifeedants, disruptants of insect growth and development (Jacobson, 1989).

In fact botanicals are in use in Indian agriculture for over a century to minimize losses caused by pests and diseases (Prakash *et al.*,1997). Applicatiom of fractions of neem seed methanolic extract on *Mythimna separata* Showed juvenile hormone mimic activity to test the insect (Schmutterer *et al*., 1983). Azadirachtin treatment interfered with the normal development of the larvae of Japanese beetle (Ladd *et al.*, 1984). Juvenile hormone activity mimicking compounds isolated from sweet basil oil (Bowers and Nishida 1980) and marigold oil produced deformities in bug species (Saxena and Srivastava 1973).

Repeated use of synthetic pesticides for pest management has disturbed natural biological control systems and led to pest resistance, pest resurgence and secondary pest out breaks. Moreover they are highly persisting accumulating themselves at various concentrations in different levels of ecosystem and are carcinogenic (Kabesh and Jalingo, 2007). Therefore, it has now become necessary to search for an alternative means of pest control, which can minimize the use of synthetic pesticides. The use of botanicals is one of the important alternatives to minimize or replace the use of synthetic pesticides as they possess an array of properties including toxicity to the pest, repellency, antifeedanty and insect growth regulatory activities against pests of agricultural importance (Prakash and Rao, 2003).

Hence, in the present study experiments were conducted to investigate the influence of neem oil on the morphogenesis of Uzifly *Exorista bombycics.*

II. MATERIAL AND METHODS

Neem oil was purchased from local Ayurvedic shop. 0.5, 1 and 1.5 µl of neem oil was dissolved in 1 µl of acetone and used for the treatments.

A. Statistical analysis

The results of the ovipositonal deterrence were expressed as mean ± standard Error (SE) and the data was subjected to ANOVA using sheffee Dunkan and Dunnet as post hoc test using SYTAT version 2011 ($P<0.05$) and ($P< 0.01$) .

B. Results

Sixty third instar maggots of Uzifly were treated topically with 0.5, 1, and 1.5 µl of neem oil with acetone as the carrier solvent with the help of Hamilton microsyrange and the experiments were replicated six times. Controls and carrier controls were maintained to compare the results.

In the neem oil treated resultant maggots of Uzifly, the neem oil, affected moulting, development and induced a wide range of morphological abnormalities over the control and carrier control (Graph: 3.3.2.1).

C. Control and carrier control

In the control all the sixty third instar maggots of Uzifly emerged as normal adults and in the carrier control adult emergence was observed and recorded as 57.17±0.601.

- *TREATED*

D. The influence of 0.5µl of neem oil/1µl of acetone on third instar maggots of Uzifly

Sixty third instar maggots of Uzifly were treated topically with neem oil 0.5µl /1µl of acetone resulted in moulting inhibition and induced various morphogenetic abnormalities in the neem oil treated resultants of the Uzifly and the experiments were replicated six times.

(i). It was recorded that on an average 2.00±0.683 of neem oil treated third instar maggots resultants developed into maggot- pupal intermediates. These forms moulted into mosaics, exhibited maggot abomen with pupal head and vice versa (Plate: 1, fig.C).

(ii). Some of the neem oil treated resultants of third instar maggots failed to pupate and their life cycle was terminated. They were recorded on an average as 9.50±0.224 (Plate:1 -fig, A and B).

(iii) Out of the sixty neem oil treated resultants of third instar maggots, 1.17±0.307 developed into pupal-adult intermediates. These intermediate forms were ruled out from further development.

(iv) O*n an average 13.50±0.224 of* neem oil *treated third instar maggots resultants metamorphosed into normal pupae which eclosed into malformed adults showing abnormal wings and malformed appendages. These deformities prevented the insects from feeding, flying and mating (Plate: 1, fig. D and E).*

(v) Among the neem oil treated resultants of third instar, on an average 29.67±1.054 emerged as normal adults and exhibited low fecundity when compared to controls.

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E. The influence of 1µl of neem oil/ 1µl of acetone on third instar maggots of Uzifly

Sixty third instar maggots of Uzifly were treated topically with neem oil with 1µl/1µl of acetone resulted in moulting inhibition and induced various morphogenetic abnormalities in the 1µl of neem oil /1µl of acetone treated resultants of the Uzifly and the experiments were replicated six times.

(i). Among third instar maggots treated with 1µl of neem oil/ 1µl of acetone, 6.33±0.494 of the treated resultants formed into maggot- pupal intermediates. These forms moulted into mosaics exhibiting maggot abdomen with pupal head and vice versa (Plate -2, Fig. C).

(ii). Some of the neem oil treated resultants failed to pupate and their life cycle was terminated and on an average they were recorded as 18.00±0.365. (Plate -2, Fig. A and B).

(iii) On an average 1.17±0.307 of neem oil treated third instar maggot resultants developed into pupal- adult intermediates. These forms failed to pupate and did not undergo subsequent developmental changes and ultimately died.

(iv) 15.17±0.307 of neem oil treated resultants of third instar maggots of Uzifly developed into adults with malformed appendages. These deformities prevented the insects from feeding flying and mating. (Plate -2, Fig. D and E).

(v) Only 16.00±0.775 of neem oil treated third instar maggots emerged as normal adults.

F. The influence of 1.5µl of neem oil/ 1µl of acetone on third instar maggots of Uzifly

Sixty third instar maggots of Uzifly were treated topically with neem oil of 1.5µl/1µl of acetone resulted in moulting inhibition and induced various morphogenetic abnormalities in the neem oil treated resultants of the Uzifly and the experiments were replicated six times.

(i) Treatment with 1.5µl of neem oil/ 1µl of acetone on third instar maggots of Uzifly caused the formation of 10.00±0.365 maggot- pupal intermediates. These forms moulted with mosaics exhibiting maggot abdomen with pupal head and vice versa.

(ii) 1.5µl of neem oil/ 1µl of acetone treated resultants of third instar maggots of Uzifly failed to pupate and their life cycle was terminated. They were recorded on an average as 21.67±0.333 (Plate:3-Fig.A).

(iii) A few of neem oil treated resultants of third instar maggots which developed into pupal- adult intermediates were observed and recorded as 2.67±0.422. These forms failed to eclose from the pupal case.

(iv) 17.50±0.500 of neem oil treated third instar maggots metamorphosed into malformed adults showing abnormal wings and legs. These deformities prevented the insects from feeding flying and mating. (Plate: 3-Fig.B and C).

(v) Only 5.17±0.601 of treated third instar maggots emerged as normal adults and these adults exhibited low fecundity.

Third instar Uzifly maggot treated resultants were more severely affected than the pupal treated resultants and led to rapid appearance of anomalies in maggots. Similarly neem oil treated larvae caused malformation in adults. It was noted that treated maggots did not pupate and formed maggot-pupal intermediates. The treated resultant maggots have blackened mouth parts and anal regions. The body size is reduced and shriveled in appearance. The pupae that were formed from the treated resultant revealed various abnormalities like abnormal cephalothorax reduced abdominal region, sometimes disproportionate pupal body and general darkening of the pupal body. In the control maggots of Uzifly, all the maggots underwent pupation and metamorphosed into normal adults. Whenever treated resultants maggots metamorphosed into pupae was either abnormal or pupae failed to emerge as normal adults.

G. Influence of neem oil on the zero hour pupae of Uzifly

Sixty zero hour pupae of Uzifly were treated topically with 0.5, 1, and 1.5 µl neem oil /1 µl acetone with the help of Hamilton microsyrange and the experiments were replicated six times. Controls and carrier controls were maintained to compare the results.

In the neem oil treated resultants of zero hour pupae of Uzifly, neem oil affected moulting, development and induced morphological abnormalities in Uzifly *Exorista bombycis* compared to the control and carrier control (Graph:3.3.2.2).

F. Control and carrier control

In the control all the *zero hour pupae* of Uzifly emerged as normal adults and in the carrier control adult emergence was recorded on average was as 57.17±0.601.

- *TREATED*

G. The influence of 0.5 µl of neem oil/ 1µl of acetone on zero hour pupae of Uzifly

Sixty zero hour pupae of Uzifly were treated topically with neem oil 0.5µl/1µl of acetone resulted in moulting inhibition and induced various morphogenetic abnormalities in the treated resultants of the Uzifly and the experiments were replicated six times.

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(i) Zero hour pupae of Uzifly, *Exorista bombycis* were treated topically with 0.5µl of neem oil /1µl of acetone. In the neem oil treated resultant pupae 15.5±0.563 failed to develop normally and did not accomplish the adult emergence (Plate: 4, fig. A).

(ii) On an average 0.83±0.167 of 0.5µl of neem oil /1µl of acetone treated resultants developed into pupal-adult intermediates. These intermediate forms remained inactive and died after few days. (Plate: 4, fig. B).

(iv) On an average 17.50±0.500 metamorphosed into malformed adults with deformed wings of the treated zero hour pupae of Uzifly with 0.5µl of neem oil /1µl of acetone. These deformed wings which prevented the insects from feeding flying and mating (Plate: 4, fig.-C, D and E).

(v) Only 38.50±0.885 of zero hour pupae of Uzifly treated with 0.5µl of neem oil /1µl of acetone emerged as normal adults and exhibited low fecundity.

H. The influence of 1 µl of neem oil/ 1µl of acetone on zero hour pupae of Uzifly

Sixty zero hour pupae of Uzifly were treated topically with neem oil with 1µl/1µl of acetone resulted in moulting inhibition and induced various morphogenetic abnormalities in the treated resultants of the Uzifly and the experiments were replicated six times.

(i) 21.83±0.601 of neem oil treated resultants of zero hour pupae failed to develop normally and did not accomplish the adult emergence (Plate: 5, fig. A).

(ii) 1µl of neem oil /1µl of acetone was topically applied on the zero hour pupae of Uzifly. On an average 1.67±0.211 of neem oil treated resultants of zero hour pupae of Uzifly developed into pupal- adult intermediates. These forms failed to eclose from the pupal case.

(iv) 17.50±0.500 of neem oil treated zero hour pupae of Uzifly emerged into malformed adults with deformed wings. These deformed wings prevented the insects from feeding flying and mating (Plate:5, fig. B and C).

(v) When 1µl of neem oil /1µl of acetone was topically applied on the zero hour pupae of Uzifly, only 29.50±0.922 of treated pupae emerged as normal adults and exhibited low fecundity when compared to the controls.

I. The influence of 1.5 µl of neem oil/ 1 µl of acetone on zero hour pupae of Uzifly

Sixty zero hour pupae of Uzifly were treated topically with neem oil with 1.5µl/1µl of acetone resulted in moulting inhibition and induced various morphogenetic abnormalities in the treated resultants of the Uzifly and the experiments were replicated six times.

(i) 1.5 µl of neem oil/ 1 µl of acetone treated resultants, 29.16±0.307 failed to develop normally and did not accomplish the adult emergence (Plate: 6, fig. A).

(ii) On an average 1.83±0.307 of neem oil of treated resultants of zero hour pupae of Uzifly developed into pupal- adult intermediates. These intermediate forms failed to eclose from the pupal case, remained inactive and died after few days.

(iv) 1.5 µl of neem oil/ 1 µl of acetone was topically applied on the thoracic region of the zero hour pupae of Uzifly. On an average 17.50±0.500 of treated zero hour pupae emerged as malformed adults with deformed wings. These deformities prevented the insects from feeding, flying and mating (Plate: 6, fig. B-D).

(v) On an average only 14.67±0.422 of neem oil treated zero hour pupae, emerged as normal adults and these adults exhibited low fecundity compared to control.

III. DISCUSSION

The present study clearly indicates the fact that neem oil disturbs the development of Uzifly, *Exorista* bombycis. Neem oil interferes with the normal development and metamorphosis of Uzifly which was manifested at different stages of the life cycle. Topical application of neem oil to the third instar maggots and zero hour pupae of Uzifly led to morphogenetic abnormalities during development.

Neem oil prevented normal development of third instar maggots of Uzifly, resulting in prolongation of maggot period, permanent maggot, death of maggot during metamorphosis and production of maggot – pupal intermediates preventing their transformation into pupae. These treated maggots which metamorphosed into pupae were also abnormal with euvium attached to the wings pads and non viable.

Pupation and adult emergence was higher in control than the treated third instar maggots and zero hour pupae of Uzifly with neem oil. According to Babu *et al.*, (1997) reduced adult emergence in *Achae janata* L. is due to the impact of 0.25% neem leaf extract. When larvae and pupae of diamond black moth were applied topically with neem seed kernal extract, it adversely affected their development and most of the treated insects surviving to the adult stage had deformed wings (Tan and Sudderuddin, 1978).

The impaired puapl-adult transformation resulting in adult deformities may be due to persistent metamorphic and morphogenetic action of neem oil as also reported by Ghoenim et al.,(2000) working with neem seed extract on Spodoptera littoralis by Koul et al.,(1987).

Neem oil resulted in marked growth and developmental disruption and inhibition of metamorphosis in the resultants of third instar maggots and zero hour pupae of Uzifly. This disruption involved a number of effects including inhibition of growth, induction of permanent maggot, death

during moulting, inhibition of pupation, deranged maggot-pupal, pupal-adult transformation, prevention of adult eclosion and reduction in adult emergence. Only a few of neem oil treated maggots and zero hour pupae developed into normal adults.

Inhibition of adult emergence in the neem oil treated resultant of third instar maggots and zero hour pupae was observed high when compared to the control and carrier control. These results are in agreement with the reports of Khaire et al., (1992) and Abdullahi et al., (2011).

The present study reveals that the morphogenic defects caused by different concentrations of neem oil in the treated resultants of third instar maggots and zero hour pupae of Uzifly were dose dependent. It was observed that at higher dose, the incidence of inhibition in the development was most prominent. The adult emergence inhibition observed can be attributed to the inherent properties of neem oil. Thus, the ability of neem oil to affect the development of Uzifly maggot/pupae after exposure to different concentrations of neem oil can be attributed to the growth disrupting effect. The results showed that the action of these neem oil treatments cause physiological disturbances leading to growth abnormalities like prolongation of pre-pupal period, incomplete metamorphosis and deformed pupae and adults. Thse results are in agreement with the reports of Tiwari et al., (2006).

In the present study the effects of administering of neem oil topically on the third instar maggots and zero hour pupae of Uzifly have detrimental effects on the development of maggot and pupae of Uzifly which led to eclosion of abnormal adults. The third instar maggot of Uzifly perished directly before moulting or in the process of moulting. Usually maggots were unable to moult normally and the exuviae was found attached to their body. These observations especially the moulting defects produced by neem oil may be due to its effects on neuroendocrine system of the Uzifly or could be due to the higher level of juvenile hormone than normally persisted in the Uzifly, allowing the growth but inhibiting metamorphosis as also reported by Grisakova et al., (2006).

Neem oil induced different morphological abnormalities in the treated resultants of maggots and zero hour pupae of Uzifly. The abnormalities include development of maggot, failure to emerge from the pupal case and incomplete development of wings in adults. This may suggest the presence of high juvenile hormone levels in the maggot or due to chemical compounds in the neem oil preventing normal pupation and normal emergence of adult. These results are in agreement with the reports of Crook et al., (2008). These morphological features may be due to the failure of the wings to expand and flatten after adult insect emergence as also been reported by Ogendo et al., (2004).

The bioactive compounds present in the neem oil may have caused pupal inhibition by direct interference with insect physiological balance in the treated resultants of third instar maggots and zero hour pupae of Uzifly as also reported by Pierce and Schmidt, (1993). The restlessness in the treated insects before death may suggest hormonal involvement in the action of neem oil as also reported by Rani and Jamil, (1989).

The present result reveals that neem oil treatments affected the larval, pupal transformation and produced intermediate forms along with adult abnormalities and affected growth of the emerged adults, which were superficially normal but with deformed elytra in the treated resultants of third instar maggots and zero hour pupae of Uzifly. All the abnormal larvae and maggot-pupal intermediates failed to survive long, and the abnormal adults were unable to mate or oviposite as also been reported by El-Domiaty et al., (2003).

In present study shrinkage of the pupae and folding of the wings of adults as a result of treatment with neem oil could be due to phytochemicals present in the neem oil as also been reported by Sabry (2004).

Neem oil may be an ecdysone agonist causes alterations to ecdysteroid and Juvenile Hormone (JH) titers by blocking the release of morphogenetic peptides from the brain, causing reduced growth, increased mortality and abnormal and delayed moults as also reported by Anuradha and Annadurai (2008) and by Nathan et al., (2007).

Compounds present in neem oil exhibited effects on developmental stages of exposed third instar maggots of Uzifly, which can produce morphological abnormalities in different developmental stages such as lack of melanization in maggot and pupal stages, dead maggot-pupal intermediate stage with the head of pupa and the abdomen of a maggot, dead adults with folded wings in pupal exuvium and emerged adults unable to escape the pupal exoskeleton, half ecdysed adults etc. The inhibition of metamorphosis as a result of neem oil application affected the emergence of adults due to hormonal disturbance and/or interference in chitin synthesis during the moulting process as also observed by Rameshwar Singh Rattan and Anuradha Sharma, (2011) working with plant secondary metabolites on Diamondback Moth (Plutella xylostella l.).

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Plate - 1

Morphological deformities of 0.5µl neem oil treated third instar maggots of uzifly resultants

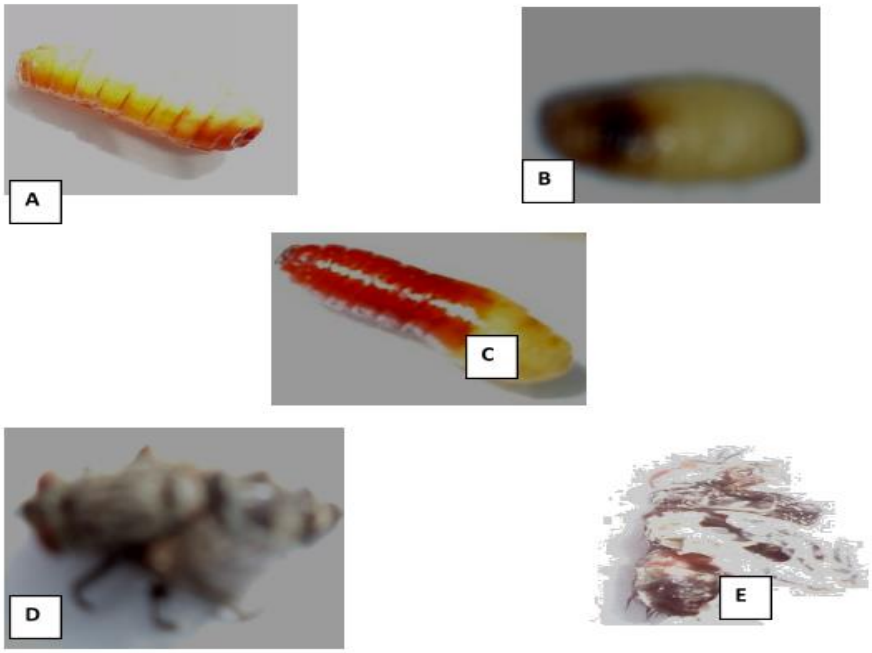

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PLATE: 1

LEGENDS

Third instar Uzifly maggots of treated resultants of Uzifly

Fig – C- Maggot- pupal intermediates

Fig –A and B - Maggots failed to pupate

Fig –D and E - Abnormal wings and malformed appendages

Plate - 2

Morphological deformities of 1µl neem oil treated third instar maggots of uzifly resultants

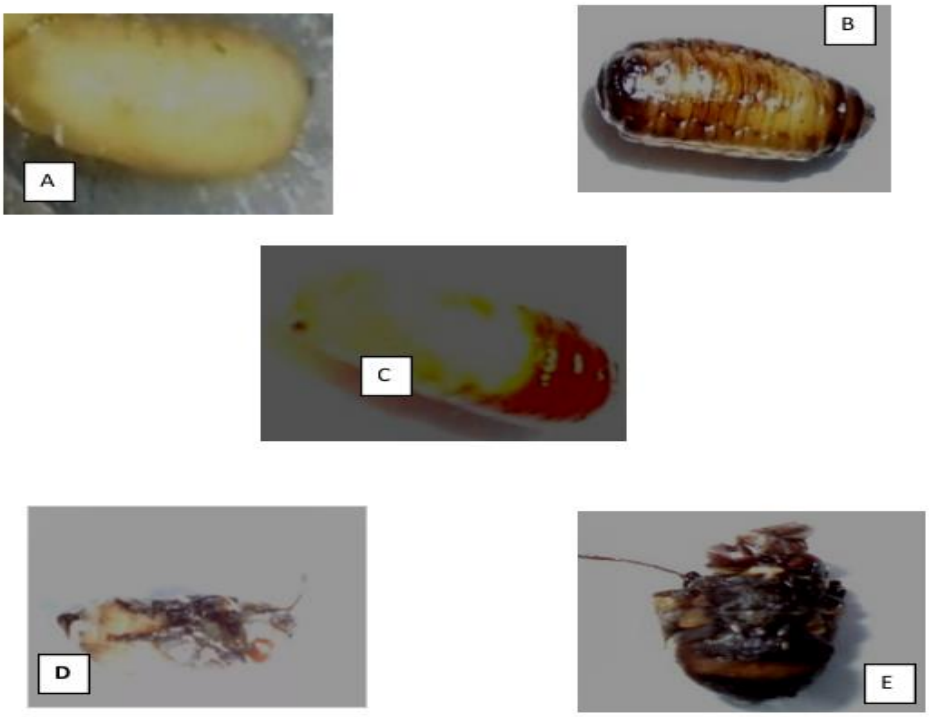

PLATE: 2

LEGENDS

Fig – A and B - Maggots failed to pupate

Fig –C- Maggot- pupal intermediates

Fig –D – Malformed adult with exuviae attached to the anterior region

Fig –E – Adults with malformed appendages

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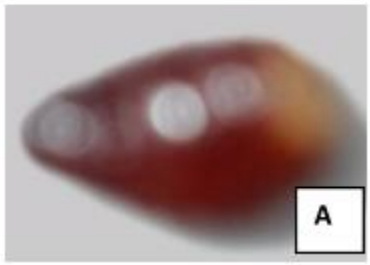
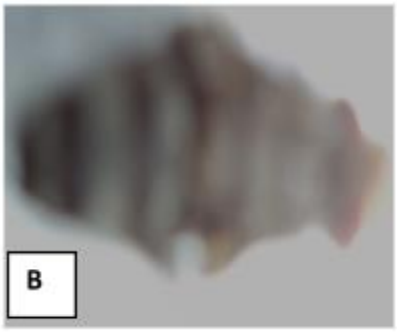
Plate - 3

Morphological deformities of 1.5µl neem oil treated third instar maggots of uzifly resultants





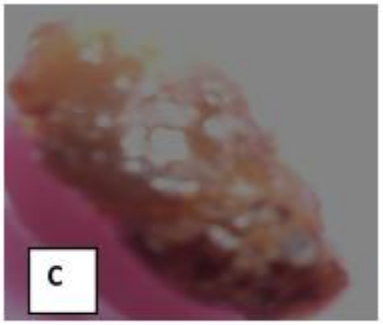


Plate: 3

LEGENDS

Fig –A - Maggot failed to pupate

Fig – B and C – Adults with malformed appendages

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Plate - 4

Morphological deformities of 0.5µl neem oil treated resultants of Uzifly of zero hour pupae

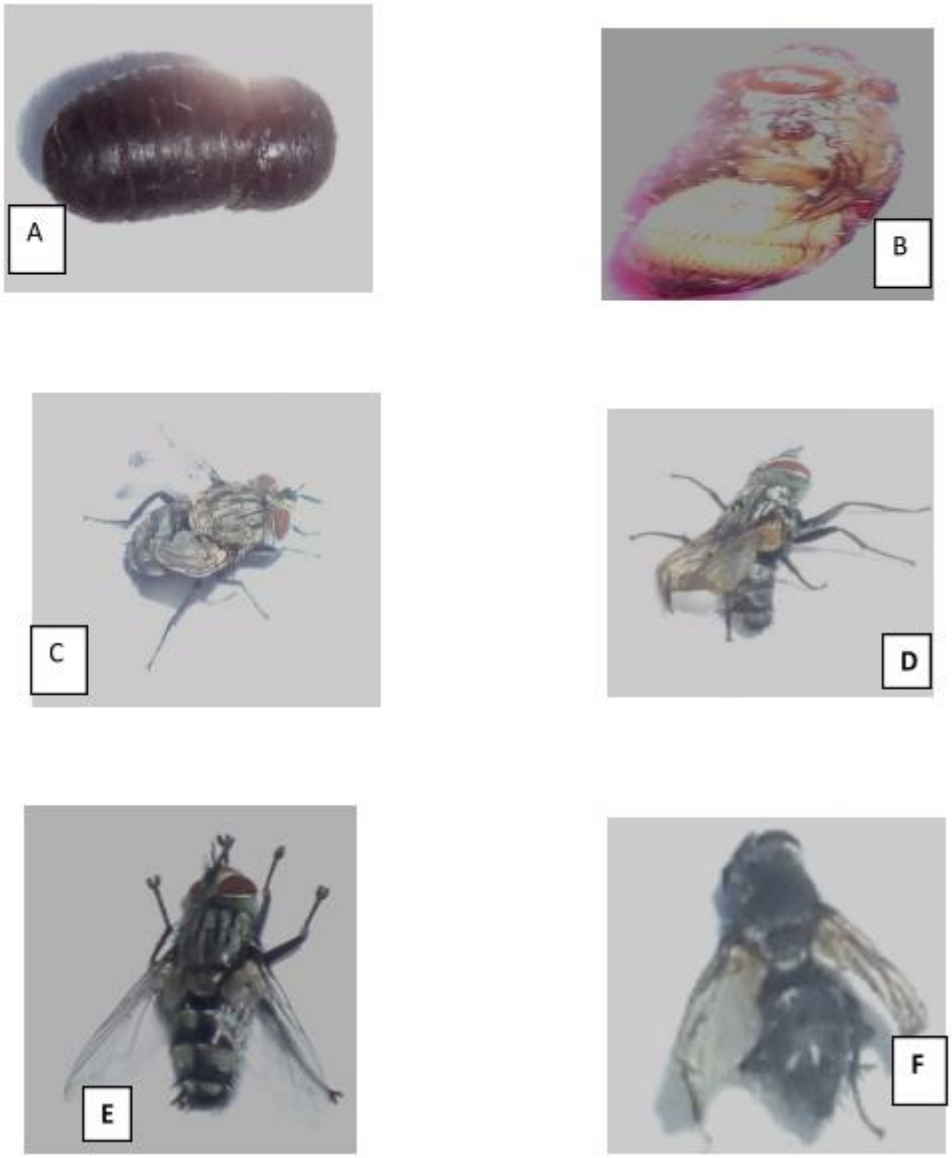


Plate - 4

LEGENDS

Zero hour pupae of Uzifly treated resultants of Uzifly

Fig -A - Malformed pupae

Fig – B - Pupal – adult intermediate

Fig – C, D& E – Adult with deformed wings and malformed appendages

Plate-5

Morphological deformities of 1µl neem oil treated zero hour pupae resultants of Uzifly

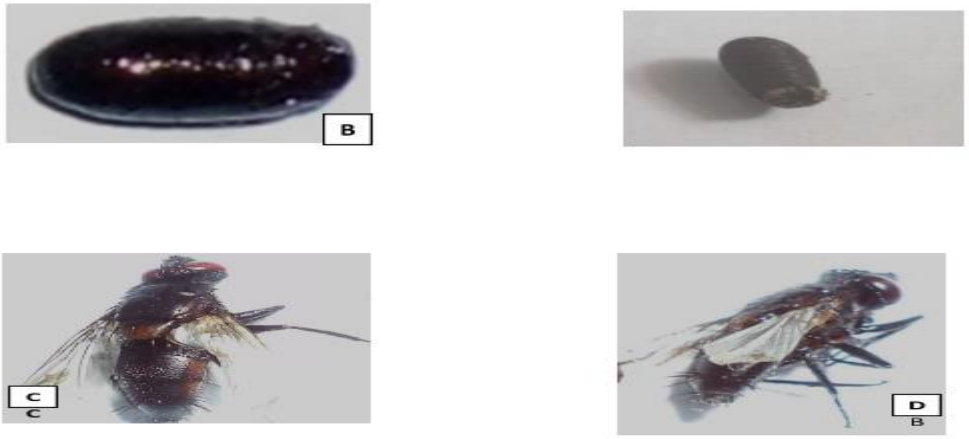


Plate - 5

LEGENDS

Fig –A- Pupa failed into adult eclosion

Fig –B- Adult unable to extricate from the pupal case

Fig- B & C– Adults with deformed wings and appendages

Plate-6

Morphological deformities of 1.5 µl neem oil treated zero hour pupae resultants of Uzifly

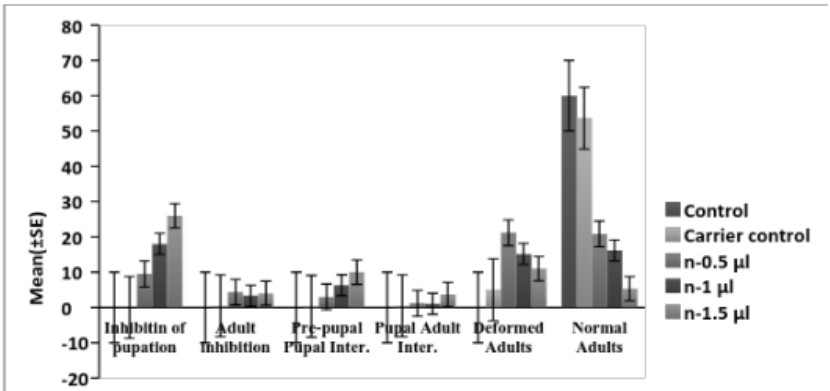
Plate - 6

LEGENDS

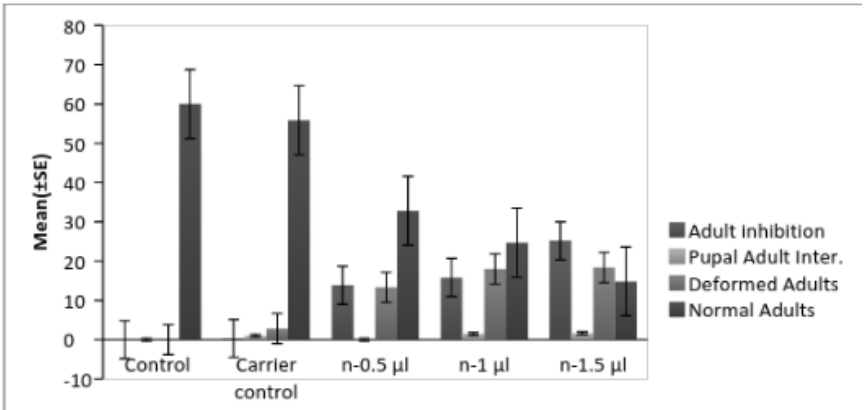
Zero hour pupae of Uzifly treated resultants of Uzifly of Uzifly

Fig –A – Pupae failed to eclose into adult and shriveled in appearance

Fig. B , C and D – Adults with deformed wings and appendages



Graph: 3.3.2.1 Influence of neem oil on third instar maggots of Uzifly



Graph: 3.3.2.2 Influence of neem oil on zero hour pupae of uzifly