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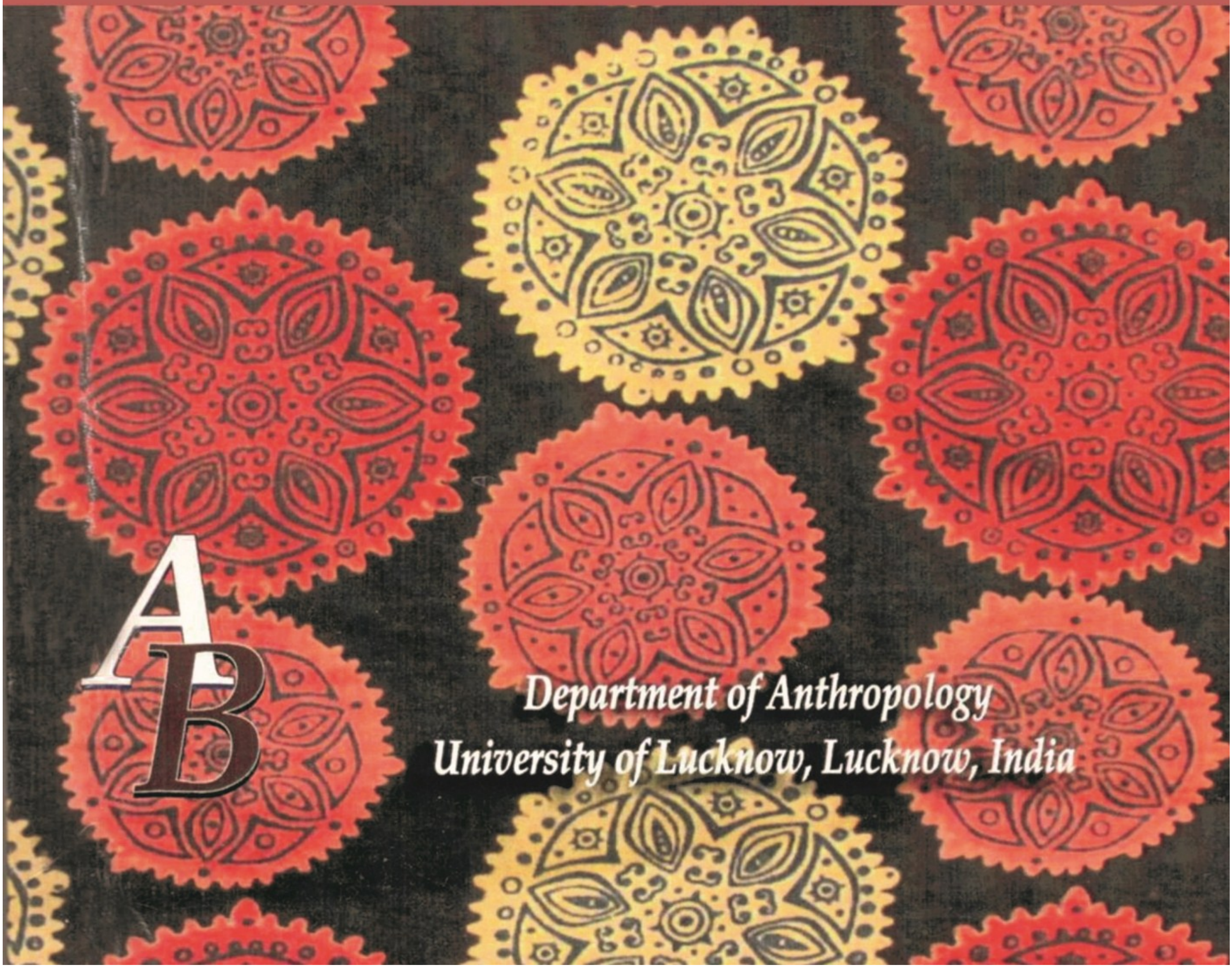

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Muristerone (Phytoecdysone) as inhibitor of growth and reproductive potential of *Spodoptera litura* Fabr. (Lepidoptera: Noctuidae)

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ABSTRACT

An experiment was conducted to evaluate the efficacy of topically applied five different doses viz., 0.5µg, 1.0µg, 2.0µg, 4.0µg, and 8.0µg of Muristerone on two advanced stages i.e. 5th and 6th instar larvae of Spodoptera litura. Following the topical application of the above said doses on the newly moulted 5th instar larvae, the total larval mortality up to pupal stage were 05%, 09%, 11%, 14% and 19% respectively as compared to control-I (00%) and control-II (00%), however the total larval pupal loss up to adult emergence were 08%, 11%, 19%, 28% and 43% respectively as compared to control-I (01%) and control-II (00%). The total number of eggs laid by the affected females emerged from the treated larvae were 1760, 1743, 1727, 1643 and 1509 respectively as compared to control-I (1765) and control-II (1781) and the total number of eggs hatched were 1729, 1701, 1661, 1523 and 1351 respectively as compared to control-I (1740) and control-II (1769). When the same doses of Muristerone were applied topically on the newly moulted 6th instar larvae, the corresponding larval loss were 02%, 05%, 04%, 13% and 17% respectively as compared to control-I (00%) and control-II (01%) however, the total larval and pupal death up to adult emergence were 06%, 11%, 11%, 25% and 33% respectively as compared to control-I (00%) and control-II (00%). The fecundity of the affected females emerged from the 6th instar treated larvae were 1771, 1763, 1733, 1658 and 1521 respectively as compared to control-I (1787) and control-II (1790) and the total fertility of the eggs were 1723, 1669, 1603, 1509 and 1301 respectively as compared to control-I (1770) and control-II (1779). The toxicity of Muristerone manifested itself by an increase in mortality, decline in larval-pupal formation, reduction in reproductive potential together with disruption of development.

Keywords: Armyworm; tobacco caterpillar, Spodoptera litura; Muristerone; Mortality; Development; Fecundity. Fertility

Introduction

The evolutionary success of arthropods, including insects, is accreditable to the development of the cuticle, which is a complex exoskeleton with diverse functions. The main structural components of the cuticle are cuticle proteins, chitin, lipids, catecholamines, and minerals. The exoskeleton supports muscle attachment for locomotion, prevents physical and

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chemical damage, and protects against infectious diseases and water loss. To meet the demands of development and metamorphosis, insects need to periodically shed old chitinous cuticles during their transformation into an adult through a process controlled by complex hormonal mechanisms known as ecdysis (Zhu *et al*., 2016).

In holometabolous insects the timing of moulting and metamorphosis is undoubtedly controlled by the moulting hormone "ecdysone" which is a prohormone secreted by the prothoracic glands of the larvae (Ishimoto *et al*., 2010) that controls the body size by regulating the duration of growth in coordination with juvenile hormone (Boulan *et al*., 2015; Nijhout *et al*., 2014; Mirth *et al*., 2005); Yamanaka *et al* (2013); Callier *et al* (2013). The ecdysones are steroidal structure therefore they are generally called ecdysteroids (Hoffmeister *et al*., 1965; Karlson and Hoffmeister, 1963).

The word ecdysteroid originates from the Ancient Greek word *ecdysis*, which means "peeling", ''the detaching of an old exoskeleton in insects". Depending on the nature of source, ecdysteroids (ECs) are subdivided into three groups: phytoecdysteroids (PEs), zooecdysteroids, and mycoecdysteroids.

Phytoecdysteroids (PEs) are naturally occurring steroid-like compounds found in various plants as secondary chemical (as a protection agent, toxins or antifeedants), primarily acting as chemical defenses against herbivores, particularly insects against herbivorous insects (Dinan *et al*., 2001). They share a similar four-ringed structure and are broadly classified based on the number of carbon atoms, 27 to 30, where Muristerone is a prominent C27 ecdysteroids found in several species of plants.

The exogenous application of ecdysone or its analogues including synthetic compounds or those extracted from plants (phytoecdysones) disorders moulting, metamorphosis and reproduction in several species of insects (Earle *et al*., 1970; Marielli, 1978; Singh & Russell, 1980; Ahmad & Khan 1982 & 1985; Khan *et al*., 1984; Kubo *et al*., 1984; Khan and Khan, 1986; Khan *et al*., 2002). The exogenous ecdysones or their analogues are generally non-toxic to non-target organisms and are environmentally safe particularly as a component of integrated pest management for the control of insect pests. With this view, investigations were carried on evaluating the toxicity of Muristerone against the leaf feeding polyphagous *Spodoptera litura.*

Spodoptera litura (Fab.) (Lepidoptera: Noctuidae), commonly known as the tobacco caterpillar or cotton leafworm, is a substantially devastating and extensively strewing agricultural pest that poses an expressive danger to world-wide crop production. Several factors support the endurance and prevalent dispersal of *S. litura*. According to Ramana *et al.* (1988), Armes *et al.* (1997), Qin *et al.* (2004), Shukla and Patel (2011), Ahmad *et al*. (2013), it is an iniquitous leaf feeding polyphagous insect having a wide host plants, invading more than 120 economically important plant species in India and worldwide such as cotton (*Gossypium* spp.), soybean (*Glycine max*), groundnut (*Arachis hypogaea*), tomato (*Solanum lycopersicum*), tobacco (*Nicotiana tabacum*), castor (*Ricinus communis*), and various vegetables from the Cucurbitaceae and Brassicaceae families. The adaptableness of *S. litura* to varied host plants, coupled with its fast developmental cycle and high fecundity, renders it a constant and austere menace to agricultural production, particularly in tropical and subtropical regions worldwide (Natikr & Balikai, 2017). This moth is mostly active at night, and because of their high mobility and capability to lay their eggs on a wide range of host plants, they help or even assures the existence of *S. litura* under a divers environmental condition (Anjali *et al*., 2014; & Naik *et al*., 2017). The larval stage shows voracious eating behaviour, devouring leaves, stems, flowers, and even fruits (Yinghua *et al*., 2017). This feeding activity leads to austere defoliation, demoted photosynthetic efficacy, and overall plant weakening, resulting in significant yield losses. Under grave infestations, plants may be totally defoliated, leading to yield reductions of up to 80% in the absence of effective pest management strategies (Patil, 2015). Furthermore, the indirect impact of *S. litura* infestations includes increased susceptibility of crops to secondary infections by pathogens, further compounding economic losses. It also becomes resistant to many commonly used insecticides, particularly pyrethroids and carbamates, resulting in failure of effective controls (Wu *et al.* 1995; Kranthi *et al*. 2002; Ahmad *et al*. 2007; Huang and Han 2007).

Materials and Methods

Adults of *Spodoptera litura* F. were collected on light during the monsoon months and pairs of adults were kept in glass rearing jars (measuring 20x15 cms) and were covered with a piece of sterilized muslin cloth which was tightly fixed by means of a rubber band to maintain the stock-culture in a B.O.D. cabinet controlled at 28 + 2°C temperature and 70-80 relative humidity. The adults were offered 5% glucose solution soaked in sterilized cotton wool. Folded sterilized paper strips 2"x20" were placed in each jar for egg laying. After hatching the larvae were provided with fresh sterilized castor (*Ricinus communis*) leaves to eat. These larvae pupated either 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 (Khowaja, 2018). For treatment one hundred newly moulted 5th and 6th instar larvae in 4 replicates were sorted out for experimental purpose. Five different doses of muristerone (viz, 0.5 µg, 1.0 µg, 2.0 µg, 4.0 µg, and 8.0 µg) were prepared in acetone solution. Freshly moulted 5th and 6th instar larvae were individually topically treated with 1 µl dose of each dilution with the help of a 2 ml tuberculin syringe fitted to a manually operated micro-applicator. A parallel acetone (Analytical grade) treated (control-I) as well as untreated (control-II) insects of the corresponding age and stage was maintained at the above-mentioned controlled conditions.

To record the egg production and egg hatching, the adult females which emerged from the treated larvae by each dose were paired with the males emerged out from untreated larval stock of same stage and age. For control, females emerged out from acetone treated larvae were similarly paired with the males emerged from untreated larvae. The response variables were subjected to one-way analysis of variance (Snedecor & Cochran, 1967; Sokal & Rohlf, 1981) to determine significance differences ($P<0.001$) among the treatments. The larval mortality was observed after 24 and the data were corrected by adjusting mortality in controls using Abbott's formula (Abbott's, 1925). 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

Following the topical application of 0.5µg, 1.0 µg, 2.0 µg, 4.0 µg, and 8.0 µg doses of Musristerone on freshly moulted 5th instar larvae, the mortality of the larvae increases linearly with increasing doses showing positive correlation ($y = 1.6586x + 6.4583$, Fig 01). Similarly, the mortality of the pupae also increases linearly showing positive correlation with increasing doses ($y = 1.5403x + 0.625$, Fig. 01). The total larval death up to pupal stage was 05%, 09%, 11%, 14% and 19% respectively as compared to control-1, whereas, the total pupal mortality was 02%, 02%, 03%, 07%, and 13% respectively as compared to control (Fig. 02). The mortality of the larvae seems to be due to the direct toxicity of the chemical rather than due to its hormonal effects on the physiological processes because just after moulting the larval skin was soft, untanned and not fully sclerotized, that may be vulnerable to the hormonal treatment. Thus, it appears that freshly moulted partially sclerotized cuticle was more permeable to the exogenous application of the chemical rather than fully sclerotized cuticle of fully grown larvae. The abnormalities in pupae and adults also increase linearly with increasing doses of Muristerone ($y= 1.4005x + 0.4583$ and $y=1.8602x + 1.8333$ respectively, Fig. 03). Out of the total pupae formed 01%, 00%, 05%, 07% and 11% pupae either died during pupal formation or could not cast off their exuviae completely leading to the total loss of 09%, 13%, 17%, 27% and 39% up to adult emergence as compared to control-1 (Fig.03). The respective adults which emerged from the affected survived pupae 02%, 07%, 09%, 16% and 21% adults were with malformed wings or legs or both (Fig. 04). Earlier Khan *et al.*, (2002) also tested 5 different doses (viz., 0.5µg, 1.0 µg, 2.0 µg, 4.0 µg, and 8.0 µg) of Polypodine-B (a phytoecdysone) on two advanced nymphal stages (4th and 5th) of *Dysdercus cingulatus* by topical and injection method and reported that the above said doses beside causing nymphal mortalities also produce the abnormalities in wings and legs of the adult.

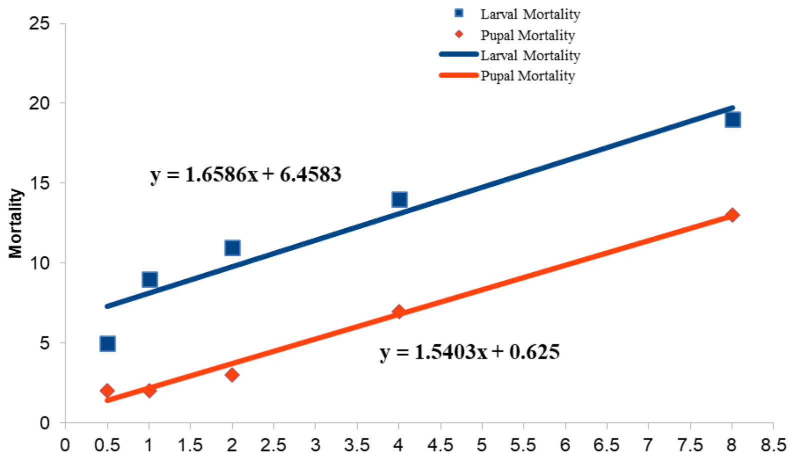

Figure 01: Larval and pupal mortality of Spodoptera litura following topical application of Muristerone on 5th instar larvae

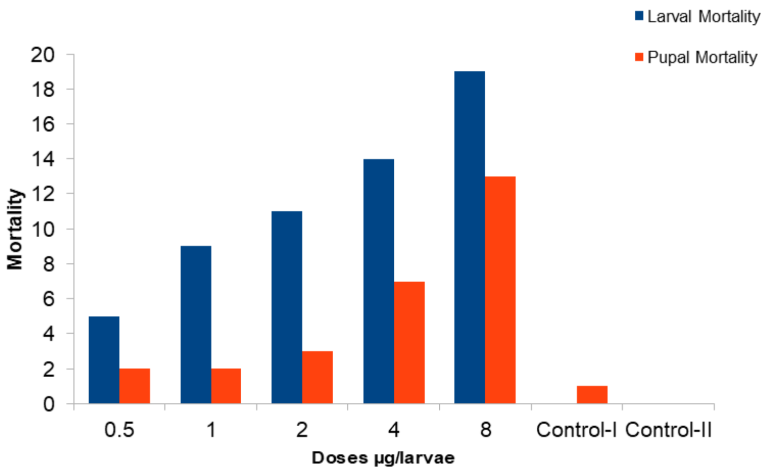

Figure 02: Larval and pupal mortality of *Spodoptera litura* following topical application of Muristerone on 5th instar larvae

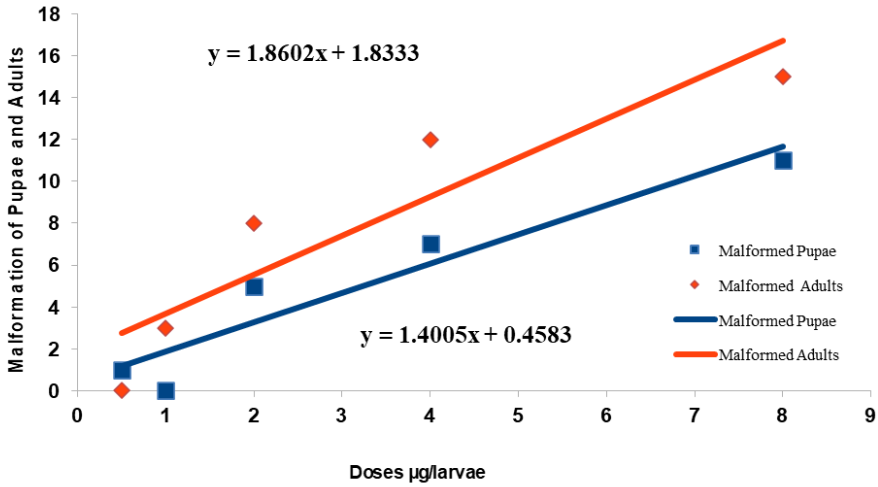

Figure 03: Malformation in pupae and adults of *Spodoptera litura* following topical application of Muristerone on 5th instar larvae.

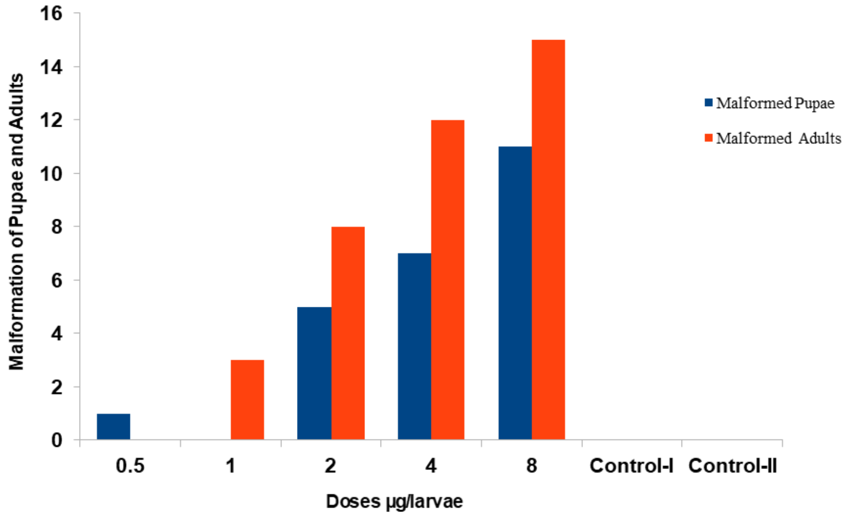

Figure 04: Malformation in pupae and adults of *Spodoptera litura* following topical application of Muristerone on 5th instar larvae.

When the same above said doses (0.5µg, 1.0 µg, 2.0 µg, 4.0 µg, and 8.0 µg doses) were applied on freshly moulted 6th instar, the mortality of the larvae and pupa also increase linearly with increasing doses (y= 2.0134x + 1.9583 and y= 0.4704x + 3.5417 respectively, Fig. 05) resulting in 02%, 05%, 04%, 13% and 17% larval loss as compared to 01% in the control-I and 04%, 03%, 05%, 06% and 07% pupal loss respectively (Fig. 06). Thus, the total larval pupal loss up to adult emergence following the topical application of the above said doses on 6th instar larvae was 06%, 08%, 09%, 19% and 24% respectively as compared to control-I

(00%). The abnormalities during pupal formation as well as the emergence of malformed adults also increase linearly with increasing doses of muristerone (y=1.1022x + 0.5833 and y=2.2043x + 0.1667 respectively, Fig. 07). The total number of deformed pupae following the exposure of various doses of muristerone was 00%, 03%, 02%, 06% and 09%, whereas 05%, 03%, 09%, 07% and 14% adults were with malformed wings or legs or both (Fig. 08).

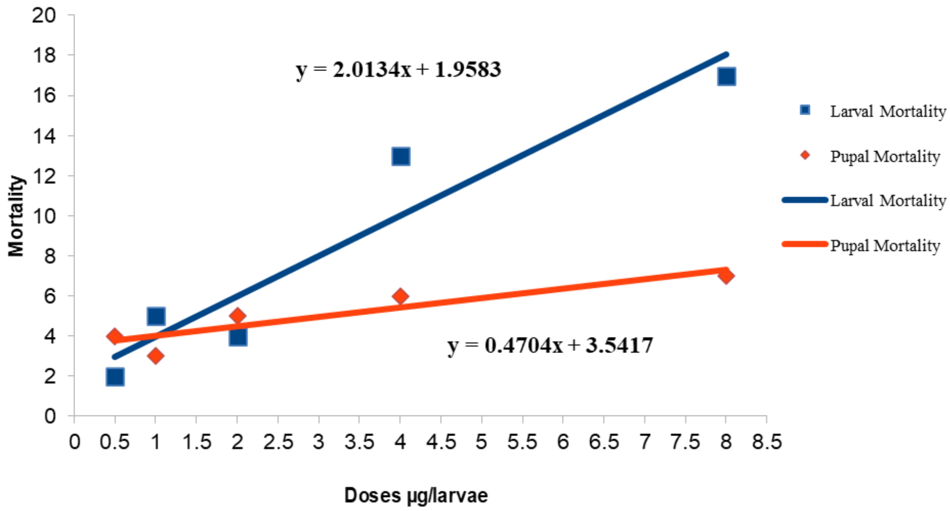

Figure 05: Larval and pupal mortality of Spodoptera litura following topical application of Muristerone on 6th instar larvae

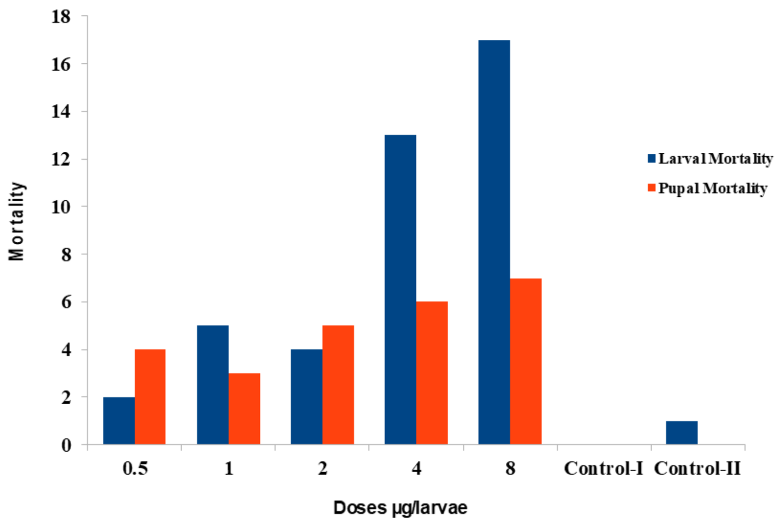

Figure 06: Larval and pupal mortality of Spodoptera litura following topical application of Muristerone on 6th instar larvae

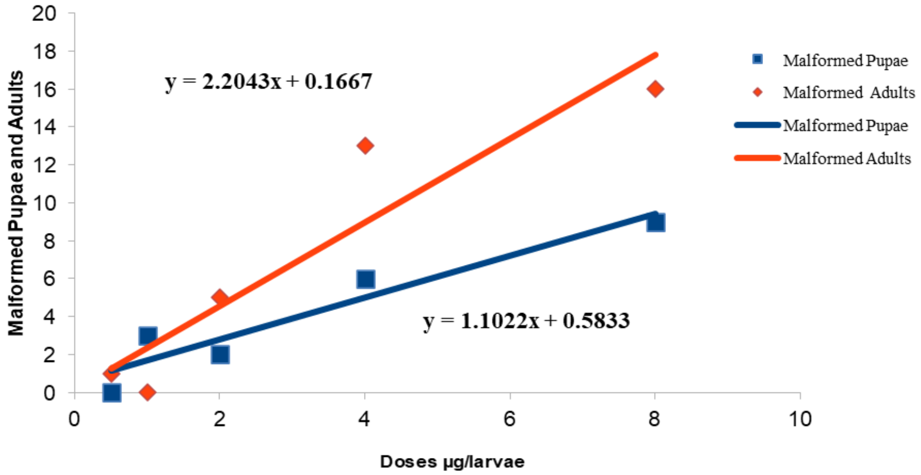

Figure 07: Malformation in pupae and adults of Spodoptera litura following topical application of Muristerone on 6th instar larvae.

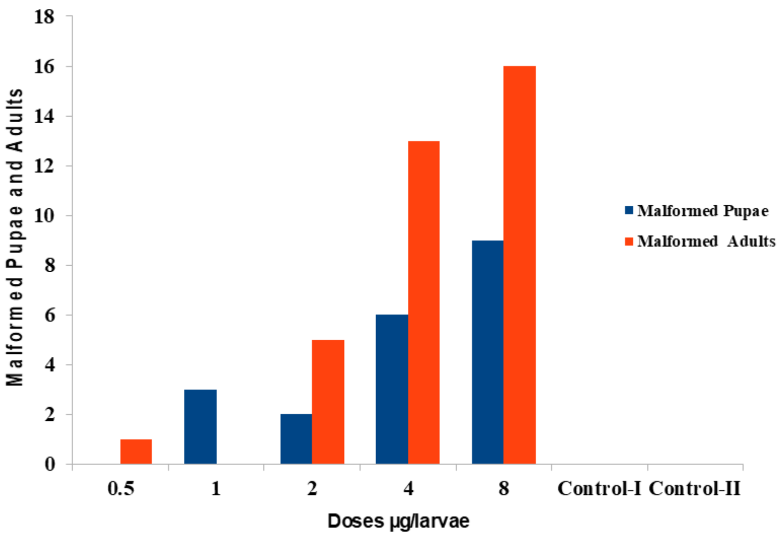

Figure 08: Malformation in pupae and adults of Spodoptera litura following topical application of Muristerone on 6th instar larvae.

Earlier Bachchan *et al*., (1992) also studied the effect of Muristerone at 8.0 µg, 04 µg, 02 µg, 01 µg and 0.5 µg doses on 4th and 5th instar larvae of *Utetheisa pulchella* by topical and injection method. The total larval death following the topical application muristerone on 4th instar larvae was 63, 44, 33, 19 and 11% respectively as compared to control, however, the injection of the same doses resulted in 68, 44, 31, 20 and 09% larval death. When the same doses of Muristerone was applied topically on the 5th instar larvae, the corresponding loss of insects were 51, 37, 25, 21 and 06% respectively as compared to control however the corresponding treatment of 5th instar larvae by injecting these doses resulted in 55, 39, 31, 18 and 12% mortality up to adult emergence. Among these two methods, injection of these doses caused little more larval death as compared to topical application and the younger larvae (4th instar) were more susceptible than those of 5th instar larvae. Ingestion of 100 ppm 20-hydroxyecdysone, Postesterone, Makisterone-A, Ponasterone-C and Dacrysterone caused 84%, 77%, 63%, 25% and 09% larval mortality respectively in houseflies (Singh & Russell, 1980). Ahmad and Khan (1985) also recorded 65% and 43% nymphal mortality of *Dysdercus cingulatus* respectively following the injection of 04µg Triol/nymph. Khan and Khan (1986) recorded 66% and 48% nymphal death of *Dysdercus cingulatus* following the injection of 8.0µg Muristerone, however, the topical application of the same dose resulted in 58% and 41% nymphal loss respectively.

The fecundity and fertility of the females following the topical application of 0.5µg, 1.0 µg, 2.0 µg, 4.0 µg, and 8.0 µg muristerone on 5th instar larvae also decreased linearly with increasing doses (y = -33.957x + 1781.7 and y = -51.183x + 1751.7 respectively, Fig. 09) and showed negative correlation**.** The average egg production by the female *Spodoptera litura* which emerged from the survived 5th instar treated larvae that mated with untreated males of the same stock and age, suffered less adversely as compared to the fertility of the eggs. After the application of higher doses 2.0µg, 4.0µg and 8.0µg of muristerone, the average egg laying of the affected females which emerged from the survived treated larvae was dropped significantly by 02.15%, 06.91% and 14.50% respectively as compared to control-I (Fig. 10). Similarly, the hatching of the eggs, due to the topical application of 2.0µg, 4.0µg and 8.0µg muristerone/larva, was reduced by 04.54%, 12.47% and 22.36% respectively as compared to the control, however, the effect of the lower doses i.e. 0.5 µg and 1.0 µg muristerone particularly on fecundity was not appreciable (Fig. 10).

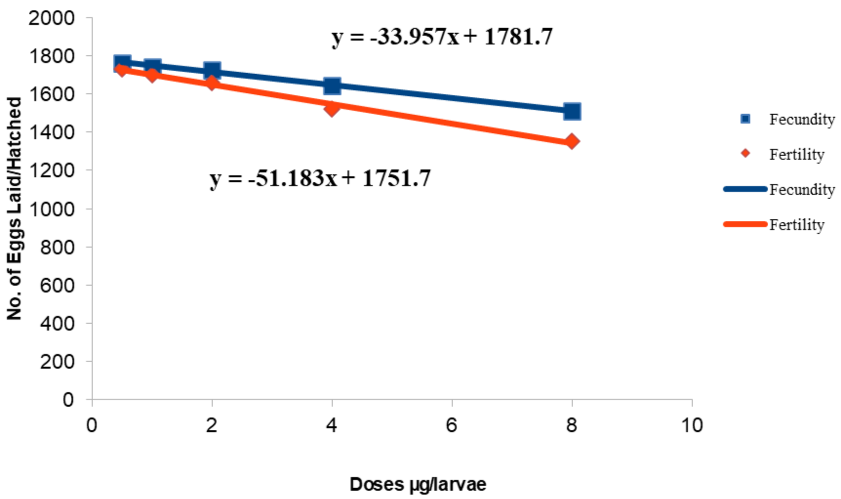

Figure 09: Fecundity and fertility of Spodoptera litura following topical application of Muristerone on 5th instar larvae

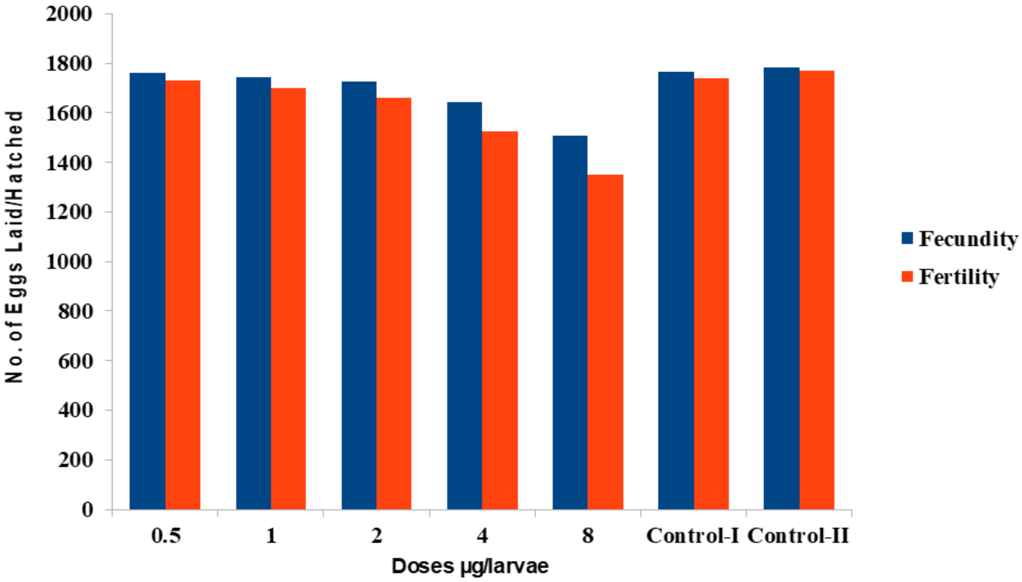

Figure 10: Fecundity and fertility of Spodoptera litura following topical application of Muristerone on 5th instar larvae

The average egg production and egg hatching of the females emerged from the 6th instar treated larvae following the topical application of 0.5µg, 1.0 µg, 2.0 µg, 4.0 µg, and 8.0 µg doses muristerone/larva, also declined linearly with increasing doses (Y= -34.089x + 1794.9 and Y= -54.167x + 1728.9 respectively, Fig. 11) showing a negative correlation coefficient. After the application of higher doses 2.0 µg, 4.0 µg, and 8.0 µg of muristerone, the average egg laying was dropped significantly by 03.02%, 07.22% and 14.89% respectively as compared to control-I, whereas the fertility of the eggs laid by the affected females was declined by 09.44%, 14.75% and 26.50% respectively as compared to the control (Fig. 12), however, the effect of the lower doses i e. 0.5 µg and 1.0 µg and muristeron particularly on fecundity and fertility was not appreciable. Earlier Khan *et al.*, (2002) also tested 5 different doses (viz., 0.5µg, 1.0 µg, 2.0 µg, 4.0 µg, and 8.0 µg) of Polypodine-B (a phytoecdysone) on two advanced nymphal stages (4th and 5th) of *Dysdercus cingulatus* by topical and injection method and reported that the effect of aforesaid doses on late nymphal instar (i.e. 5th instar) were less fatal as compared to the earlier instar.

Based on present observations, it can be concluded that Muristerone is toxic to *Spodoptera litura* when applied during the larval stages. Thus, it can be recommended for use in the control of this species.

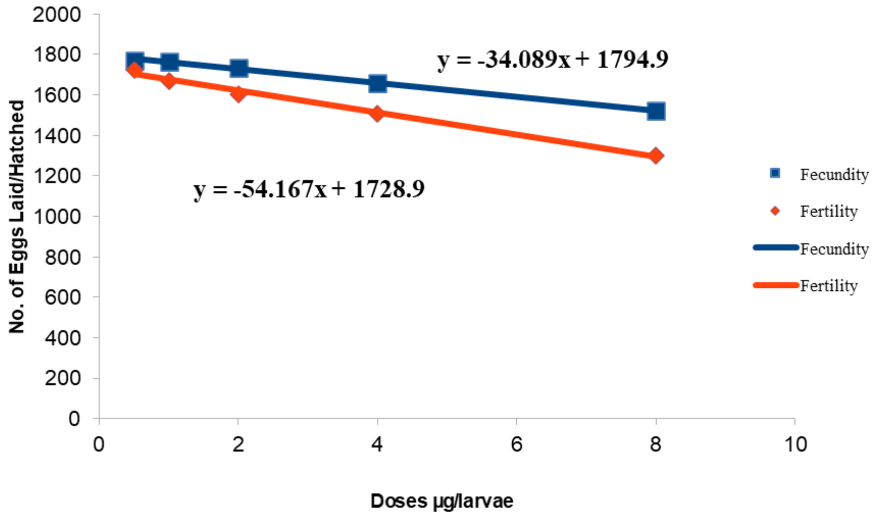

Figure 11: Fecundity and fertility of Spodoptera litura following topical application of Muristerone on 6th instar larvae

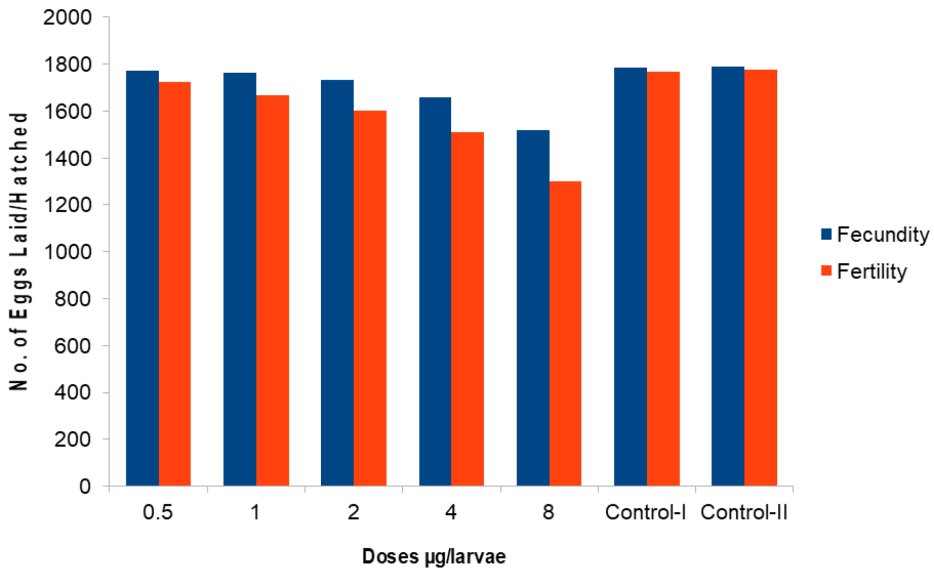

Figure 12: Fecundity and fertility of Spodoptera litura following topical application of Muristerone on 6th instar larvae

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