THE EFFECT OF CERTAIN BIOPESTICIDES AND IRRADIATION ON THE DEVELOPMENTAL STAGES OF MYIASIS PRODUCING FLIES

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that this thesis entitled "**THE EFFECT OF CERTAIN BIOPESTICIDES AND IRRADIATION ON THE DEVELOPMENTAL STAGES OF MYIASIS PRODUCING FLIES**" is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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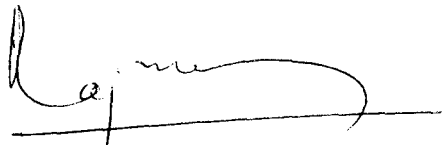

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Dr. SUBRAMANIAN, H.

Dedicated to
Radha and Sachin

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Introduction

INTRODUCTION

Man has long been intrigued by insects by bringing him both joy and sorrow. Insects contribute to substantial benefits by way of serving as significant pollinators, honey gatherers and producers of commercially valuable materials like silk and lac. Many of them destroy valuable crops and spread certain fatal diseases of man and animals particularly in tropics and sub tropics.

Substantial economic loss has been attributed to insects due to various effects. This include creation of nuisance and morbidity in animals and dissemination of diseases in animals and man. The morbid actions include skin reactions, blood loss, reduced feed conversion efficiency, poor weight gain and decreased production performance in livestock. According to Sen and Fletcher (1962), the loss due to the depreciation of hide value in cattle and loss in quality and quantity of wool in sheep were estimated to the tune of 1.5 crores in a year.

While some of the fatal diseases have been tackled by our advanced knowledge in science and improved control techniques, the rest have not yet received adequate attention. Cutaneous myiasis is one such condition. It is the infestation of dipterous larvae in the dermal and subdermal tissues and certain other organs of animals, which at least for a certain

period feed on the host's living or dead tissue to complete growth. Myiasis producing larvae do harm to the host by producing extensive deep wounds which may at times lead to toxaemic conditions culminating in death. Attack of organs like udder and uterine cervix may hit hard on dairy production. Damage to sense organs like eye and ear is also very common in domestic animals.

Majority of cutaneous myiasis is caused by the larvae of *Chrysomya bezziana* (Villeneuve - Diptera, Calliphoridae) and *Lucilia cuprina* (Weidemann - Diptera, Calliphoridae). In certain occasions *Calliphora erythrocephala* (Meigen - Diptera, Calliphoridae), *Chrysomya megacephala* (Fabricus - Diptera, Calliphoridae), *Musca domestica* (Linnaeus - Diptera, Muscidae) and *Sarcophaga ruficornis* (Fabricus - Diptera, Sarcophagidae) are also found involved.

The hazardous effects of chemical pesticides on both man and animals have been in the forefront of public attention for years. The indiscriminate use of chemical pesticides without consideration of the complexities of nature has been a major cause of ecological disruption. In view of higher mammalian toxicity, development of resistance and ill effects on non targeted and beneficial insects by chlorinated hydrocarbon, organophosphate, carbamate and synthetic pyrethroid groups of insecticides, the adoption of safer pest management programmes

with the use of biopesticides in controlling harmful insects, has gained importance.

Biopesticides are environment friendly chemicals or plant derived products which act on different stages of the insect and destroy them. Each of them have specific action on the developmental stages of insects. Among the biopesticides, the most important are the synthetic juvenile hormone analogue (Methoprene) which prevents the maturation of larvae, the antichitin agent (Diflubenzuron) which inhibit the deposition of chitin in larval cuticle, the endotoxin of *Bacillus thuringiensis* causing larval mortality, the neem ingredient (Azadirachtin) causing profound antifeedant and repellant effects and extract of *Acorus calamus* producing sterility effect on insects. The significance of biopesticides over conventional pesticides were described by Nayar *et al.* (1976). Notwithstanding these, the advantages of sterilization in controlling insects by irradiation is significant in the sense that the effect is produced only in the targeted species without any deleterious effects of conventional pesticides.

Cutaneous myiasis is a condition highly prevalent in animals in Kerala. The efficacy of biopesticides and irradiation in controlling the flies producing cutaneous myiasis appears to be worthy of investigation. Hence a study was undertaken to

a. assess the efficacy of certain biopesticides against propagation of flies producing cutaneous myiasis.

b. evaluate the efficacy of Gamma radiation on the development and reproductive performance of myiasis producing flies.

c. study the efficacy of biopesticides on natural cases of cutaneous myiasis.

Review of Literature

REVIEW OF LITERATURE

2.1 Prevalence of cutaneous myiasis

A review on the prevalence of cutaneous myiasis reveals that it is a common condition found in domestic animals in India. It was Patton (1920a) who studied for the first time in detail about cutaneous myiasis and its causative agents. He stated that *Chrysomyia* and *Lucilia* are the two important genera of flies which cause cutaneous myiasis in domestic animals in India. He (1922e) recorded 87 cases of cutaneous myiasis of which *C. bezziana* (92 per cent) was the main myiasis producer, while *C. megacephala* (5.75 per cent) and *L. cuprina* (2.25 per cent) were also involved with the condition. A survey on cutaneous myiasis in domestic animals in Madras presidency was conducted by Rao and Pillay (1936). Out of 404 positive cases, they recorded 335 (83 per cent) cases in cattle including buffaloes, 19 (4.7 per cent) in goats, 21 (5.2 per cent) in dogs and the rest in other domestic animals. Senior White *et al.* (1940) gave a detailed description on the morphology of myiasis producing flies in India.

Sen Gupta *et al.* (1951) studied the seasonal prevalence of cutaneous myiasis at Indian Veterinary Research Institute, Izatnagar, during 1948 and found the maximum prevalence of 84 per cent from November to March. Zumpt (1965) described the

various species of flies responsible for producing myiasis in man and domestic animals. Nachiappan (1971) studied the incidence of cutaneous myiasis in domestic animals in Tamil Nadu and observed that 98.5 per cent of the condition were caused by *C. bezziana* followed by 0.75 per cent of *C. megacephala* and *L. cuprina* each. Subramanian and Rajamohanan (1980) recorded 155 cases of cutaneous myiasis in Trichur from June 1977 to May 1978. They observed a maximum prevalence of 68 cases in February followed by 41 and 31 cases in March and January respectively. *C. bezziana* larvae were recorded from 93.5 per cent of the condition followed by *C. megacephala* (4.52 per cent), *L. cuprina* (1.3 per cent) and *C. rufifacies* (0.65 per cent). Among the different hosts for cutaneous myiasis 78.7 per cent of cases were in cattle including buffaloes, 12.3 per cent in goats and 9 per cent in dogs. Valandiker (1980) gave a detailed description on the morphology and biology of *C. megacephala*.

In a study of 45 myiasis cases in dogs at Madras Veterinary College Clinic, Thilager *et al.* (1989) observed only *C. bezziana* larvae and noticed the infection between January to April with March being the peak of infection. Thilager *et al.* (1991) also observed that 63 per cent of the myiasis cases in cattle and buffaloes occurred during the summer season. Martin Hall (1995) mentioned the clinical complications due to cutaneous myiasis in humans and domestic animals.

2.2 Effect of Methoprene

The inhibitory effect of juvenile hormone analogue methoprene on adult emergence in *Musca domestica* was first demonstrated by Cerf and Georghion (1972). They observed that methoprene is an extremely powerful inhibitor of adult development which prevented 100 per cent fly emergence when applied to larval breeding medium at 5 ppm concentration. Further evidence was given by Jakob (1973b) who noted complete inhibition of housefly emergence at 10 ppm concentration in poultry manure. Miller and Ubel (1974) reported that methoprene at 1 and 10 ppm concentration prevented 100 per cent fly emergence in *M. autumnalis* and 50 per cent in *M. domestica* respectively. Morgan *et al.* (1975) found that methoprene when incorporated in moistened poultry droppings at 10 ppm caused complete larval mortality of *M. domestica* over a period of seven days. Moreover, they also observed underdevelopment of antennae, compound eyes and mouth parts in the adult flies on larval treatment at lesser concentrations.

Adams *et al.* (1976) bioassayed the concentration of methoprene in poultry manure after giving as feed additive and stated that 10 ppm concentration of methoprene arrested housefly emergence by 77 per cent in manure buckets. Miller *et al.* (1976) did not observe any larval mortality in horn flies at 20 and 30 ppm of methoprene in cattle dung after

administering methoprene in drinking water but prevented fly emergence from pupa @ 94.5 per cent and 100 per cent respectively. Sehnal and Zadarek (1976) noticed that methoprene at 1 to 5 ppm when fed to larvae of *M. domestica*, *Sarcophaga crassipalpis* and *Calliphora vomitoria* produced 10 to 30 per cent larval to pupal intermediaries. Moreover, they also described the inability of the affected flies to crawl out of the puparium and nonpigmented eyes and reduced number of bristles on the body of the few emerged flies. Nosec *et al.* (1977) observed a reduction of 98 to 100 per cent of fly emergence in *M. domestica* when methoprene at 10 to 40 ppm was mixed with breeding medium containing prepupal stage. Palaniswamy and Sivasubramanian (1977) showed that methoprene at 5 ppm concentration in larval medium inhibited 70 per cent of adult eclosion. They also observed morphological defects like reduction in number of bristles, irregular orientation of bristles and partial failure in the development of abdomen. A reduction of 93.5 per cent in adult emergence of *Haematobia irritans* was noted by Paysinger and Adkins (1977) on application of methoprene at 20 ppm concentration in cattle dung.

Taen *et al.* (1977) reported that at 1 ppm of methoprene, the total growth period of larval instars prolonged by 10 hours in *Musca* and *Stomoxys* flies. Miller *et al.* (1978) observed a reduction of 80 per cent in the population of

M. autumnalis at 0.54 ppm in cattle dung after allowing the cattle to lick methoprene containing mineral blocks. At 0.13 ppm concentration, the adult population was controlled by 40 per cent only. Buci *et al.* (1979) noticed 95 per cent egg mortality in *M. domestica* at 5 ppm of methoprene treatment. They also observed a stronger inhibitory effect on adult emergence in *M. domestica*, *Lucilia illustris*, *Sarcophaga similis* and *S. crassipalpis* when methoprene was applied to older larvae than younger ones.

Complete inhibition on adult emergence in *Stomoxys calcitrans* at 3 ppm of methoprene in larval breeding medium was observed by Matsumara (1979) though the pupation was totally unaffected. He noticed incomplete metamorphosis of pupa to adult fly stage on dissection of puparia. At lower concentrations malformed adults with smaller wings and bodies were also demonstrated.

The effects of two highly active juvenile hormone analogues JHA 147 and 148 containing methoprene was compared by Styczynska (1979) in *M. domestica*. Treatment of the larva with 1 ppm methoprene did not affect the pupation process but adult emergence was 7 times lower in treated larvae compared to untreated ones. Lineva and Chunina (1980) compared the effects of IGR methoprene with hydroprene on adult development of *M. domestica* and observed that methoprene at 5 ppm

concentration resulted in complete impairment of pupal development which failed to give rise to adults.

Styczynska *et al.* (1980) determined the effects of juvenile hormone analogues cycloprene and methoprene against *M. domestica* and revealed that impregnation of methoprene at 5 ppm concentration in larval substrate caused 82.9 per cent reduction in fly population with 46.2 per cent morphological defects in resulting adults.

Mohiuddin and Qureshi (1982) tested methoprene at 1 to 10 ppm in larval and pupal stages of *M. domestica* and found that newly formed pupa were more sensitive than younger stages. At the highest dose, adults failed to emerge completely and dissection of pupae revealed a gradient of pupal adult intermediary stages. A significant percentage of larval to pupal intermediaries and morphological deformities in adults with reduced lifespan on *M. domestica* at 10 ppm of methoprene treatment were observed by Qureshi *et al* (1983). Asano *et al.* (1984) recorded 88 per cent reduction in adult emergence of *M. domestica* for seven days on single application of methoprene at 50 ppm concentration in poultry droppings.

Azad and Mulla (1985) discussed the effect of methoprene causing significant morphological aberrations in treated 3rd instar larvae and subsequent developmental stages of *M. domestica* and found that the commonest effect at 1 to

1.5 ppm in the rearing medium was the formation of larviform puparia about twice as long as normal ones.

El-Ela *et al.* (1990) noticed 82 per cent in fly emergence in *M. domestica* on application of 0.5 ppm of methoprene in larval rearing medium containing first instar larvae. Fincher (1991) observed an efficacy of 99.4 per cent in the emergence of *Haematobia irritans* at 3 ppm concentration of methoprene in cattle dung.

2.3 Effect of Diflubenzuron

The first report on the potent larvicidal property of the antichitin agent diflubenzuron with regard to agricultural pests was given by Van Daalen (1972). Later on, Jakob (1973a) studied the inhibitory effect of urea analogue diflubenzuron on the development of mosquitoes and houseflies. He observed that newly hatched larvae of *M. domestica* reared in medium containing 1 and 5 ppm of diflubenzuron produced 90 and 100 per cent mortality respectively. Ishaaya and Casida (1974) reared third stage larvae of *M. domestica* in medium containing 0.4, 1 and 2.5 ppm of diflubenzuron and noticed that the chitin content were 55.5, 42.3 and 26.4 per cent respectively, when compared to the untreated larvae. Miller (1974) observed that 0.01 to 0.1 ppm of diflubenzuron caused 70 to 100 per cent mortality of *M. atumnalis* larvae in cow dung, while in

M. domestica it caused 71 to 100 per cent mortality with 25 per cent adult development in the lowest concentration. Albes *et al.* (1975) reported that topical application of 10 to 100 ppm of diflubenzuron on *M. domestica* eggs showed 0 to 12 per cent mortality, while feeding of old larvae with diflubenzuron at 1.25 ppm caused 90 per cent mortality. Bijloo (1975) showed that on ingestion of diblubenzuron at toxic levels of 5 ppm, the diptera larvae were unable to complete next moult and died of cuticle rupture which he attributed to reduction in chitin formation. Barker and Jones (1976) noted that the larvae of *M. domestica* were sensitive to diflubenzuron and at 0.13 and 0.52 ppm concentrations, the material prevented larval development by 75 and 83 per cent respectively. It was found by Grosscurt (1976) that diflubenzuron has absolutely no contact ovicidal effect on the eggs of housefly *M. domestica*. Hayakawa (1976) observed that diflubenzuron at 1 ppm in the breeding medium of mature larvae of stomoxys calcitrans completely affected pupal metamorphosis. Buci and Okabe (1977) recorded that the lethal concentration 50 of diflubenzuron in the second and third instar larval diet were 0.12 ppm for *M. domestica*, 0.32 ppm for *Phormia regina*, 1.1 ppm for *Sarcophaga crassipalpis* and 5 ppm for *Sarcophaga similis*. They also mentioned that topical treatment of diflubenzuron at 2.5 ppm afforded 97 per cent control of *M. domestica* population. It was revealed by Rupes *et al.* (1977) that diflubenzuron is a potent larvicidal agent in

M. domestica, predominantly affecting the immature stages by interferring in the larval moulting and they believed that chitin deficient cuticle as the cause for the mortality of the larvae.

Grosscurt (1978) noted that diflubenzuron at 10 and 100 ppm produced 100 per cent larvicidal and 90 per cent ovicidal effect respectively in *M. domestica* with higher susceptibility to younger ones. Kunz and Harris (1978) observed that diflubenzuron on topical application at 5 and 10 ppm respectively prevented the egg hatching and larval development completely in *Haematobia irritans*. Wright (1978) attributed the inhibition of chitin production as reason for high mortality of larvae of *M. domestica* and *Stomoxys calcitrans* on diflubenzuron treatment. El-Khodary *et al.* (1979) stated that diflubenzuron at 0.56 to 5.6 ppm in larval diet of *M. domestica* produced 50 to 100 per cent mortality. At 2.8 ppm level, the mortality was found to be 100 per cent, 96 per cent and 75 per cent respectively for the first, second and third instar larvae. It was Grosscurt (1980) who observed a significant reduction in pupal weight when the larvae of *M. domestica* were grown in diflubenzuron treated culture medium.

Turnbull *et al.* (1980) observed significant reduction of chitin content on treatment of diflubenzuron in *L. cuprina*.

They recorded that at an increasing dose of 1 to 5 ppm, the chitin deposition reduced from 50 to 28 per cent. Webb and Wildey (1986) found that treatment of slurry pits of pig weaning house with diflubenzuron at 4.16 and 6.25 ppm reduced the *M. domestica* population by 80 and 92.5 per cent respectively. It was Kandasamy (1987) who explained the complete mortality obtained at 5 ppm concentration in diptera, due to interference of chitin deposition in larval cuticle. Demeny (1989) observed that 0.1 ppm diflubenzuron treatment to larvae of *M. domestica* produced abnormally thin shelled pupa. Miller *et al.* (1990) recorded that diflubenzuron at 3 ppm concentration in cow dung obtained by feeding Dimilin boluses orally, controlled 80 per cent of immature stages of *M. autumnalis* and *Haematobia irritans*.

2.4 Effect of *Bacillus thuringiensis*

The first reports of larvicidal effect caused by the toxin of *Bacillus thuringiensis* on diptera were published by Korzh *et al.* (1977), who observed that the first stage larvae of *M. domestica* and *S. calcitrans* did not develop further in manure heaps containing 100 to 500 ppm of *B. thuringensis*. Larget and Barjac (1981) reported the extremely toxic effects of *B. thuringiensis* (israelensis) suspension on mosquitoes, simulids and sandflies but found no effect on *M. domestica* larvae. The fact that larvae of *M. domestica* were not

susceptible to *B. thuringiensis* (israelensis) was revealed by Vankova (1981). Singh *et al.* (1986) noticed that *B. thuringiensis* var israelensis at 4 ppm in larval medium caused death of *M. domestica* larvae due to damage in body wall musculature. An evidence of moderate toxicity to larval stages of *Lucilia cuprina* due to application of *B. thuringiensis* (israelensis) suspension in the culture medium was given by Arellano *et al.* (1990). A detailed illustration on the biocontrol of arthropods affecting livestock and poultry was given by Rudge and Patterson (1990).

2.5 Effect of Azadirachtin

Schmutterer (1976) expressed that azadirachtin is one of the most promising materials of plant origin exerting antifeedant and growth regulatory effect in insects. The profound ovicidal and larvicidal effects of neem extracts containing azadirachtin on diamond black fly *Putella xylostella* were observed by Mong Ting Tan and Sudderuddin (1978). Moreover they also noted inhibition of pupal development and deformed wings in surviving adults. Warthen (1979) studied the feeding inhibitory and growth regulatory effects of neem *Azadirachta indica* and opined that certain components of neem were non toxic and harmless to the environment but has profound antifeedant and growth regulatory property on larvae and adults of stored grain pests. Interference in larval and pupal development, high rate of pupal mortality and less adult emergence with malformations in *Apis melliferra* flies were

noticed by Rambold *et al.* (1980). Schmutterer and Rambold (1980) observed strong growth disruptive effect on larvae of *M. domestica* resulting in death with application of extracts of *Azadirachta indica*. It was Gaaboub and Hayes (1984a) who studied in detail about the effects of azadirachtin on larvae, pupae and adult flies of *M. autumnalis*. They found 20 to 100 per cent mortality on larvae or pupae at 1 to 100 ppm concentration of azadirachtin. Moreover at 0.1 ppm, a significant reduction in pupal weight, dimension, and adult size were also noticed. Gaaboub and Hayes (1984b) also observed that treatment at 0.39 ppm of azadirachtin to third instar larvae of *M. domestica* caused 11 per cent larval death, 52 per cent inhibition of adult formation, 85 per cent reduction in egg production and 60 per cent reduction in egg hatch. Rice *et al.* (1985) reported that in *Lucilia cuprina* flies, the potent antifeedant azadirachtin showed complete oviposition deterrence at 200 ppm while the effect started at lower concentration of 20 ppm itself. Schmutterer (1988) noted 70 per cent reduction of food consumption in *M. domestica* larvae at 3 ppm concentration of azadirachtin in the breeding medium. Miller and Chamberlain (1989) observed lethal concentration 50 and 90 at 0.151 and 0.268 ppm of azadirachtin in larvae of *Haematobia irritans*, 7.7 and 18.7 ppm in *Stomoxys calcitrans*, and 10.5 and 20.2 ppm in *M. domestica* respectively. Subrahmanyam (1990) noticed profound growth disruptive action for azadirachtin against dipterous larvae.

The properties and potentialities of azadirachtin for pest control were discussed by Schmutterer (1990). He observed 100 per cent repellant and ovipositional deterrent effect and 75 per cent antifeedant effect on treatment of azadirachtin at 5 ppm concentration in *Lucilia serricata*. Govindachari (1992) gave an excellent report on the biological activity of azadirachtin and observed that the compound is highly toxic, potent antifeedant and repellant on diptera.

Mishra (1994) opined that neem based product azadirachtin exhibited potent toxic, antifeedant and repellent properties against livestock infesting flies.

2.6 Effect of *Acorus calamus*

Mathur and Saxena (1975) observed for the first time that feeding or exposure to 1 per cent oil of *Acorus calamus* induced 98 to 100 per cent sterility in house fly *M. domestica*. Chavan *et al.* (1976) tried different extracts of *Acorus calamus* and found that petroleum ether extract was highly toxic to the fourth stage larvae of *Culex fatigans*. It was Pandey *et al.* (1977) who showed that 0.5 per cent of petroleum ether extract of *Acorus calamus* produced 91 per cent mortality and 72 per cent antifeedant effect on larvae and 82 per cent repellant effect on adults of *Athalia proxima* flies. Sudhakar *et al.* (1978) noticed that 0.5 per cent concentration

of petroleum ether extract of *Acorus calamus* exibitted 69 per cent antifeedant effect in *Athalia proxima* larvae, while 2 per cent concentration caused complete mortality. The complete failure of ovarian follicular development in adult flies of *Athalia proxima* due to the exposure to 1 per cent oil of *Acorus calamus*, was observed by Tikku *et al.* (1978). Chavan *et al.* (1979) reported 100 per cent mortality of 4th instar larvae of *Culex fatigans* when exposed to 0.01 per cent of petroleum ether extract of *Acorus calamus*. Theotia and Pandey (1979) observed 48.5 per cent mortality on Rice weevil, *Sitophylus oryzae* larvae with 1.5 per cent of petroleum ether extract in 24 hours. The insecticidal property of *Acorus calamus* was investigated by Ahmed *et al.* (1981). They found that 1 per cent petroleum ether extract produced 90 per cent mortality in 3rd instar larvae of *M. domestica*. Banerji *et al.* (1982) observed 100 per cent anti feedant effect with 1 to 2.5 per cent petroleum ether extract of *Acorus calamus* against mustard saw fly larvae *Athalia proxima*. Deshmukh *et al.* (1982) observed that petroleum ether extract at 0.2 and 0.5 per cent killed 50 and 100 per cent larvae of *M. domestica* respectively. Khan and Borle (1985) prevented 100 per cent attack of pulse beetle *Callosobrunchus chinensis* using 0.5 per cent oil of *Acorus calamus*.

2.7 Effect of irradiation

The possibility of control of insect population by sterile insect release was first mentioned by Labrecque and Keller (1965). The effect of gamma irradiation on the larvae of *M. domestica* was first studied in detail by Abdu and Razik (1975). They observed varied rates of pupation of 74 and 46 per cent at an exposure of 400 and 1400 rads respectively to one day old larvae. They also observed 62 and 78 per cent adult emergence at 400 rads exposure to one and six day old larvae respectively but fly emergence was absent at 1200 rads exposure, demonstrating that younger larvae were more susceptible to radiation than older larvae. The effect of gamma irradiation on pupae of various ages in *M. domestica* was studied by Ganeidy *et al.* (1975). There was no fly emergence in one day old pupae at 5000 to 25000 rads but 23.5 and 85 per cent emergence were noted at the lowest exposure dose on 3 and 6 days old pupa respectively. Whitfield *et al.* (1978) induced complete sterility on exposure of 6 days old pupa of *Stomoxys calcitrans* to 2000 rads of cobalt 60 without any other deleterious effect on the adult flies. Crystal (1979) observed excellent fly emergence, survival and sterility when 3 day old pupae of blow fly *Cochliomyia hominivorax* were irradiated at 2500 rads. Progressive reduction in lifespan and mating competitivenes were noted in higher doses. Donald *et al.* (1977) obtained total sterilization of both sexes of

Cochliomyia hominivorax at 6000 rads exposure to 6 day old pupae using cobalt 60. Higher mortality in younger larvae of *M. domestica* was observed by Srinivasan and Kesavan (1979) when a range of 40 to 72 hours old larvae were exposed at 500 to 10000 rads. When older larvae were irradiated at an exposure of 4000 rads and above, the developed pupae showed abnormality in shape. Abdu *et al.* (1982) observed 96.6 per cent reduction of fly emergence in *M. domestica* when 2 day old pupae were irradiated at 1500 rads. They also noticed 77.5 per cent reduction in fecundity at 1100 rads and 90 per cent reduction in egg hatch at 1300 rads in the resultant adults. Spradberry *et al.* (1983) noticed that adult *Chrysomyia bezziana* was rendered totally infertile without any other deleterious effects when pupae, one day before fly emergence were exposed to cesium 137 radiation at 4000 rads. Huda *et al.* (1983) reported 100 per cent infecundity in *Lucilia cuprina* on exposure of pupa one day before emergence at an exposure dose of 3000 rads. Spradberry (1992) has successfully sterilized *Cochliomyia hominivorax* flies by pupal irradiation at 4000 rads.

Materials and Methods

MATERIALS AND METHODS

3.1 Prevalence, identification and rearing of myiasis producing fly and larvae

3.1.1 Prevalence of cutaneous myiasis

Cattle, goats and dogs brought to the Kerala Agricultural University Veterinary Hospitals at Thrissur and Mannuthy for various ailments constituted the main source of study on the prevalence of cutaneous myiasis. The prevalence was studied for a period of one year, from September 1994 to August 1995. The myiasis condition was detected by the presence of dipterous larvae in the wound on the animals body.

3.1.2 Collection and identification of the larvae

The larvae were collected mainly with the use of scoop and forceps. In certain cases a cotton plug dipped in oil of turpentine was applied on the wound for 5 minutes and the larvae were collected using forceps after removing the plug. At the end of the collection the wound was carefully examined for the presence of any more larvae. The larvae collected from the maggot wound were washed in distilled water and identified by examining under a binocular dissection microscope.

3.1.3 Development and identification of adult flies

The larvae collected from the maggot wound were placed in a 500 ml beaker containing sand. The mouth of the beaker was closed using a muslin cloth and kept in the laboratory at room temperature. After pupation, the pupae were separated to a petri dish and placed in acrylic jars measuring 24x16x36 cms, the mouth of which was closed with a muslin cloth and tied around. On emergence of the flies from the pupae, they were anaesthetised using chloroform vapour and mounted on thermocool sheets using entomological pins. The flies were carefully examined under binocular dissection microscope and speciated according to the characters mentioned by Senior White *et al.* (1940).

3.1.4 Rearing and colonisation of adult flies in the laboratory (Fig.4,5)

The flies which emerged on development from the larvae collected from maggot wounds were reared in acrylic jars mentioned earlier in an atmospheric temperature ranging from 25-35°C and relative humidity of 72-92 per cent. Sand was provided at the bottom of the jar and the mouth, closed using a muslin cloth. Ten grams each of glucose powder and fresh meat moistened with blood in a separate petri dish and 20 ml of distilled water in a glass vial were provided daily. Each jar contained 10 female and 5 male flies. The flies of

Chrysomyia megacephala, *Chrysomyia bezziana* and *Lucilia cuprina* were reared in the laboratory.

3.1.5 Rearing of larvae in the laboratory

The eggs were laid by the adult flies of *C. megacephala* and *L. cuprina* on the meat provided in side the fly rearing jars as mentioned earlier (Fig.7). The eggs collected were placed in 50 gms of moistened meat in a 500 ml beaker and the beaker was placed over sand in an acrylic jar. The mouth of the acrylic jar was closed using muslin cloth to pave way for the escape of gases of putrifaction of meat and to prevent attack of meat infesting flies from outside. In *C. bezziana* the flies were bred according to the method followed by Spradberry *et al.* (1983). The flies were allowed to lay eggs and hatch on meat in test tubes (3x20 cms) kept in a waterbath at 37°C (Fig.6). The meat containing larvae were transferred to 500 ml beaker and kept in water bath maintaining the temperature between 30-37°C and allowed to develop till maturation.

The larvae of all the flies after maturation migrated out of the beaker and pupated in the sand. The pupae were collected and kept for adult emergence as mentioned earlier.

3.2 Biopesticides used and their application

The biopesticides, Methoprene 5 per cent (Altosid, Zoecon Corporation, USA), Diflubenzuron 25 per cent WP (Dimilin, Coromandel Indag Products India Pvt. Ltd., Madras), Azadirachtin 300 PPM (Achook, Godrej Agrovet Pvt. Ltd., Bombay), Bacillus thuringiensis var israelensis, 1.6 per cent (Teknar, Zoecon corporation, USA) and Petroleum ether extract, alcoholic extract and steam distillate of sweet flag, *Acorus calamus* were used in the present investigation (Fig.8). The required concentrations of the biopesticide were prepared by diluting the materials in distilled water. The various effects of each biopesticide in different concentration on eggs (2 hours after oviposition) and three larval stages (2,14 and 30 hours after hatching respectively) of *C. megacephala*, *C. bezziana* and *L. cuprina* were studied. In each study the number of eggs and larvae taken were 50 and 20 respectively. The required number of eggs and different stages of larvae from different flies were obtained from the colonised flies reared as mentioned earlier. The eggs were soaked with different concentrations of each biopesticide for 5 minutes and placed on 10 gms of meat for further observations. The different stages of the larvae were also placed over 10 gms of meat having different effective concentration of biopesticides. All the experiments were repeated once, observations made and the mean values were recorded.

The control experiments were conducted separately for each biopesticide, soaking of eggs in distilled water for 5 minutes, or adding 1 ml of distilled water to the larval rearing medium and observing the development of the respective stages.

3.2.1 **Methoprene (Altosid)**

The eggs and larval stage I (L-I) of the different species of flies were treated with 1, 5, 10, 30 and 50 parts per million (ppm) of methoprene. The observations made were percentage of mortality of eggs or larvae, pupation of the developed larvae, adult development and malformed adults. The longevity of adult male and female flies and the average number of eggs laid by female flies in their life span were also studied.

The larval stage II (L-II) and larval stage III (L-III) of the three different species were also allowed to develop in meat with the above concentration of methoprene. The observation taken were percentage of mortality of the larvae, period of larval development, percentage of larval to pupal intermediaries and pupation, length of the pupae, percentage of adult development and malformed adults, longevity of adult male and female flies, average number of eggs laid by female flies in their life span and the percentage of egg hatch and their subsequent development to adult flies. The various

morphological aberrations appeared as deformities in the adult flies developed from the treated larvae were also studied in detail.

3.2.2 Diflubenzuron (Dimilin)

The eggs and L-I stage of different species of flies were treated with 0.5, 1, 2.5, 5 and 10 ppm concentrations of diflubenzuron. The observations noted were percentage of mortality of eggs or larvae, pupation of the developed larvae, adult development and malformed adults, longevity of adult male and female flies and the average number of eggs laid by female flies in their lifespan.

The L-II and L-III stages of three different species were also allowed to develop in meat with the above concentration of diflubenzuron. The observations noted were percentage of mortality of larvae, of pupation, average weight of pupae, percentage of adult development and malformed adults, longevity of adult male and female flies, average number of eggs laid by female flies in their life span and the percentage of egg hatch and their subsequent development to adult flies. The various morphological aberrations in the adult flies developed from treated larvae were also studied.

The damages caused by diflubenzuron on the cuticular surface of the larvae of the 3 different species were also

studied by boiling the dead larvae in 10 per cent potassium hydroxide, dehydrating in ascending grades of alcohol and mounting them in Canadabalsm.

3.2.2.1 Effect on chitin production

Each group of 100 larvae of L-I, L-II and L-III stages of the 3 different species of flies were allowed to develop with an effective concentration of 0.5, 1, 2.5, 5 and 10 ppm of diflubenzuron in 50 gms of meat. After 24 hours the live and dead larvae recovered were washed in distilled water and mopped using filter paper to remove the water content from the surface of the larvae. The larvae were processed further for the estimation of chitin content by following the procedure adopted by Hackman and Goldberg (1971). The larvae were boiled in 50 ml of 3 per cent Sodium hydroxide solution for 30 minutes, decanted the clear solution and washed the residue 3 times with distilled water to remove the alkali content. The residue was treated again with 1.25 N hydrochloric acid for one hour at room temperature, decanted the clear solution, washed the residue 3 times in distilled water, rinsed with ethyl alcohol and dried at room temperature. The weight of the residue is taken to account for the chitin content. The larvae in the control group developed without addition of diflubenzuron were also processed similarly. The quantity of chitin obtained in diflubenzuron treated group were compared

with control group to assess the efficacy of diflubenzuron in inhibiting chitin production.

3.2.3 *Bacillus thuringiensis israelensis* (Teknar)

The eggs, L-I, L-II and L-III stages of the 3 different species of flies were treated with 160, 320 and 800 ppm concentration of Bacillus thuringiensis suspension and allowed to develop further. The percentage of mortality of eggs and of the respective stages of the larvae in the treated and untreated control groups were recorded.

3.2.4 Azadirachtin (Achook)

Ovicidal and larvicidal effect

The eggs, L-I, L-II and L-III stages of the 3 different sp. flies were treated with 6, 10.5 and 15 ppm concentrations of azadirachtin and allowed to develop further and the percentage of mortality of eggs and the respective stages of the larvae in the treated and untreated control groups were recorded.

3.2.4.1 Effect on development and performance

Larval stage III

The L-III stage of different species were allowed to develop on meat containing 1.5, 3 and 6 ppm concentrations of

azadirachtin concentration. Higher concentrations over 6 ppm was not used since further development was impossible hindering any valuable observation. The observations recorded were percentage of pupation, average weight of pupae, percentage of adult development and malformation, longevity of adult male and female flies, average number of eggs laid by female flies, and the percentage of eggs hatched. The morphological aberrations in the adult flies developed from the treated larvae were also studied.

Adult flies

Ten female and 5 male flies of the three different species were allowed to feed on meat containing 1.5, 3 and 6 ppm concentration of azadirachtin from day 1 to day 5. Untreated meat was provided for the subsequent days for feeding and egg laying.

The observations noted were longevity of adult male and female flies, average number of eggs laid by female flies, percentage of egg hatch, adult development and malformations.

3.2.4.2 Antifeedant effect on larvae

The larvae of L-I stage of the 3 different species of flies were placed on meat containing 1.5, 3 and 6 ppm concentrations of azadirachtin. The quantity of treated meat

consumed by the larvae to complete their growth phase was recorded and compared with control group to assess the antifeedant effect of azadirachtin.

3.2.4.3 Ovipositional deterrant effect on adult flies

Ten female and 5 male flies of the 3 species of flies were reared by feeding them with normal untreated meat for the first 5 days to meet the protein requirement for ovarian development. Subsequently only glucose powder and water were provided up to 11th day for survival. On the 12th day, the gravid females of *C. megacephala* and *L. cuprina* were exposed for 5 hours to 10 g of 24 hours putrified meat with uniform application of 1.5, 3 and 6 ppm of azadirachtin over the surface of the meat. In the case of *C. bezziana* the meat was incubated at 37°C for the laying of eggs. The average number of eggs laid by the flies in the treated meat were recorded and compared with the control group to assess the ovipositional deterrant effect of azadirachtin.

3.2.4.4 Repellant effect on adult flies

Petridishes (10 cm diameter) containing 100 g of 24 hours putrified meat were exposed outside for 5 hours with the uniform application of 1.5, 3 and 6 ppm concentration of azadirachtin on the surface of meat. The time of fly arrival and sitting on the bait, time of oviposition or larviposition

and the number of eggs or larvae deposited in the treated and control bait by meat infesting flies were recorded and compared to understand the repellant effect of azadirachtin.

3.2.5 Extracts of *Acorus calamus*

Preparation of extracts

The petroleum ether and alcoholic extracts of *Acorus calamus* were prepared separately by the cold method following the procedure adopted by Teotia (1979). The dried rhizomes of *Acorus Calamus* obtained from the market were ground to powder form using pestle and mortar and sieved. Three hundred grams of fine powder was taken in a 2 litre capacity conical flask and 900 ml of petroleum ether (B.P 60°C) or absolute alcohol was added. The mixture was stirred thoroughly for 15 minutes and the mouth of the flask was plugged tightly with cotton. The solution was kept away from light and stirred from time to time. The solution was then filtered with whatman filter paper and the filtrate was evaporated in a vacuum evaporator to obtain the stock solution of residual extract. The steam distillate was prepared using a steam distillation apparatus by condensing the steam passed through 300 gms of fine powder of *Acorus calamus* and evaporating the water content at 40°C in hot air oven.

3.2.5.1 Ovicidal and larvicidal effect

The eggs, L-I, L-II and L-III stages of the three different species flies were treated with petroleum ether extract, alcoholic extract and steam distillate of *Acorus calamus* at 1.5, 2 and 2.5 per cent concentrations. The respective stages were allowed to develop further and the mortality of eggs and larvae in the treated and untreated groups were recorded.

3.2.5.2 Effect on development and performance

Larval stage-III

The L-III stage of the different species were allowed to develop on meat containing 0.1, 0.5 and 1 per cent concentration of petroleum ether and alcoholic extracts and steam distillate of *Acorus calamus*. The observations recorded were percentage of pupation and adult emergence, longevity of adult male and female flies, average number of eggs laid by female flies and the percentage of egg hatch.

Adult flies

Ten female and 5 male flies of three different species were allowed to feed on meat containing the above mentioned extracts in the same concentration from day 1 to day 5. Untreated meat was provided for subsequent days for feeding

and egg laying. The observations taken were similar to that of azadirectin treatment on adult flies.

3.2.5.3 Antifeedant effect on larvae

The L-I stage of the larvae of 3 different flies were placed on meat containing 0.1, 0.5 and 1 per cent concentration of petroleum ether and alcoholic extracts and steam distillate of *Acorus calamus* and the antifeedant effect was studied as in the case of azadirectin.

3.2.5.4 Ovipositional deterrant effect on adult flies

Ten female and 5 male flies of the 3 different species of flies were reared as mentioned in azadirachtin treatment and exposed to different extracts of *Acorus calamus* with 1.5, 2.0 and 2.5 per cent concentrations. The ovipositional deterrant effect was studied as mentioned with respect to azadirectin.

3.2.5.5 Repellant effect on adult flies

The repellant effect of different extracts of *Acorus calamus* were studied by exposing putrified meat containing 1.5, 2.0 and 2.5 per cent and the study was conducted as in the case of azadirectin.

3.3 Effect of Gamma Irradiation

Fifty numbers each of eggs (6 hrs old), the first, second and third stage larvae of 10, 20 and 40 hrs old after hatch respectively and pupae of 1 and 3 day old of *C. megacephala*, *C. bezziana* and *L. cuprina* were irradiated in gamma chamber using Cobolt=60 at the Radiotracer laboratory, Kerala Agricultural University. The stages were exposed to 500, 1000, 2000, 3000, 4000, 5000 and 6000 rads and were allowed to develop further. Untreated control groups of similar stages were also maintained. In the case of eggs, the percentage of hatch and in L-I, L-II and L-III stages, the percentage of pupation and adult emergence, longevity of male and female flies and the average number of eggs laid by female flies were observed and recorded. In 1 and 3 day old pupa, the percentage of adult emergence, life span of male and female flies, average number of eggs laid by female flies, the percentage of egg hatch and subsequent adult development were recorded. The experiment was repeated once and the mean values were expressed.

3.4 Treatment trials with chosen biopesticides

Diflubenzuron, Azadirachtin and petroleum ether extract of Acous calamus which showed significant larvicidal effect in the *in vitro* studies conducted earlier were mixed with

paraffinum molle album at 5 ppm, 15 ppm and 2.5 per cent respectively and tried in natural cases of cutaneous myiasis to find out their efficacy. For each biopesticide tested, 10 cases in cattle, 5 cases in goats and 5 cases in dogs were selected. Before the application of the biopesticide, the edges and surface of the maggot wound were cleaned using moist cotton to remove the dead tissue and pus material. After 24 hours of application the number of live and dead larvae recovered were counted to calculate the percentage of efficacy of each biopesticide.

Fig.1. *Chrysomyia bezziana* - adult male and female fly

Fig.2. *Lucilia cuprina* - adult male and female fly

Fig.3. Chrysomyia megacephala - adult male and female fly

Fig.4. Breeding of myiasis producing flies in the laboratory

Fig.5. **Breeding of *C. megacephala* in the laboratory - individual cage**

Fig.6. ***C. bezziana* adult female flies in glass tubes kept in water bath for egg laying**

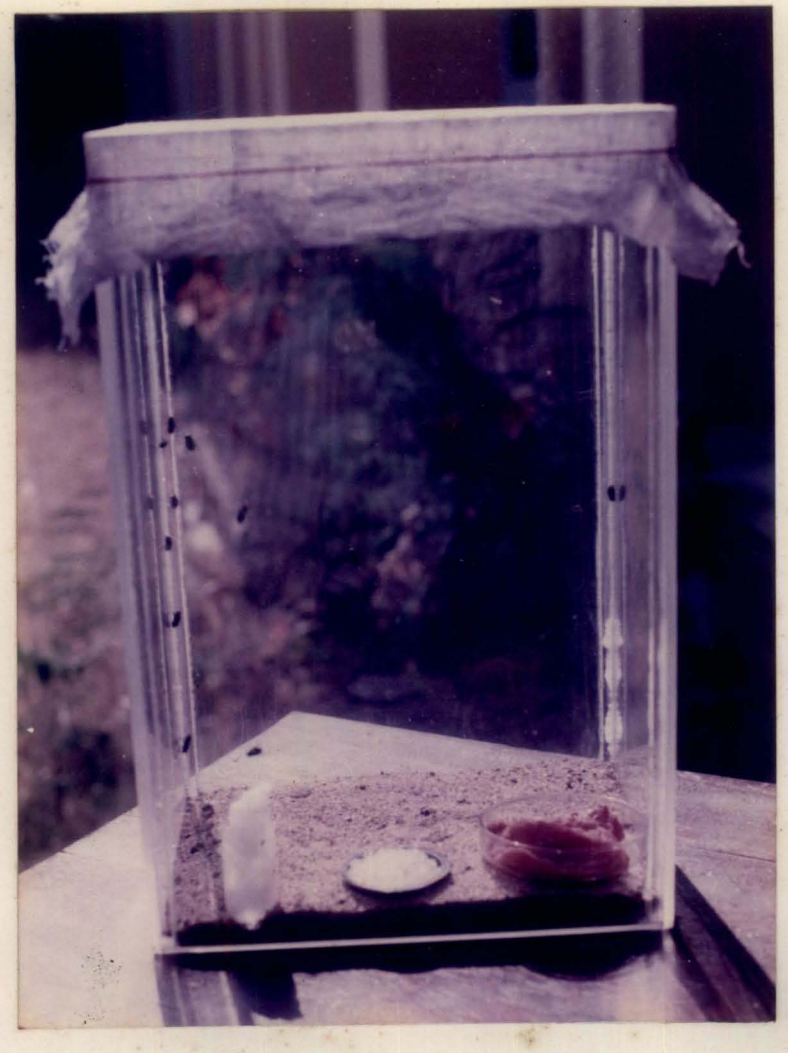


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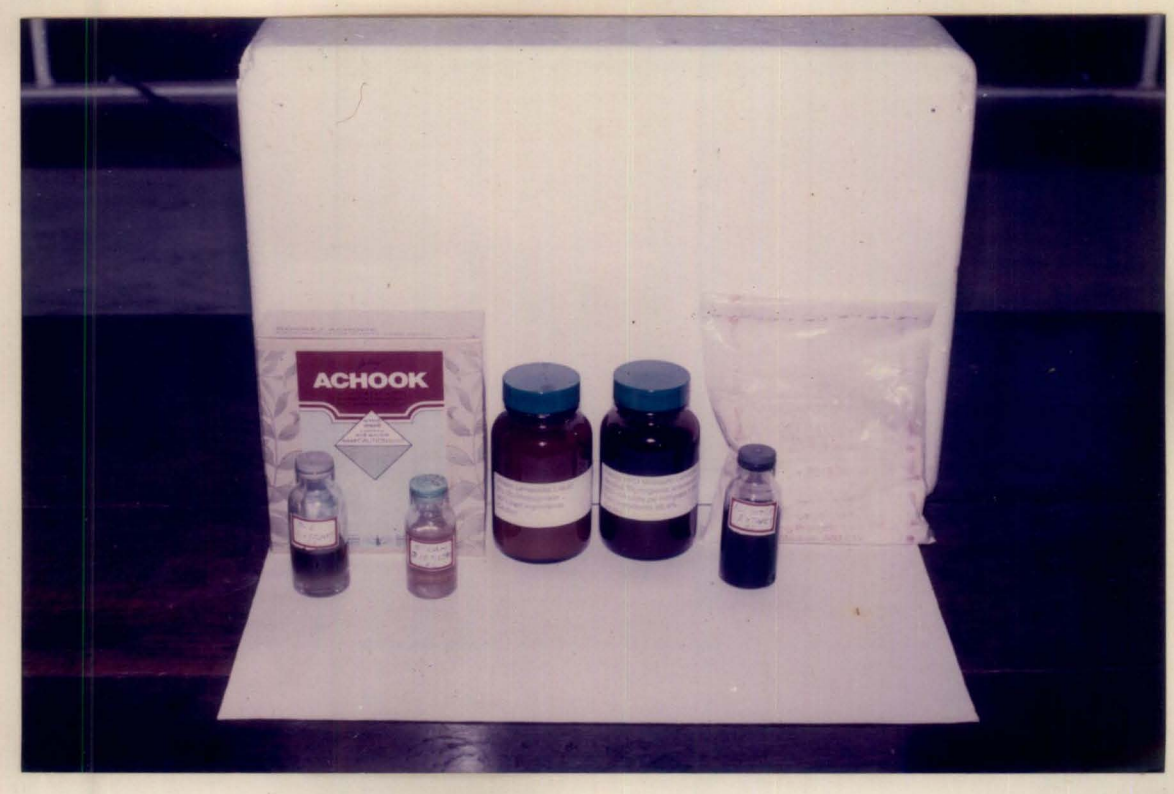
Fig.7. *C. megacephala* - Eggs laid in partly putrified meat

Fig.8. Biopesticides used in studying the effects in developmental stages of myiasis producing flies


ACHOOK

Results

RESULTS

4.1 PREVALENCE AND IDENTIFICATION OF MYIASIS PRODUCING FLIES AND LARVAE

4.1.1 Prevalence of cutaneous myiasis

Out of a total number of 9861 animals comprising cattle, goats and dogs examined for cutaneous myiasis, over a period of one year from September 1994 to August 1995 at University Veterinary hospitals, Kokkalai and Mannuthy, 205 cases were recorded as positive , the prevalence being 2.08 per cent (Table 1). The maximum prevalence was noted in the month of January (10.25 per cent), followed by February (6.08 per cent) and December (4.58 per cent) (Chart 1). The prevalence was lower during March (1.18 per cent) followed by November (0.99 per cent), October (0.32 per cent) and April (0.15 per cent). The condition was not reported from May to September. Out of the 205 positive cases 130 (63.41 per cent) were in cattle, 24 (11.71 per cent) in goats and 51 (24.88 per cent) in dogs (Table 5 and Chart 2).

Cattle

The prevalence of the condition in 5929 cattle were studied at both the hospitals. The number of positive cases were 130 giving a prevalence rate of 2.19 per cent (Table 2).

The prevalence was highest during January (10.10 per cent) and lowest during April (0.25 per cent). At University Veterinary hospital, Kokkalai alone, out of 4966 animals examined, 88 cases were positive, giving a prevalence rate of 1.77 per cent. The maximum prevalence was observed in January (7.81 per cent), followed closely by February (6.71 per cent) and the minimum was noted in April (0.34 per cent).

At University Veterinary hospital, Mannuthy, a total number of 42 cases were recorded out of 963 cattle examined giving a prevalence rate of 4.36 per cent. The maximum prevalence was noticed in January (20.45 per cent) and the minimum in November (2.33 per cent).

Goats

A total number of 1586 goats were examined at both the hospitals of which 24 were found positive giving a prevalence rate of 1.51 per cent (Table 3). The prevalence was noted from December to February, the highest being in January (10.16 per cent) followed by February (4.14 per cent) and December (2.94 per cent). At University Veterinary hospital, Kokkalai, 1280 animals were examined, out of which 16 positive cases were recorded giving a prevalence rate of 1.25 per cent. The prevalence was highest in January (9.30 per cent) followed by 3.79 per cent in February and 2.68 per cent in December. Out of 306 animals examined at University Veterinary hospital,

Mannuthy, 8 animals were positive giving a prevalence rate of 2.61 per cent. The maximum prevalence was noted in January (11.90 per cent), followed by February (5.41 per cent) and December (4.17 per cent).

Dogs

Among 2346 dogs examined for cutaneous myiasis at both the hospitals, put together, the number of positive cases were 51 with a prevalence rate of 2.17 per cent (Table 4). The maximum prevalence was noted during January (10.62 per cent) and the minimum in March (0.50 per cent). Out of 2222 animals examined at University Veterinary hospital, Kokkalai, 46 were positive, giving a prevalence rate of 2.07 per cent. The highest prevalence was seen during January (10.09 per cent) and the lowest in March (0.52 per cent).

At University Veterinary hospital, Mannuthy 5 positive cases were recorded from 124 animals examined with a prevalence rate of 4.03 per cent. The prevalence was highest in January (25 per cent) and lowest in February (7.14 per cent).

4.1.2 Species of larvae obtained

The species of larvae obtained from cutaneous myiasis cases recorded above, belonged to *Chrysomyia bezziana*, *Lucilia cuprina* and *Chrysomyia megacephala* (Fig.1,2 and 3). Out of

205 positive cases 186 (90.73 per cent) were of *Chrysomyia bezziana*, 15 cases (7.32 per cent) were of *Lucilia cuprina*, followed by *Chrysomyia megacephala* in 4 cases (1.95 per cent) (Table 5 and Chart 3). In the 186 *C. bezziana* infected cases 114 (61.29 per cent) were in cattle, 23 (12.37 per cent) in goats and 49 (26.34 per cent) in dogs. In the 15 cases of *L. cuprina*, 12 cases (80 per cent) were seen in cattle 1 (6.67 per cent) in goats, and 2 (13.33 per cent) in dogs. All the 4 cases infected with *C. megacephala* were noted in cattle only.

4.1.3 Morphology of adult flies and larvae

4.1.3.1 Chrysomyia bezziana

Third stage larvae

The larva measured 11-18 mm in length, 2-3.6 mm in breadth and was creamy white in colour. The body was composed of 12 segments, the first 4 segments telescoped at their posterior margins. The anterior most segment carried 2 small thick fleshy antennae, each of which were guarded by a pair of dark nob-like structures placed one below the other. The anterior spiracles were fan shaped and the common stalk carried 6 papillae. Thoracic segments had backwardly directed spine bands, 3-4 such rows on the prothorax, 5-6 rows on the mesothorax and 7-8 rows on the metathorax. Abdominal segments had prominent spine bands composed of 7-9 rows of irregularly

alligned thorn like spines. The peritreme of the spiracle was incomplete at the postero-inferior aspect with both ends pointed and the button was obsolete.

Adult fly

The flies measured a length of 9-11 mm. The parafascialia, antennae and palpi were orange in colour. The thorax was green to bluish purple in colour. Chetotaxy on the thorax was, acrostichals 0:1, dorsocentrals 2:3, intra alars 1, supra alars 2, post alars 2, presutural intra alars 0, humerals 2, post humerals 1, and sternopleurals 1:1. The abdomen was also green to bluish purple in colour and the posterior margins of the second and third visible segments were dark margined.

4.1.3.2 *Chrysomyia megacephala*

Third stage larvae

The larvae measured 12.5 to 20 mm in length and 2.5 to 3.5 mm in breadth. The larvae were whitish yellow in colour and the prothorax was provided with 6 rows of recurved brown spines. The anterior spiracle carried 13 papillae. The girdle of spines present on the abdominal segments were simple with single or bifid tips. The peritreme of the posterior spiracle was incomplete, thin and lightly chitinised with one

of its ends bearing a cleft and the other end pointed. The button was faintly visible.

Adult fly

The fly measured a length of 10-12 mm. The parafrontalia in male was reduced to a fine line and in female, it was slightly narrower than the width of frons, which appeared black towards the vertex. Thorax was greenish blue in colour and the prothoracic spiracles was dark brown. Abdomen was also coloured greenish blue. The wings were slightly darkened at its base.

4.1.3.3 *Lucilia cuprina*

Third stage larvae

The larvae measured 10-16 mm in length, 1.5 to 2.5 mm in breadth and was creamy white in colour. The prothorax was not provided with spinules and was smooth. The anterior spiracles carried 8 papillae. The mesothoracic, metathoracic and abdominal segments possessed 6-7 rows of minute yellow recurved spines along their anterior margins. The abdominal segments had thick ventral pads with inconspicuous spines on them. The posterior spiracles were placed at a wider angle with complete peritreme and a button.

Adult flies

The adult flies measured 6.0-8.0 mm in length. The eyes in males were separated by a distance equal to double the width of the third antennal segment. Thorax was coppery green in colour, the males had much more coppery colouration than the females. Chetotaxy on the thorax was, humerals 3:4, notopleurals 2, supra alars 2:4, intra alars 2:2, post alars 3, achrostichals 2:3:3, dorsocentrals 3:3 and the marginal scutellar 4. Abdomen was also coppery green in colour and was arched in profile.

Table 1. Monthly prevalence of cutaneous myiasis in animals in University Veterinary Hospitals from September 1994 to August 1995

Period	Number of animals examined	Number found positive	Prevalence percentage
September '94	745	0	0
October '94	944	3	0.32
November '94	1114	11	0.99
December '94	830	38	4.58
January '95	839	86	10.25
February '95	937	57	6.08
March '95	765	9	1.18
April '95	685	1	0.15
May '95	577	0	0
June '95	685	0	0
July '95	795	0	0
August '95	945	0	0
Total	9861	205	2.08

Table 2. Monthly prevalence of cutaneous myiasis among cattle

Period	Veterinary Hospital Kokkalai			Veterinary Hospital Mannuthy			Total		
	Number of animals examined	Number of positive cases	Pervalence percentage	Number of animals examined	Number of positive cases	Pervalence percentage	Number of animals examined	Number of positive cases	Pervalence percentage
September '94	416	0	0	86	0	0	502	0	0
October '94	519	3	0.58	92	0	0	611	3	0.49
November '94	677	8	1.18	43	1	2.33	720	9	1.25
December '94	423	17	4.02	112	7	6.25	535	24	4.49
January '95	397	31	7.81	88	18	20.45	485	49	10.10
February '95	328	22	6.71	107	14	13.08	435	36	8.28
March '95	333	6	1.80	67	2	2.99	400	8	2
April '95	297	1	0.34	97	0	0	394	1	0.25
May '95	248	0	0	69	0	0	317	0	0
June '95	349	0	0	62	0	0	411	0	0
July '95	411	0	0	93	0	0	504	0	0
August '95	568	0	0	47	0	0	615	0	0
Total	4966	88	1.77	963	42	4.36	5929	130	2.19

Table 3. Monthly prevalence of cutaneous myiasis among goats

Period	Veterinary Hospital Kokkalai			Veterinary Hospital Mannuthy			Total		
	Number of animals examined	Number of positive cases	Pervalence percentage	Number of animals examined	Number of positive cases	Pervalence percentage	Number of animals examined	Number of positive cases	Pervalence percentage
September '94	111	0	0	12	0	0	123	0	0
October '94	92	0	0	18	0	0	110	0	0
November '94	172	0	0	36	0	0	208	0	0
December '94	112	3	2.68	24	1	4.17	136	4	2.94
January '95	86	8	9.30	42	5	11.90	128	13	10.16
February '95	132	5	3.79	37	2	5.41	169	7	4.14
March '95	145	0	0	19	0	0	164	0	0
April '95	90	0	0	21	0	0	111	0	0
May '95	69	0	0	28	0	0	97	0	0
June '95	74	0	0	31	0	0	105	0	0
July '95	83	0	0	20	0	0	103	0	0
August '95	114	0	0	18	0	0	132	0	0
Total	1280	16	1.25	306	8	2.61	1586	24	1.51

Table 4. Monthly prevalence of cutaneous myiasis among dogs

Period	Veterinary Hospital Kokkalai			Veterinary Hospital Mannuthy			Total		
	Number of animals examined	Number of positive cases	Pervalence percentage	Number of animals examined	Number of positive cases	Pervalence percentage	Number of animals examined	Number of positive cases	Pervalence percentage
September '94	110	0	0	10	0	0	120	0	0
October '94	211	0	0	12	0	0	223	0	0
November '94	176	1	0.57	10	1	10	186	2	1.08
December '94	151	9	5.96	8	1	12.5	159	10	6.29
January '95	218	22	10.09	8	2	25	226	24	10.62
February '95	319	13	4.08	14	1	7.14	333	14	4.20
March '95	192	1	0.52	9	0	0	201	1	0.50
April '95	167	0	0	13	0	0	180	0	0
May '95	149	0	0	14	0	0	163	0	0
June '95	162	0	0	7	0	0	169	0	0
July '95	179	0	0	9	0	0	188	0	0
August '95	188	0	0	10	0	0	198	0	0
Total	2222	46	2.07	124	5	4.03	2346	51	2.17

Table 5. Species of larvae obtained from cutaneous myiasis

Species of larvae	Cattle		Goats		Dogs		Total	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
C. megacephala	4	100.00	0	0	0	0	4	1.95
C. bezziana	114	61.29	23	12.37	49	26.34	186	90.73
L. cuprina	12	80.00	1	6.67	2	13.33	15	7.32
Total	130	63.41	24	11.71	51	24.88	205	100.00

Chart.1 MONTHLY (SEASONAL) PREVALENCE OF CUTANEOUS MYIASIS IN THRISSUR FROM SEPTEMBER '94 TO AUGUST '95

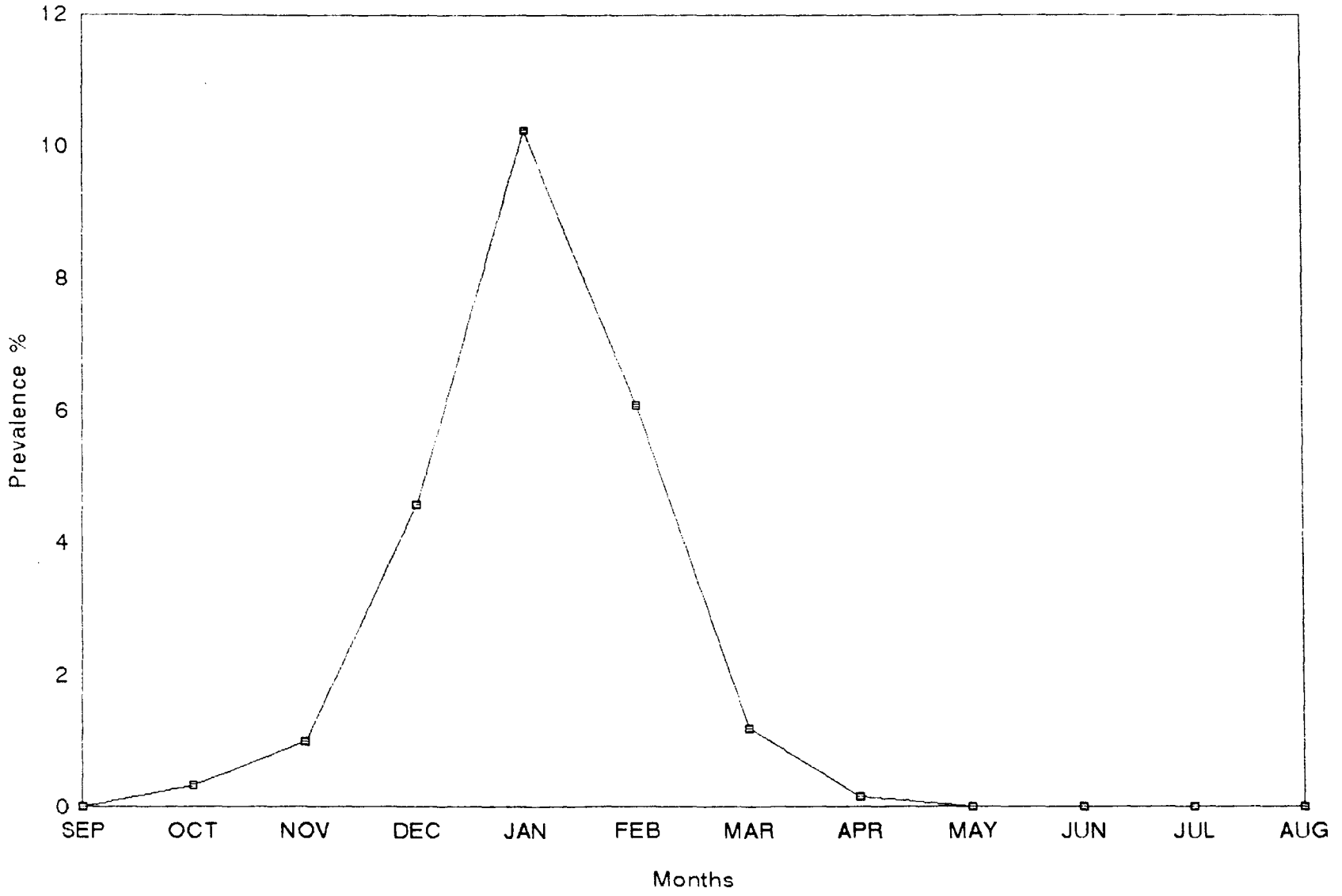


Chart.2 PREVALENCE OF CUTANEOUS MYIASIS IN DIFFERENT HOSTS

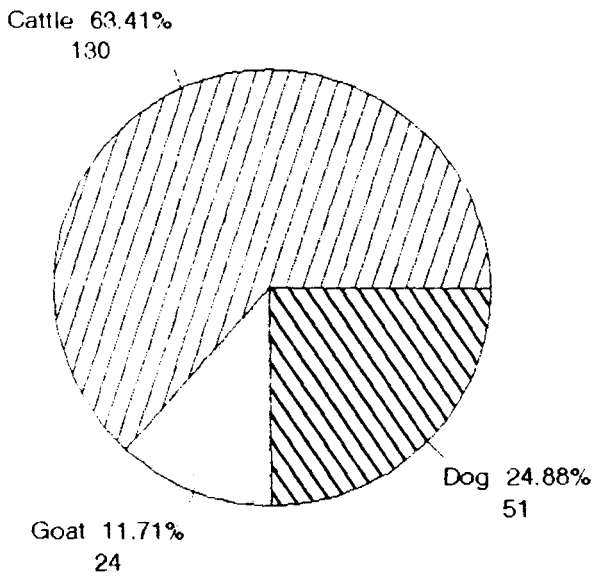
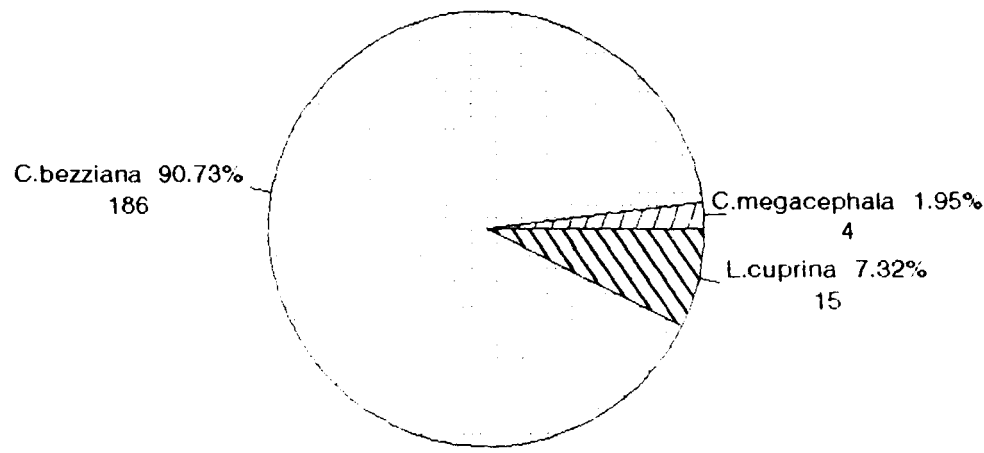


Chart.3 SPECIES OF LARVAE ENCOUNTERED IN CUTANEOUS MYIASIS



4.2 EFFECT OF METHOPRENE

4.2.1 Eggs (Table 6)

C. megacephala

The mortality rates of eggs at 1, 5 and 10 ppm were 74, 87 and 96 per cent respectively and 100 per cent at higher concentrations of 30 and 50 ppm, while in the control, only 2 per cent mortality was noticed. Further development of the larvae was noted in 1 and 5 ppm concentrations only. At these respective concentrations mentioned, reduced rate of pupation (21 and 6 per cent), adult development (11 and 2 per cent), malformed adults (6 and 2 per cent) and adult longevity (7 days and 1 day in males and 13 and 2 days in females) were also noticed. The average number of eggs laid were 180 at 1 ppm and egg laying was totally absent at higher concentrations. The malformed adults showed difficulty to unfold wings properly. The life cycle could not be completed at 5 ppm and above concentrations of methoprene. In the controls, the corresponding data obtained were pupation (95 per cent), adult development (90 per cent), malformed adults (0 per cent) and adult life span (15 days in males and 62 days in females). The flies laid 2239 eggs on an average.

C. bezziana

The mortality rates of eggs treated at 1, 5 and 10 ppm concentrations were 81, 92 and 98 per cent respectively and 100 per cent at higher concentrations. At 10 ppm and above, further development was not observed in the hatched out larvae. At 1 and 5 ppm reduced rate of pupation (9 and 3 per cent), adult development (3 and 1 per cent) and malformed adults (1 and 1 per cent) respectively were observed in the treated cases. The longevity of male flies was 3 days and 1 day and of female flies was 6 days and 1 day respectively. Eggs were not laid in any of the treatments mentioned. Permanently folded wings were the only morphological deformity noted in the malformed adults. The life cycle could not be completed even at the lowest concentration of methoprene tested. In the control, the mortality (10 per cent), pupation (85 per cent), adult development (82 per cent), malformed adults (0 per cent) and the life span of males (18 days) and females (49 days) were observed as noted against them. The average number of eggs laid by adult female flies was 1927.

L. cuprina

The mortality rates of eggs at 1 and 5 ppm were 85 and 94 per cent respectively and 100 per cent at higher concentrations. The percentages of pupation were 9 and 2 at

1 and 5 ppm respectively. Further development was seen only at 1 ppm level. The adult development (6 per cent), malformed adults with permanently folded wings (3 per cent) and longevity of adult male and female flies (2 and 5 days respectively) were also observed at the above level. Eggs were not laid in any of the concentrations tested. Even at 1 ppm concentration life cycle could not be completed. In the controls, the mortality (5 per cent), pupation (87 per cent) adult development (83 per cent), malformed adults (0 per cent), life span of males (17 days) and females (61 days) and the average number of eggs laid by adult flies (1380) were recorded as shown against them.

4.2.2 **Larval stage I** (Table 6)

C. megacephala

The mortality of the larvae ascended from 52.5 to 92.5 per cent and the pupation descended from 40 to 2.5 per cent at 1 to 50 ppm. The adult development descended from 35 to 2.5 per cent, malformed adults with permanently folded wings, 12.5 to 2.5 per cent, and the longevity of flies (10 days to 1 day) in males and 36 to 4 days in females at 1 to 30 ppm concentrations. The average numbers of eggs laid by flies were 836 and 42 at 1 and 5 ppm respectively and no eggs were laid at higher concentrations. No adult development was observed at 50 ppm concentration. In the controls except for

the mortality of 0 per cent, values of other parameters were similar to the development of eggs in control group of *C. megacephala*.

C. bezziana

The mortality of the larvae ascended from 57.5 to 97.5 per cent at 1 to 50 ppm and the pupation descended from 35 to 2.5 per cent at 1 to 30 ppm concentrations. The adult development descended from 30 to 5 per cent, malformed adults with permanently folded wings 10 to 5 per cent and the longevity of flies (9 days to 1 day) in males and 18 to 3 days in females at 1 to 10 ppm. The average number of eggs laid at 1 ppm was 187 and egg laying was not observed at higher concentrations. No pupation and adult development were observed at 50 and 30 ppm respectively and the life cycle could not be completed at 5 ppm of methoprene. In the controls, except for the mortality of 0 per cent, values of other parameters were similar to the development of eggs in control group in *C. bezziana*.

L. cuprina

The mortality of larvae ascended from 67.5 to 100 per cent at 1 to 50 ppm of methoprene. The pupation descended from 27.5 to 7.5 per cent and adult development from 20 to 2.5 per cent at 1 to 10 ppm. At the above concentrations malformed adults with permanently folded wings decreased from

10 to 2.5 per cent and the longevity of flies from 6 days to 1 day in males and 12 to 2 days in females. The average number of eggs laid at 1 ppm was 49 while eggs were not seen laid in higher concentrations. No pupation was noticed at 30 ppm and the life cycle could not be completed at 5 ppm concentrations. In the controls, except for the mortality of 0 per cent, values of other parameters were similar to the development of eggs in control group of *L. cuprina*.

4.2.3 Larval stage II (Table 7)

C. megacephala

In 1 to 50 ppm concentrations, the larval mortality increased from 27.5 to 32.5 per cent, and the period of larval development from 18 to 25 hours. An increase of larval pupal intermediaries from 5 to 42.5 per cent were also observed. The pupation descended from 67.5 to 27.5 per cent, and pupal length increased from 9 to 10.5 mm in 1 to 50 ppm concentrations (Fig.11). The adult development decreased from 60 to 17.5 per cent, malformed adults increased from 17.5 to 20 per cent and the longevity of flies reduced from 8 days to 1 day in males and 22 to 2 days in females at 1 to 10 ppm concentrations. The average numbers of eggs laid were 398 and 44 at 1 and 5 ppm respectively. The subsequent egg hatch was 38 per cent and adult development 7.5 per cent at 1 ppm. All the flies emerged at 10 ppm had morphological deformities.

The observations were failure of fly to emerge fully out of the puparium, incomplete regression of ptilinal sac after emergence, failure to form metallic colour on the body, permanently folded wings (Fig.18), incomplete unfolding of one or both the wings (Fig.19) and the abnormally flexed legs. No adult development, egg laying and egg hatch were noted at 30, 10 and 5 ppm concentration respectively. The life cycle could not be completed at 5 ppm concentration. In the control, the period of larval development was 15 hours, larval pupal intermediaries 5 per cent, length of the pupae 9 mm, egg hatch 92 per cent and the subsequent development of adults 87.5 per cent. All the values of other parameters in the controls were similar to the development of larval stage I of *C. megacephala*

C. bezziana

The mortality increased from 32.5 to 40 per cent, the period of larval development from 18 to 24 hours and larval pupal intermediaries from 15 to 52.5 per cent at 1 to 50 ppm concentrations. The pupation decreased from 52.5 to 15 per cent and pupal length increased from 8 to 9.5 mm at 1 to 30 ppm (Fig.10). The adult development descended from 47.5 to 12.5 per cent, malformed adults (20 to 12.5 per cent), and longevity of flies 7 days to 1 day in males and 20 days to 1 day in females at 1 to 10 ppm. The average numbers of eggs laid were 246 and 12 at 1 and 5 ppm respectively. There was

no egg hatch at 5 ppm, and at 1 ppm an egg hatch rate of 24 per cent and adult development of 2.5 per cent were recorded. All the flies emerged at 10 ppm concentration had morphological deformities similar to the flies developed from treated larval stage II of *C. megacephala* (Fig.12-17). No pupation, adult development, egg laying and egg hatch were noted at 50, 30, 10 and 5 ppm respectively. The life cycle could not be completed at 5 ppm concentration of methoprene. In the control, the larval development of 16 hours, larval pupal intermediaries of 15 per cent, pupal length of 8 mm (Fig.9), the egg hatch of 85 per cent and the subsequent development of adults 82.5 per cent from the hatched eggs were observed. All the values of other parameters in the controls were similar to the development of larval stage I of *C. bezziana*.

L. cuprina

The mortality increased from 37.5 to 45 per cent, the period of larval development from 20 to 25 hours and the larval pupal intermediaries from 15 to 55 per cent at 1 to 50 ppm. The pupation rate descended from 52.5 to 7.5 per cent, and pupal length increased from 6.5 to 8 mm at 1 to 30 ppm. The rates of adult development were 32.5 and 17.5 per cent at 1 and 5 ppm respectively. Malformed adults were 17.5 per cent at both the above concentrations. Longevity rates of 7 days

and 1 day in males and 16 and 4 days in females at 1-5 ppm were also noted. The number of eggs laid were 92 with a hatch of 12 per cent and adult development was 0 per cent at 1 ppm. All the flies emerged at 5 ppm concentration had morphological deformities similar to the flies developed from treated larval stage II of *C. megacephala* (Fig.20). No pupation, adult development, egg laying and subsequent development occurred at 50, 10, 5 and 1 ppm respectively. The life cycle was arrested at 1 ppm concentration. In the control, a larval development of 16 hours, larval pupal intermediaries of 12.5 per cent, pupal length of 6.5 m.m, egg hatch of 88 per cent and subsequent adult development of 80 per cent were noted. All the values of other parameters in the controls were similar to the development of larval stage I of *L. cuprina*.

4.2.4 Larval stage III (Table 8)

C. megacephala

The rate of mortality of the larvae increased from 0 to 7.5 per cent, the period of larval development from 23 to 38 hrs and larval pupal intermediaries from 10 to 60 per cent at 1 to 50 ppm concentrations. At the above concentrations the pupation rate descended from 90 to 32.5 per cent while the length of the pupae increased from 9 to 10.5 mm. The adult development decreased from 75 to 12.5 per cent, malformed

adults from 20 to 12.5 per cent and the longevity of flies from 9 days to 1 day in males and from 20 days to 1 day in females. The average numbers of eggs laid were 412 and 40 at 1 and 5 ppm respectively. The rate of egg hatch and subsequent adult development were 36 and 5 per cent respectively at 1 ppm concentration only. All the flies emerged at 10 ppm concentrations had morphological deformities similar to the deformed flies developed from the treated second stage larvae of *C. megacephala*. Adult development, egg laying and egg hatching were not observed at 30, 10 and 5 ppm respectively. The life cycle was arrested at 5 ppm itself. In the controls, except for the period of larval development of 22 hours, values of other parameters were similar to those obtained in the second stage larvae of *C. megacephala*.

C. bezziana

The mortality of the larvae increased from 0 to 10 per cent, the period of larval development from 26 to 36 hours and the larval pupal intermediaries from 15 to 52.5 per cent at 1 to 50 ppm concentrations. At the same concentrations the pupation decreased from 85 to 37.5 per cent while the length of the pupae increased from 8 to 9.5 mm. The adult development descended from 67.5 to 10 per cent, malformed adults from 22.5 to 10 per cent and the longevity of the flies from 7 days to 1 day in males and from 18 to 4 days in females

at 1 to 10 ppm concentration. The numbers of eggs laid were 213 and 13 at 1 and 5 ppm concentration respectively. Subsequent egg hatching and adult development were 18 and 2.5 per cent at 1 ppm only. All the flies emerged at 10 ppm concentrations had morphological deformities similar to that of flies developed from treated second stage larvae of *C. megacephala*. The adult development, egg laying and hatching were absent at 30, 10 and 5 ppm concentrations respectively. The life cycle was arrested at 5 ppm concentration of methoprene. In the controls, except for the period of larval development of 24 hrs, values of other parameters were similar to the second stage larvae of *C. bezziana*.

L. cuprina

The mortality of the larvae enhanced from 2.5 to 17.5 per cent, the period of larval development from 29 to 42 hrs and the larval pupal intermediaries from 15 to 55 per cent at 1 to 50 ppm concentrations. At similar concentrations, the pupation descended from 82.5 to 27.5 per cent while the length of the pupae increased from 6.5 to 8 mm. The adult development descended from 70 to 5 per cent, malformed adults from 27.5 to 5 per cent, longevity of the flies from 6 days to 1 day in males and from 15 to 3 days in females at 1 to 10 ppm concentrations. The number of eggs laid, rate of egg hatch and subsequent adult development were 118, 12 per cent and 2.5 per cent respectively at 1 ppm concentration. All the flies

emerged at 10 ppm concentrations had morphological deformities similar to that of the flies developed from the treated second stage larvae of *C. megacephala*. Adult development and egg hatching were not recorded at 30 and 5 ppm concentrations respectively. The lifecycle was blocked at 5 ppm concentration. In the controls, except for the period of larval development of 28 hrs, values of other parameters were similar to second stage larvae of *L. cuprina*.

The mortality effect of methoprene on eggs and different larval stages of *C. megacephala* and adult development of eggs and different larval stages on methoprene treatment are graphically represented in charts 4 and 5 respectively. Such effects on *C. bezziana* (Charts 6 and 7) and *L. cuprina* (Charts 8 and 9) are also given.

Table 6. Effect of Methoprene on eggs and larval stage-I of myiasis producing flies (Mean values)

Species	Concentration ppm	Eggs							Larval stage-I						
		Mortality per cent	Pupation per cent	Adult development per cent	Malformed adults per cent	Adult life span days M	Adult life span days F	Eggs laid average	Mortality per cent	Pupation per cent	Adult development per cent	Malformed adults per cent	Adult life span days M	Adult life span days F	Eggs laid average
C. megacephala	1	74	21	11	6	7	13	180	52.5	40	35	10	10	36	836
	5	87	6	2	2	1	2	0	65	25	22.5	12.5	6	14	42
	10	96	0	0	0	0	0	0	77.5	12.5	7.5	5	3	6	0
	30	100	0	0	0	0	0	0	82.5	7.5	2.5	2.5	1	4	0
	50	100	0	0	0	0	0	0	92.5	2.5	0	0	0	0	0
	Control	2	95	90	0	15	62	2239	0	95	90	0	15	62	2239
C. bezziana	1	81	9	3	1	3	6	0	57.5	35	30	10	9	18	187
	5	92	3	1	1	1	1	0	70	22.5	12.5	7.5	2	5	0
	10	98	0	0	0	0	0	0	80	10	5	5	1	3	0
	30	100	0	0	0	0	0	0	92.5	2.5	0	0	0	0	0
	50	100	0	0	0	0	0	0	97.5	0	0	0	0	0	0
	Control	10	85	82	0	18	49	1927	0	85	82.5	0	18	49	1927
L. cuprina	1	85	9	6	3	2	5	0	67.5	27.5	20	10	6	12	49
	5	94	2	0	0	0	0	0	72.5	17.5	7.5	5	3	4	0
	10	100	0	0	0	0	0	0	87.5	7.5	2.5	2.5	1	2	0
	30	100	0	0	0	0	0	0	92.5	0	0	0	0	0	0
	50	100	0	0	0	0	0	0	100	0	0	0	0	0	0
	Control	5	87	83	0	17	61	1380	0	87.5	82.5	0	17	61	1380

M: Male F: Female

Table 7. Effect of Methoprene on larval stage-II of myiasis producing flies (Mean values)

Species	Concentration ppm	Mortality per cent	Larval development period (hours)	Larval pupal intermediaries per cent	Pupation per cent	Pupal length (mm)	Adult development per cent	Malformed adults per cent	Adult life span days		Eggs laid (average)	Eggs hatch per cent	Adult development per cent
									M	F			
	1	27.5	18	5	67.5	9	60	17.5	8	22	398	38	7.5
	5	30	21	10	60	9.5	32.5	20	5	12	44	0	0
C. megacephala	10	27.5	24	12.5	60	10	17.5	17.5	1	2	0	0	0
	30	32.5	24	27.5	40	10.5	0	0	0	0	0	0	0
	50	30	25	42.5	27.5	10.5	0	0	0	0	0	0	0
	Control	0	15	5	95	9	90	0	15	62	2239	92	87.5
	1	32.5	18	15	52.5	8	47.5	15	7	20	246	24	2.5
	5	37.5	19	20	42.5	8.5	27.5	20	5	12	12	0	0
C. bezziana	10	37.5	23	22.5	40	9.5	12.5	12.5	1	1	0	0	0
	30	40	24	45	15	9.5	0	0	0	0	0	0	0
	50	37.5	24	52.5	0	-	0	0	0	0	0	0	0
	Control	0	16	15	85	8	82.5	0	18	49	1927	85	82.5
	1	42.5	20	15	52.5	6.5	32.5	17.5	7	16	92	12	0
	5	37.5	21	15	47.5	7	17.5	17.5	1	4	0	0	0
L. cuprina	10	42.5	23	32.5	25	7.5	0	0	0	0	0	0	0
	30	45	25	47.5	7.5	8	0	0	0	0	0	0	0
	50	45	25	55	0	-	0	0	0	0	0	0	0
	Control	0	16	12.5	87.5	6.5	82.5	0	17	61	1380	88	80

M: Male F: Female

Table 8. Effect of Methoprene on larval stage-III of myiasis producing flies (Mean values)

Species	Concentration ppm	Mortality per cent	Larval development period (hours)	Larval pupal intermediaries per cent	Pupation per cent	Pupal length (mm)	Adult development per cent	Malformed adults per cent	Adult life span days		Eggs laid (average)	Eggs hatch per cent	Adult development per cent
									M	F			
	1	0	23	10	90	9	75	20	9	20	412	36	5
	5	2.5	27	12.5	85	9.5	37.5	17.5	5	14	40	0	0
C. megacephala	10	2.5	30	17.5	80	10	12.5	12.5	1	1	0	0	0
	30	5	36	42.5	52.5	10.5	0	0	0	0	0	0	0
	50	7.5	38	60	32.5	10.5	0	0	0	0	0	0	0
	Control	0	22	5	95	9	90	0	15	62	2239	92	87.5
	1	0	26	15	85	8	67.5	22.5	7	18	213	18	2.5
	5	2.5	28	20	77.5	8.5	22.5	7.5	6	12	13	0	0
C. bezziana	10	5	30	25	70	9	10	10	1	4	0	0	0
	30	7.5	36	40	52.5	9.5	0	0	0	0	0	0	0
	50	10	36	52.5	37.5	9.5	0	0	0	0	0	0	0
	Control	0	24	15	85	8	82.5	0	18	49	1927	85	82.5
	1	2.5	29	15	82.5	6.5	70	27.5	6	15	118	12	2.5
	5	5	32	17.5	77.5	7	17.5	10	4	8	0	0	0
L. cuprina	10	10	38	22.5	67.5	7.5	5	5	1	3	0	0	0
	30	15	42	37.5	47.5	7.5	0	0	0	0	0	0	0
	50	17.5	42	55	27.5	8	0	0	0	0	0	0	0
	Control	0	28	12.5	87.5	6.5	82.5	0	17	61	1380	88	80

M: Male F: Female

Chart.4 MORTALITY EFFECT OF METHOPRENE ON DIFFERENT DEVELOPMENTAL STAGES OF C.megacephala

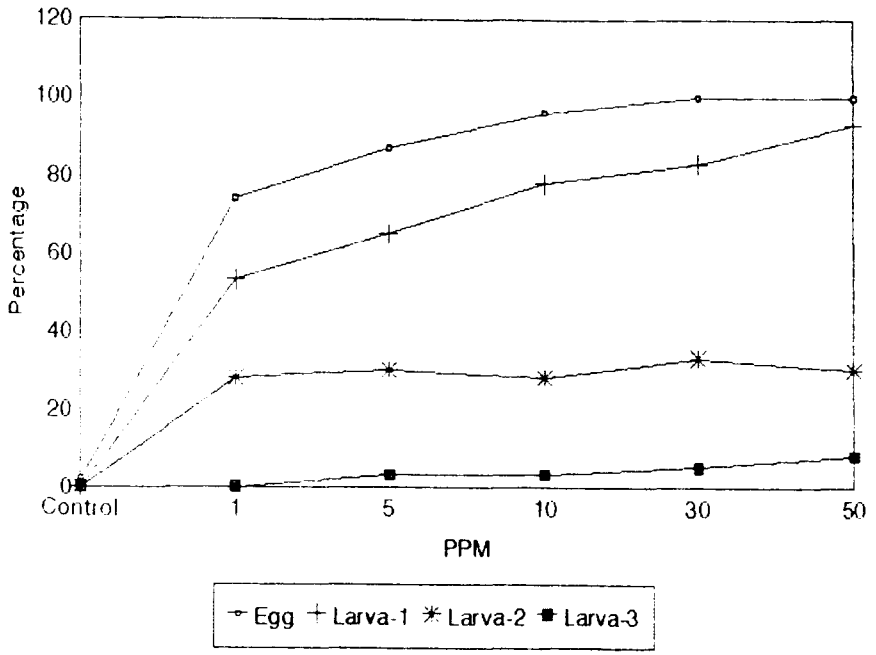


Chart.5 ADULT DEVELOPMENT OF DIFFERENT STAGES OF C.megacephala ON METHOPRENE TREATMENT

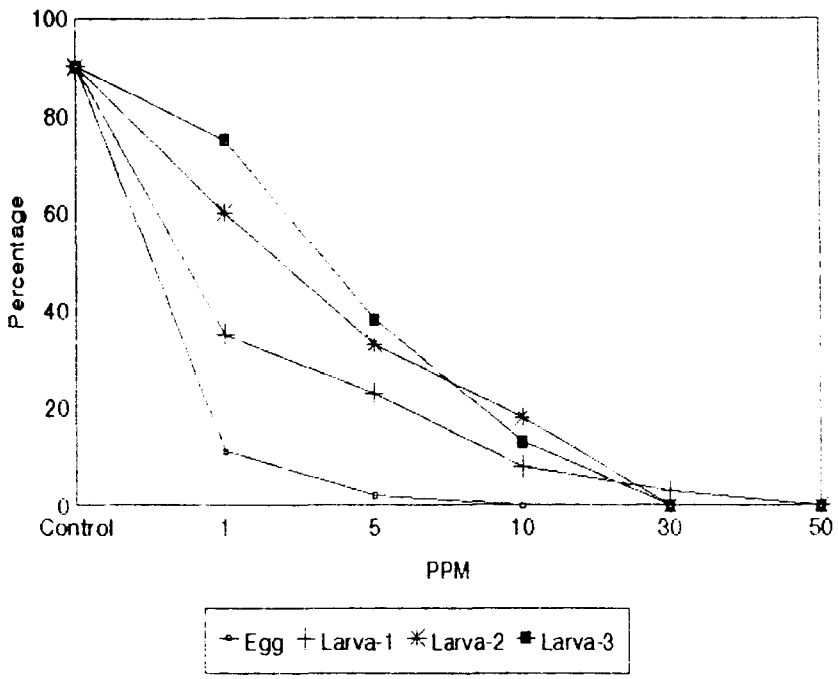


Chart.6 MORTALITY EFFECT OF METHOPRENE ON DIFFERENT DEVELOPMENTAL STAGES OF C.bezziana

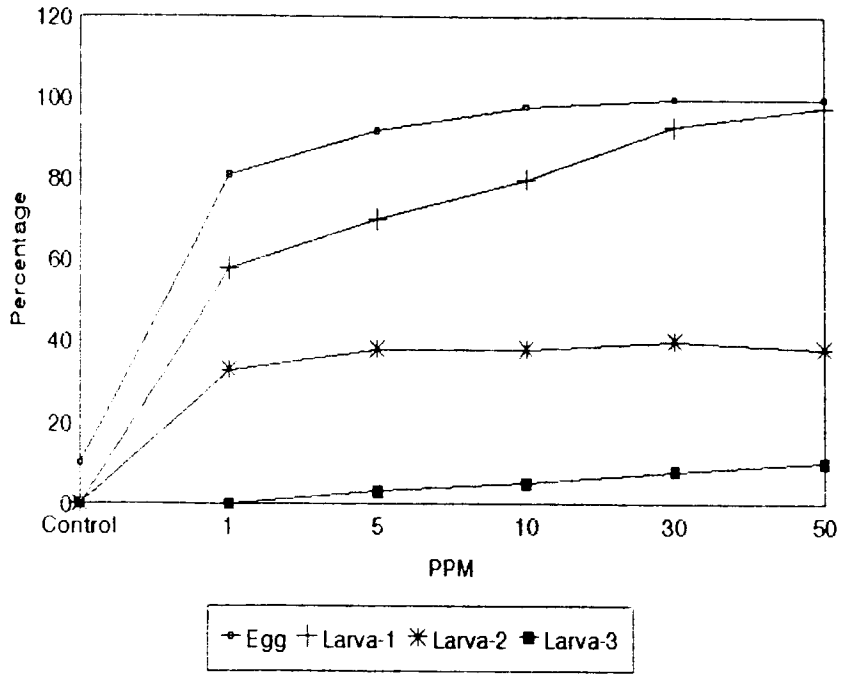


Chart.7 ADULT DEVELOPMENT OF DIFFERENT STAGES OF C.bezziana ON METHOPRENE TREATMENT

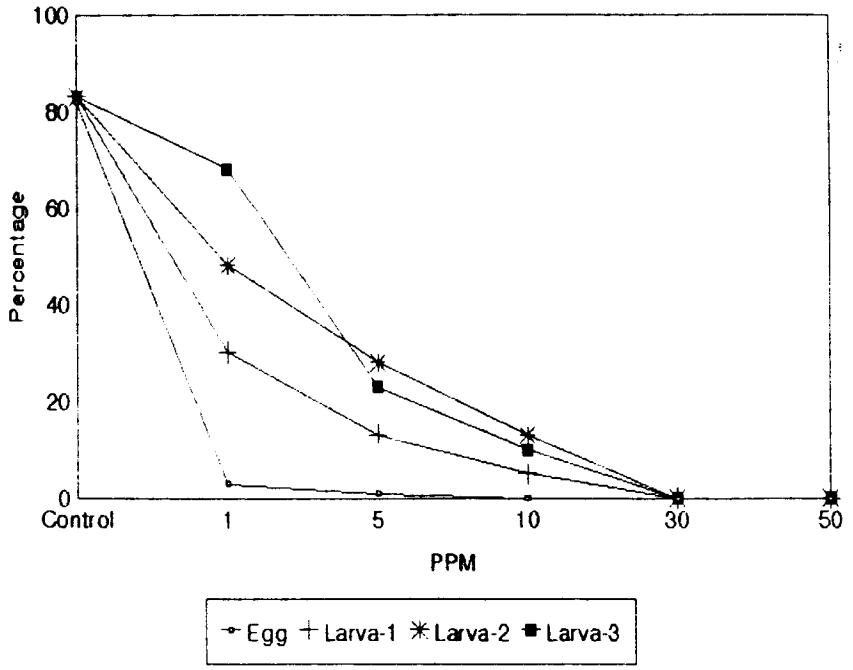


Chart.8 MORTALITY EFFECT OF METHOPRENE ON DIFFERENT DEVELOPMENTAL STAGES OF L.cuprina

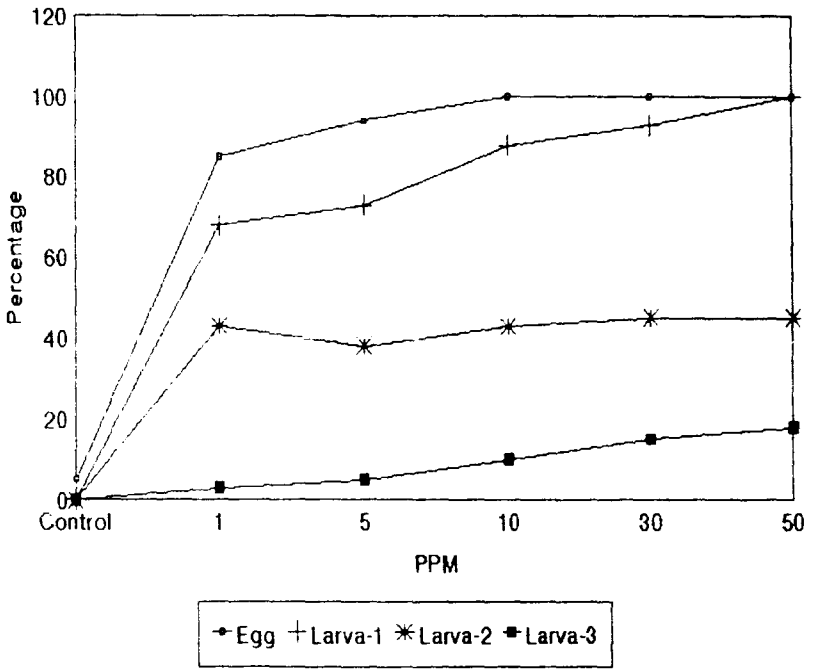


Chart.9 ADULT DEVELOPMENT OF DIFFERENT STAGES OF L.cuprina ON METHOPRENE TREATMENT

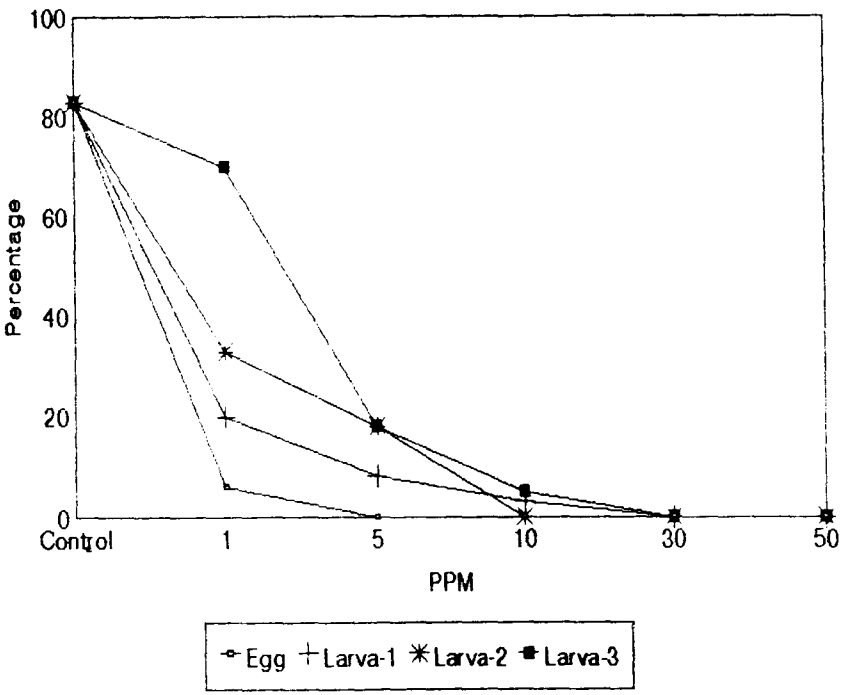


Fig.9. *C. bezziana* - Normal pupae

Fig.10. *C. bezziana* - Pupae developed on treatment of methoprene in larval stages

Fig.11. *C. megacephala* - pupae developed on treatment of methoprene in larval stages

Fig.12. *C. bezziana* - Partly emerged flies from puparium on treatment of methoprene in larval stages

Fig.13. *C. bezziana* - Partly emerged flies from puparium on treatment of methoprene in larval stages

Fig.14. *C. bezziana* - Emerged flies showing absence of metallic colour and flexed legs on treatment of methoprene in larval stages

Fig.15. *C. bezziana* - Emerged flies showing totally folded wings on methoprene treatment in larval stages

Fig.16. *C. bezziana* - Emerged flies showing partially folded wings on methoprene treatment in larval stages

Fig.17. *C. bezziana* - Emerged flies showing abnormally flexed legs on methoprene treatment in larval stages

Fig.18. *C. megacephala* - Emerged fly showing totally folded wings on methoprene treatment in larval stages

Fig.19. *C. megacephala* - Emerged flies showing partially folded wings on methoprene treatment in larval stages

Fig.20. *L. cuprina* - Emerged flies showing totally folded wing on methoprene treatment in larval stages

4.3 EFFECT OF DIFLUBENZURON

4.3.1 Egg (Table 9)

C. megacephala

The mortality of eggs was found to be very low ranging from 4 to 13 per cent at 0.5 to 10 ppm concentrations. The pupation rates were 16, 14 and 9 per cent at 0.5, 1 and 2.5 ppm concentrations respectively. The adult developments were 9 and 3 per cent with a longevity of 6 and 2 days in males and 12 and 4 days in females at 0.5 and 1 ppm concentrations respectively. At 0.5 ppm, the malformed adults were 1 per cent and the flies laid an average of 15 eggs. Pupation, adult development and egg laying were not noticed at 5, 2.5 and 1 ppm respectively and the life cycle was not completed at 1 ppm. In the malformed adults, functional incapacities of the legs and wings were the only observations noted. In the controls the corresponding data obtained were mortality (2 per cent), pupation (94 per cent), adult development (91 per cent) and malformed adults (0 per cent). Adult life spans of 14 days in males and 58 days in females were also observed. An average of 2386 eggs were laid by the flies.

C. bezziana

The rates of mortality of eggs were slightly higher than that of *C. megacephala* and ranged from 20 to 27 per cent at

0.5 to 10 ppm concentrations. The rates of pupation were 15 and 9 per cent, adult development 6 and 1 per cent, and longevity 3 days and 1 day in males and 7 and 2 days in females at 0.5 and 1 ppm respectively. The adult deformities and egg laying by flies were not observed in any of the concentration tested. There was no pupation at 2.5 ppm and the life cycle was not completed even at the lowest concentration of 0.5 ppm.

In the controls, the mortality was 11 per cent, pupation 86 per cent, adult development 81 per cent and malformed adults 0 per cent. Adult life span of 17 days in males and 50 days in females were observed and an average of 1969 eggs were laid by the flies.

L. cuprina

The mortality rates were higher than that of *C. megacephala* but lower than that of *C. bezziana*, and ranged from 10 to 19 per cent at 0.5 to 10 ppm concentrations. The pupation rates were 11 and 4 per cent, adult development 3 and 1 per cent and a longevity of 3 days and 1 day in males and 5 days and 1 day in females at 0.5 and 1 ppm respectively. Malformed adults and egg laying by the flies were not noticed in any of the concentrations tested. There was no pupation at 2.5 ppm and the life cycle could not be completed even at 0.5 ppm concentration. In the control, mortality was 4 per cent,

pupation 90 per cent, adult development 88 per cent and malformed adults 0 per cent. Adult life spans of 16 days in males and 57 days in females were observed and an average of 1519 eggs were laid by the females.

4.3.2 Larval stage-I (Table 9)

C. megacephala

A very high mortality effect was noticed by the treatment of diflubenzuron on larval stage-I. The mortality rates were 90 and 97.5 at 0.5 and 1 ppm respectively and 100 per cent at higher concentrations. The rate of pupation was 5 per cent, adult development 2.5 per cent, male longevity of 1 day and females longevity 4 days at 0.5 ppm. Eventhough no adult deformities were noted, there was no egg laying by adult flies in any of the concentrations. The pupation was blocked at 1 ppm and the life cycle could not be completed at 0.5 ppm concentration.

In the controls, the mortality was 0 per cent, pupation 92.5 per cent and adult development 90 per cent. Values in other parameters were similar to that of the development of eggs in *C. megacephala*.

C. bezziana

The effects of mortality on *C. bezziana* were higher than that on *C. megacephala*. The mortality rates were 92.5

per cent at 0.5 ppm and 100 per cent at higher concentrations. Though 2.5 per cent pupation was noted at 0.5 ppm, further development was not noticed. In the controls, mortality was 0 per cent, pupation 85 per cent and adult development 80 per cent. Values in other parameters were similar to the development of eggs in *C. bezzina*.

L. cuprina

The effect of mortality was the highest compared to that on *C. megacephala* and *C. bezziana*. The mortality rates were 95 per cent at 0.5 ppm and 100 per cent at higher concentrations. Though the pupation was 2.5 per cent at 0.5 ppm further development was not observed. In the controls mortality was 0 per cent, pupation 90 per cent and adult development 87.5 per cent. Values in other parameters were similar to that on the development of eggs in *L. cuprina*.

4.3.3 Larval stage-II (Table 10)

C. megacephala

The mortality rates were 72.5, 80 and 92.5 per cent at 0.5, 1 and 2.5 ppm respectively and 100 per cent at higher concentrations. The pupation rates of 15 and 7.5 per cent, average pupal weight of 39 and 34 mgs, adult development of 10 and 5 per cent and a longevity of 8 and 3 days in males and

19 and 7 days in females were noted at 0.5 and 1 ppm concentrations respectively. At 0.5 ppm, 135 eggs were laid on an average, with 12 per cent egg hatch and 5 per cent subsequent development to adults. The pupation was totally blocked at 2.5 ppm and the lifecycle could not be completed at 1 ppm concentration. In the controls, the observations were mortality 0 per cent, pupation 92.5 per cent, average pupal weight 52 mgs, adult development 90 per cent, adult deformities 0 per cent and the lifespan 14 days in males and 58 days in females. On an average the flies laid 2386 eggs with 98 per cent hatchability and 87.5 per cent of subsequent development to adult flies.

C. bezziana

The mortality effect observed was higher than that in *C. megacephala*. The mortality rates were 77.5, 92.5 and 97.5 per cent at 0.5, 1 and 2.5 ppm respectively and 100 per cent at higher concentrations. The rates of pupation were 12.5 and 7.5 per cent, average pupal weight 41 and 37 mgs, adult development 7.5 and 2.5 per cent, malformed adults 0 and 0 per cent, longevity of males 6 days and 1 day and of females 13 and 4 days at 0.5 and 1 ppm concentrations respectively. Though the flies laid 43 eggs on an average at 0.5 ppm, further development was not noticed. The pupation was arrested at 2.5 ppm of diflubenzuron. In the controls, the mortality was 0 per cent, pupation 85 per cent, average pupal

weight 56 mgs adult development 80 per cent, malformed adults 0 per cent and the lifespan of 17 days in males and 50 days in females. The flies laid 1969 eggs on an average with 89 per cent hatchability and 77.5 per cent of subsequent adult development.

L. cuprina

The mortality effect on L-II was the highest when compared to that on *C. megacephala* and *C. bezziana*. The mortality rates were 87.5 and 95 per cent at 0.5 and 1 ppm and 100 per cent higher concentrations. The rates of pupation were 7.5 and 2.5 per cent and average pupal weight 32 and 28 mgs at 0.5 and 1 ppm respectively due to thin shelled pupae (Fig.29). At 0.5 ppm the adult development was 5 per cent and longivity 2 days in males and 6 days in females, while the emerged flies did not lay any eggs. The pupation was totally arrested at 2.5 ppm concentration. In the controls, the mortality was 0 per cent, pupation 90 per cent, average pupal weight 41 mgs, adult development 87.5 per cent, and life span 16 days in males and 57 days in females. The flies laid 1519 eggs on an average with 92 per cent hatchability and 82.5 per cent subsequent development to adults.

4.3.4 Larval stage-III (Table 11)

C. mugacephala

The mortality rates were 55, 77.5 and 87.5 per cent at 0.5, 1 and 2.5 ppm respectively and 100 per cent at higher concentrations. The pupation rates were 40, 22.5 and 7.5 per cent, with an average pupal weight of 41, 35 and 34 mgs and adult development rates of 32.5, 15 and 2.5 per cent at 0.5, 1 and 2.5 concentrations respectively. At the above concentrations, longevity of 9, 7 and 6 days in males and 26, 20 and 15 days in females were observed. The number of eggs laid were 523, 191 and 27 at 0.5, 1 and 2.5 ppm respectively. The egg hatch were 42 and 18 per cent, and subsequent adult development rates were 32.5 and 7.5 per cent at 0.5 and 1 ppm respectively. At 0.5 ppm, the malformed adults (2.5 per cent) showed functional incapacities of legs and wings. The pupation and lifecycle were blocked at 5 ppm and 2.5 ppm respectively. In the controls, values of all the parameters were similar to those of the II stage larvae of *C. megacephala*.

C. bezziana

The mortality effect was slightly higher than that in *C. megacephala*. The mortality rates were 62.5, 85 and 92.5 per cent at 0.5, 1 and 2.5 ppm respectively and 100 per cent at higher concentrations. The pupation rates were 32.5, 17.5

and 5 per cent, average pupal weight 43, 40 and 38 mg♀ and adult development 27.5, 10 and 2.5 per cent at 0.5, 1 and 2.5 ppm concentrations respectively. The longevity recorded were 8, 7 and 2 days in males and 18, 12 and 6 days in females at 0.5, 1 and 2.5 ppm respectively. An average number of 349 and 21 eggs were laid at 0.5 and 1 ppm respectively. The egg hatch 18 per cent and subsequent adult development 7.5 per cent were noted at 0.5 ppm concentration. The pupation and lifecycle were arrested at 5 and 1 ppm concentration respectively. In the controls, the values in all the parameters were similar to those of II stage larvae of *C. bezziana*.

L. cuprina

The mortality effect was the highest when compared to that on *C. megacephala* and *C. bezziana*. The mortality rates were 67.5, 87.5 and 97.5 per cent at 0.5, 1 and 2.5 ppm and 100 per cent at higher concentrations. The pupation rates were 22.5 and 7.5 per cent, average pupal weight 36 and 33 mg♀ and adult development 17.5 and 2.5 per cent, at 0.5 and 1 ppm concentrations. A longevity of 6 days and 1 day in males and 16 and 3 days in females at 0.5 and 1 ppm concentration respectively were also observed. The flies laid 146 eggs with 11 per cent hatchability and 5 per cent subsequent development to adults at 0.5 ppm. The pupation was blocked at 2.5 ppm and

lifecycle could not be completed at 1 ppm concentration. In the controls, the values in all the parameters were similar to those of the II stage larvae of *L. cuprina*.

4.3.5 Effect on chitin production

The chitin content of the larvae were reduced from half to one fifth on treatment ranging from 0.5 to 10 ppm concentrations of diflubenzuron when compared to the controls. The values mentioned below represent the quantity and percentage of chitin obtained from 100 larvae of the respective stages.

C. megacephala

In larval stage-I, the quantity of chitin ranged in the descending order from 24 to 10 mgs (48 to 20 per cent) while in larval stage-II from 74 to 37 mgs (47.44 to 23.72 per cent) and in larval stage-III from 1095 to 497 mgs (51.48 to 23.36 per cent) on treatment at 0.5 to 10 ppm of diflubenzuron. In the controls, the quantity of chitin content were 50, 156 and 2127 mgs for the respective larval stages.

C. bezziana

In the larval stage I, the quantity of chitin ranged in the descending order from 32 to 13 mgs (51.61 to 20.97 per cent) while in larval stage-II from 94 to 45 mgs (52.51 to

25.14 per cent) and in larval stage-III 1218 to 612 mgs (52.07 to 26.17 per cent). In the controls, the quantities of chitin were 62, 179 and 2339 mgs for the respective larval stages.

L. cuprina

In the larval stage I, the quantity of chitin ranged in the descending order from 16 to 7 mgs (42.11 to 18.42 per cent), while in larval stage-II from 67 to 28 mgs (47.52 to 19.86 per cent) and in larval stage-III from 867 to 401 mgs (47.77 to 22.09 per cent). In the controls, the quantities of chitin were 38, 141 and 1815 mgs for the respective larval stages.

The mortality effect of diflubenzuron on eggs and different larval stages of *C. megacephala* and the effect of diflubenzuron on chitin production in *C. megacephala* larvae are graphically represented in charts 10 and 11 respectively. Such effects on *C. bezziana* (Charts 12 and 13) and *L. cuprina* (Charts 14 and 15) are also presented.

The chitin deficiency in the cuticle caused severe damage and rupture of the cuticle wall irrespective of the stage and species of myiasis producing larvae on diflubenzuron treatment (Fig.21-28).

Table 9. Effect of Diflubenzuron on eggs and larval stage-I of myiasis producing flies (Mean values)

Species	Concentration ppm	Eggs							Larval stage-I						
		Mortality per cent	Pupation per cent	Adult development per cent	Malformed adults per cent	Adult life span days M	F	Eggs laid average	Mortality per cent	Pupation per cent	Adult development per cent	Malformed adults per cent	Adult life span days M	F	Eggs laid average
C. megacephala	0.5	4	16	9	1	6	12	15	90	5	2.5	0	1	4	0
	1	5	14	3	0	2	4	0	97.5	0	0	0	0	0	0
	2.5	8	9	0	0	0	0	0	100	0	0	0	0	0	0
	5	13	0	0	0	0	0	0	100	0	0	0	0	0	0
	10	12	0	0	0	0	0	0	100	0	0	0	0	0	0
	Control	2	94	91	0	14	58	2386	0	92.5	90	0	14	58	2386
C. bezziana	0.5	20	15	6	0	3	7	0	92.5	2.5	0	0	0	0	0
	1	20	9	1	0	1	2	0	100	0	0	0	0	0	0
	2.5	24	0	0	0	0	0	0	100	0	0	0	0	0	0
	5	27	0	0	0	0	0	0	100	0	0	0	0	0	0
	10	26	0	0	0	0	0	0	100	0	0	0	0	0	0
	Control	11	86	81	0	17	50	1969	0	85	80	0	17	50	1969
L. cuprina	0.5	10	11	3	0	3	5	0	95	2.5	0	0	0	0	0
	1	12	4	1	0	1	1	0	100	0	0	0	0	0	0
	2.5	17	0	0	0	0	0	0	100	0	0	0	0	0	0
	5	17	0	0	0	0	0	0	100	0	0	0	0	0	0
	10	19	0	0	0	0	0	0	100	0	0	0	0	0	0
	Control	4	90	88	0	16	57	1519	0	90	87.5	0	16	57	1519

M: Male F: Female

Table 10. Effect of Diflubenzuron on larval stage-II of myiasis producing flies (Mean values)

Species	Concentration ppm	Mortality per cent	Pupation per cent	Pupal weight average (mgs)	Adult development per cent	Malformed adults per cent	Adult life span days		Eggs laid (average)	Eggs hatch per cent	Adult development per cent
							M	F			
C. megacephala	0.5	72.5	15	39	10	0	8	19	135	12	5
	1	80	7.5	34	5	0	3	7	0	0	0
	2.5	92.5	0	-	0	0	0	0	0	0	0
	5	100	0	-	0	0	0	0	0	0	0
	10	100	0	-	0	0	0	0	0	0	0
	Control	0	92.5	52	90	0	14	58	2386	98	87.5
C. bezziana	0.5	77.5	12.5	41	7.5	0	6	13	43	0	0
	1	92.5	7.5	37	2.5	0	1	4	0	0	0
	2.5	97.5	0	-	0	0	0	0	0	0	0
	5	100	0	-	0	0	0	0	0	0	0
	10	100	0	-	0	0	0	0	0	0	0
	Control	0	85	56	80	0	17	50	1969	89	77.5
L. cuprina	0.5	87.5	7.5	32	5	0	2	6	0	0	0
	1	95	2.5	28	0	0	0	0	0	0	0
	2.5	100	0	-	0	0	0	0	0	0	0
	5	100	0	-	0	0	0	0	0	0	0
	10	100	0	-	0	0	0	0	0	0	0
	Control	0	90	41	87.5	0	16	57	1519	92	82.5

M: Male F: Female

Table 11. Effect of Diflubenzuron on larval stage-III of myiasis producing flies (Mean values)

Species	Concentration ppm	Mortality per cent	Pupation per cent	Pupal weight average (mgs)	Adult development per cent	Malformed adults per cent	Adult life span days		Eggs laid (average)	Eggs hatch per cent	Adult development per cent
							M	F			
C. megacephala	0.5	55	40	41	32.5	2.5	9	26	523	42	32.5
	1	77.5	22.5	35	15	0	7	20	191	18	7.5
	2.5	87.5	7.5	34	2.5	0	6	15	27	0	0
	5	100	0	-	0	0	0	0	0	0	0
	10	100	0	-	0	0	0	0	0	0	0
	Control	0	92.5	52	90	0	14	58	2386	98	87.5
C. bezziana	0.5	62.5	32.5	43	27.5	0	8	18	349	18	7.5
	1	85	17.5	40	10	0	7	12	21	0	0
	2.5	92.5	5	38	2.5	0	2	6	0	0	0
	5	100	0	-	0	0	0	0	0	0	0
	10	100	0	-	0	0	0	0	0	0	0
	Control	0	85	56	80	0	17	50	1969	89	77.5
L. cuprina	0.5	67.5	22.5	36	17.5	0	6	16	146	11	5
	1	87.5	7.5	33	2.5	0	1	3	0	0	0
	2.5	97.5	0	-	0	0	0	0	0	0	0
	5	100	0	-	0	0	0	0	0	0	0
	10	100	0	-	0	0	0	0	0	0	0
	Control	0	90	41	87.5	0	16	57	1519	92	82.5

M: Male F: Female

Table 12. Effect of Diflubenzuron on chitin production in larvae of myiasis producing flies

Species	Stages of larva	Concentration (ppm)										
		0.5		1		2.5		5		10		Control
		A	B	A	B	A	B	A	B	A	B	A
	L-1	24	48	17	34	12	24	10	20	10	20	50
C. megacephala	L-2	74	47.44	52	33.33	40	25.64	37	23.82	37	23.72	156
	L-3	1095	51.48	710	33.38	551	25.90	501	23.55	497	23.36	2127
	L-1	32	51.61	20	32.26	15	24.19	13	20.97	13	20.97	62
C. bezziana	L-2	94	52.51	69	38.55	48	26.82	46	25.70	45	25.14	179
	L-3	1218	52.07	976	41.73	640	27.36	621	26.55	612	26.17	2339
	L-1	16	42.11	11	28.95	8	21.05	7	18.42	7	18.42	38
L. cuprina	L-2	67	47.52	43	30.50	34	24.11	30	21.28	28	19.86	141
	L-3	867	47.77	612	33.72	437	24.08	411	22.64	401	22.09	1815

L-1: Larval stage I
L-2: Larval stage II
L-3: Larval stage III

A: mgs of chitin per 100 larvae
B: percentage of chitin with respect to control

Chart.10 MORTALITY EFFECT OF DIFLUBENZURON ON DIFFERENT DEVELOPMENTAL STAGES OF C.megacephala

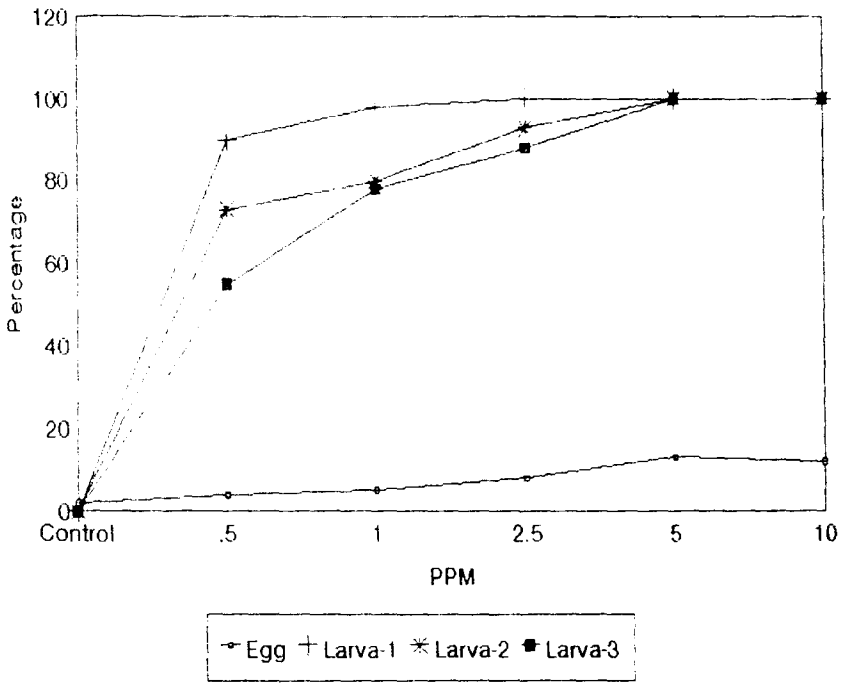


Chart.11 EFFECT OF DIFLUBENZURON ON CHITIN PRODUCTION OF C.megacephala LARVAE

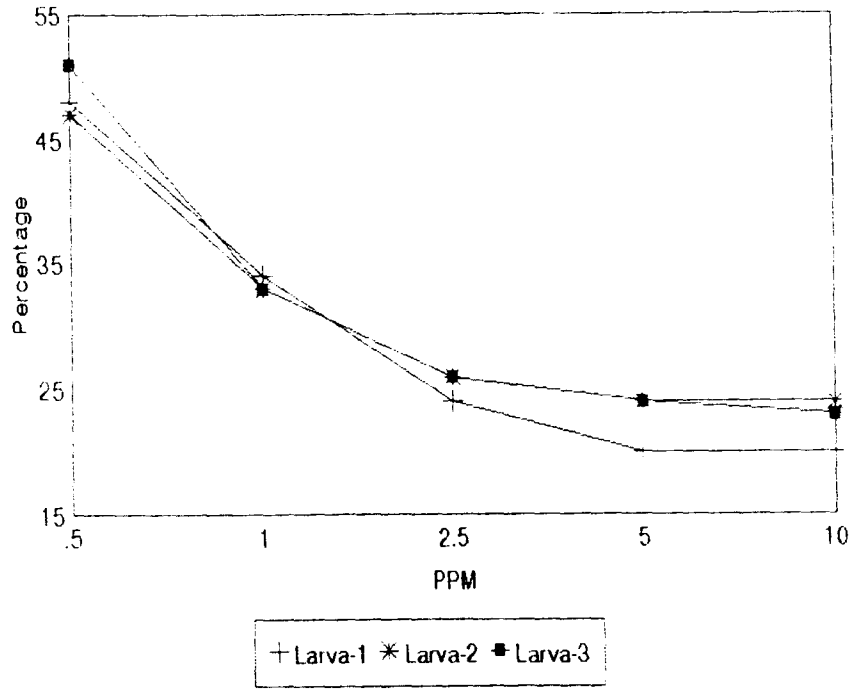


Chart.12 MORTALITY EFFECT OF DIFLUBENZURON ON DIFFERENT DEVELOPMENTAL STAGES OF C.bezziana

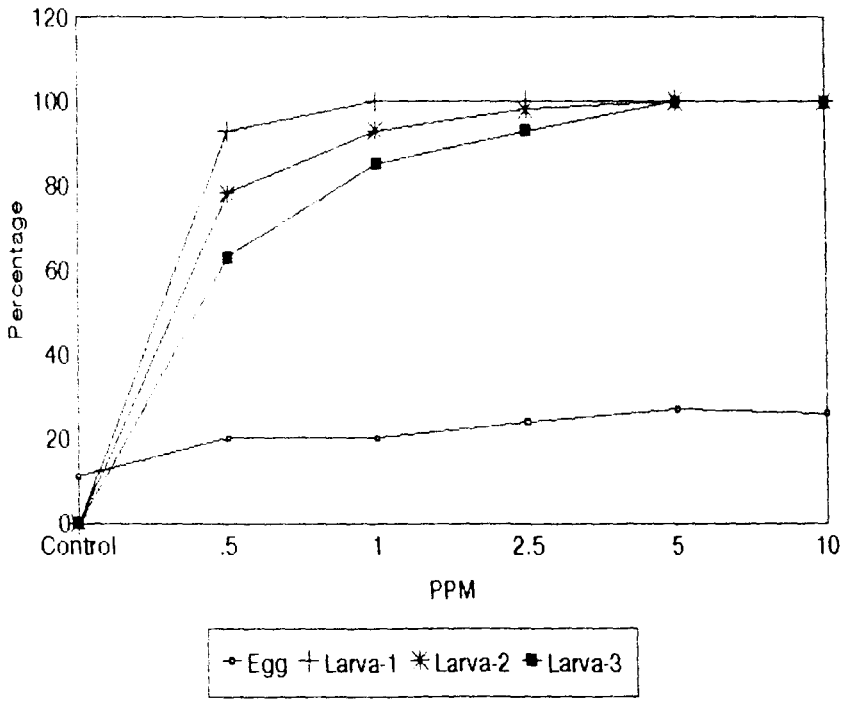


Chart.13 EFFECT OF DIFLUBENZURON ON CHITIN PRODUCTION OF C.bezziana LARVAE

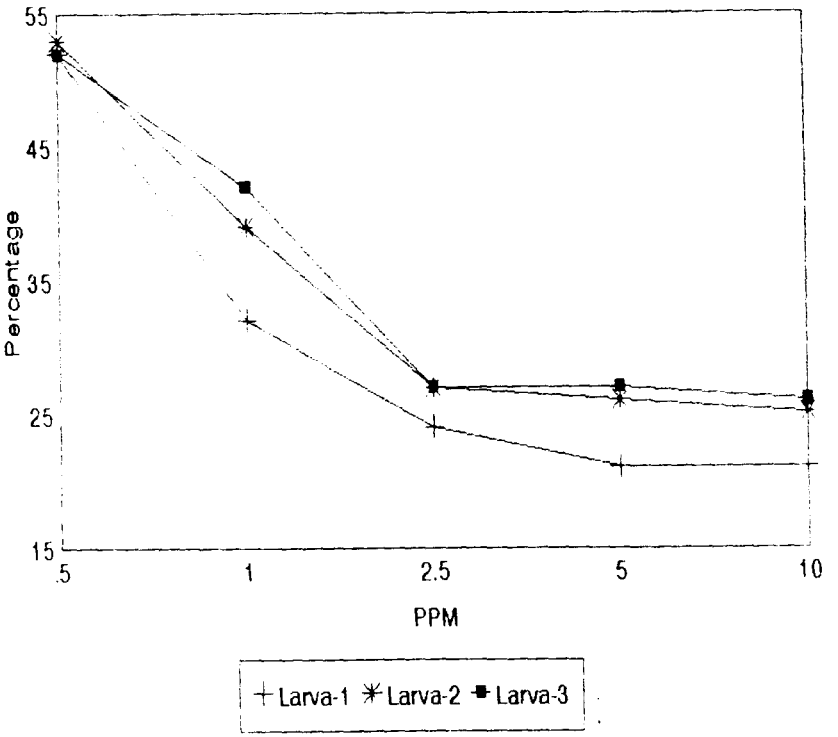


Chart.14 MORTALITY EFFECT OF DIFLUBENZURON ON DIFFERENT DEVELOPMENTAL STAGES OF L.cuprina

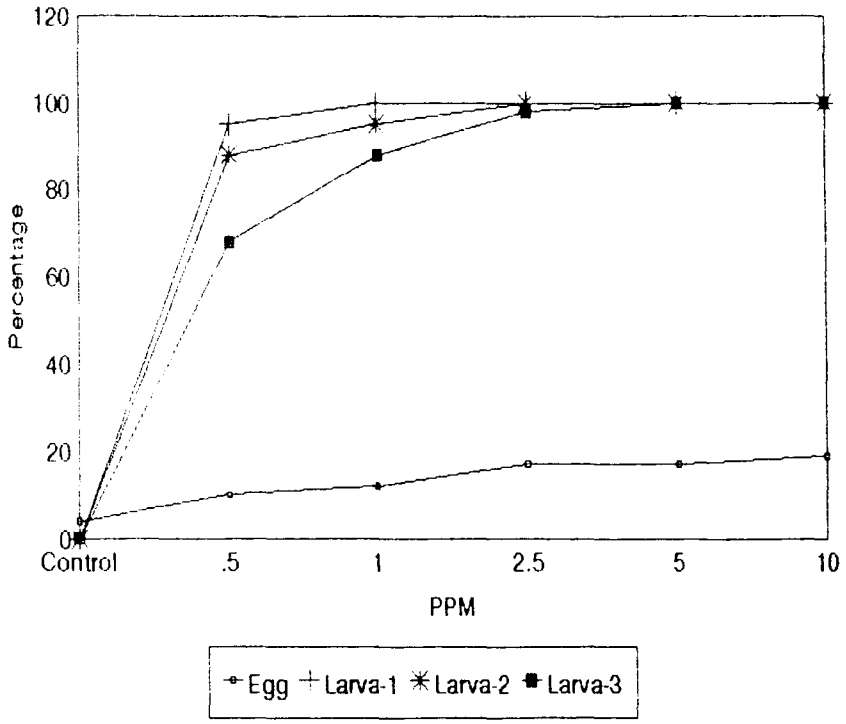


Chart.15 EFFECT OF DIFLUBENZURON ON CHITIN PRODUCTION OF L.cuprina LARVAE

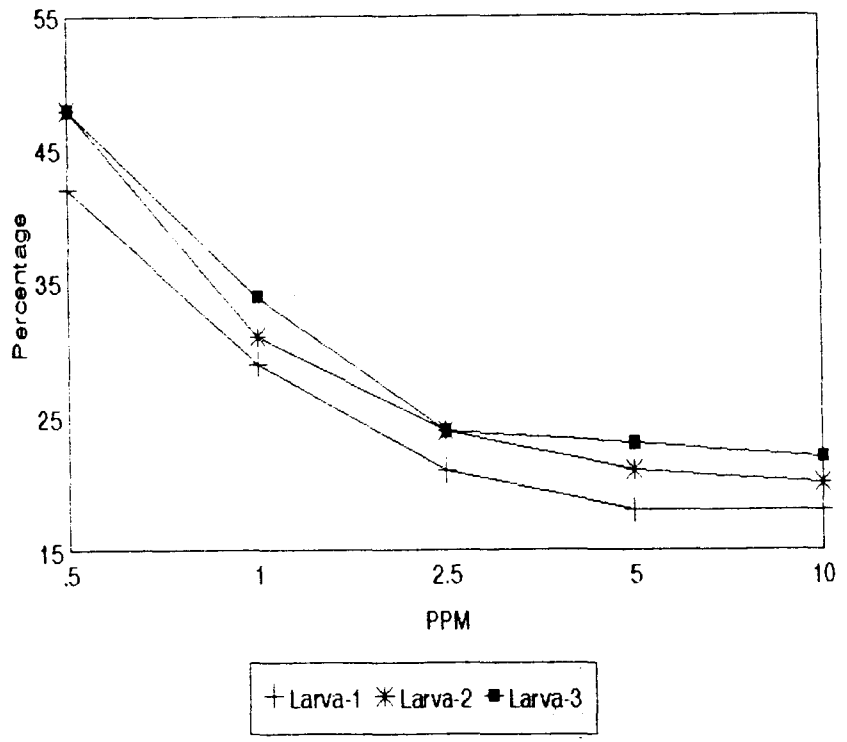
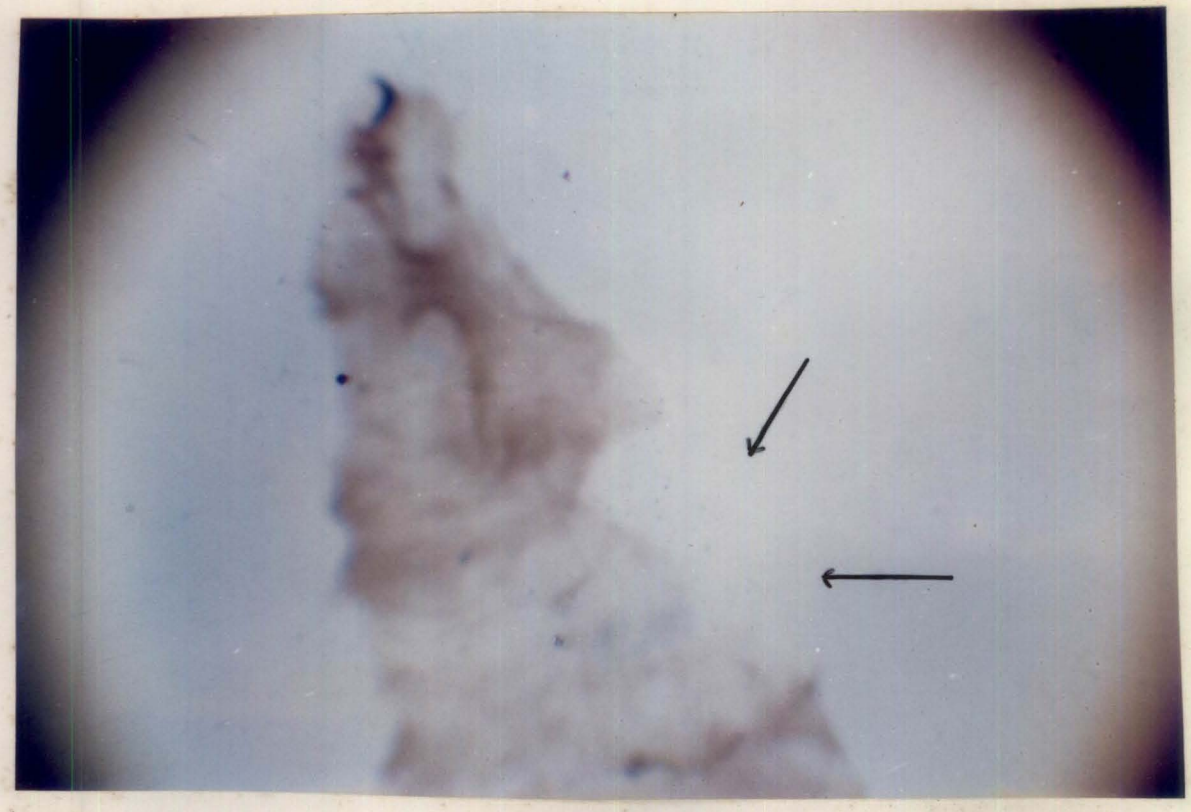


Fig.21. *C. bezziana* - First stage larva - rupture of cuticle on diflubenzuron treatment - 40x

Fig.22. *C. bezziana* - Third stage larva - rupture of cuticle on diflubenzuron treatment - 40x



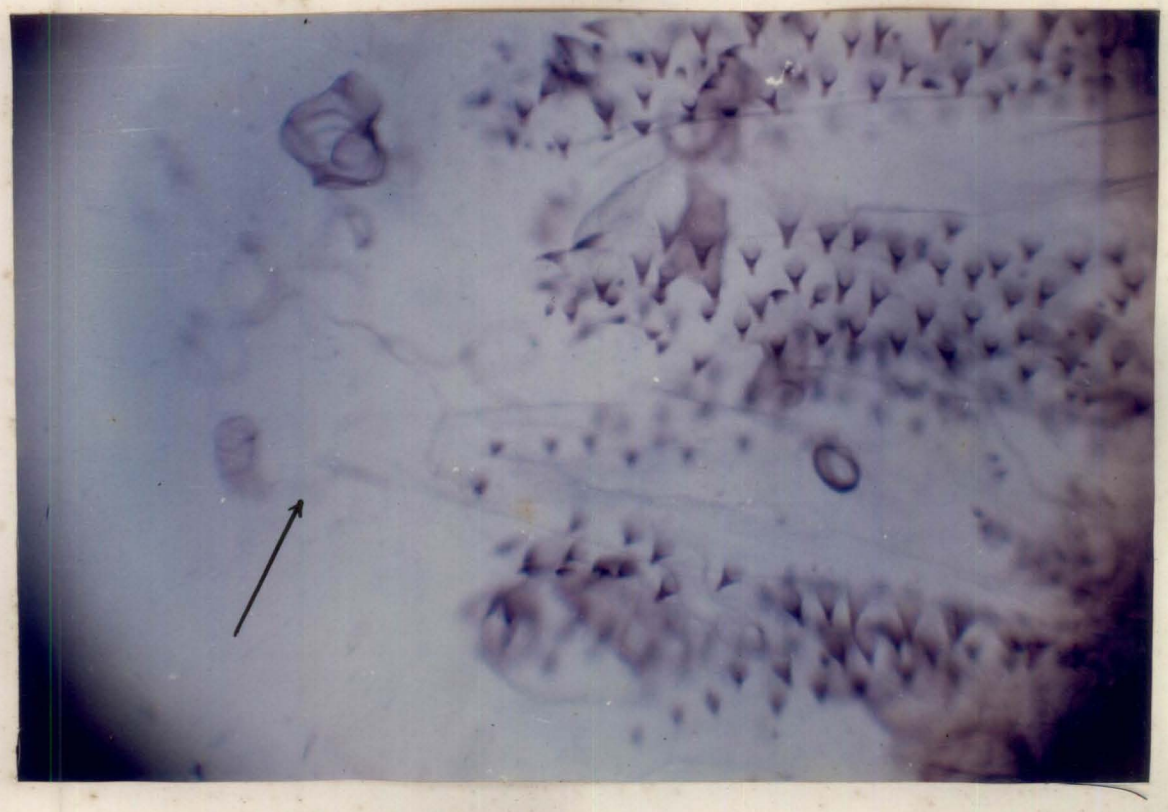
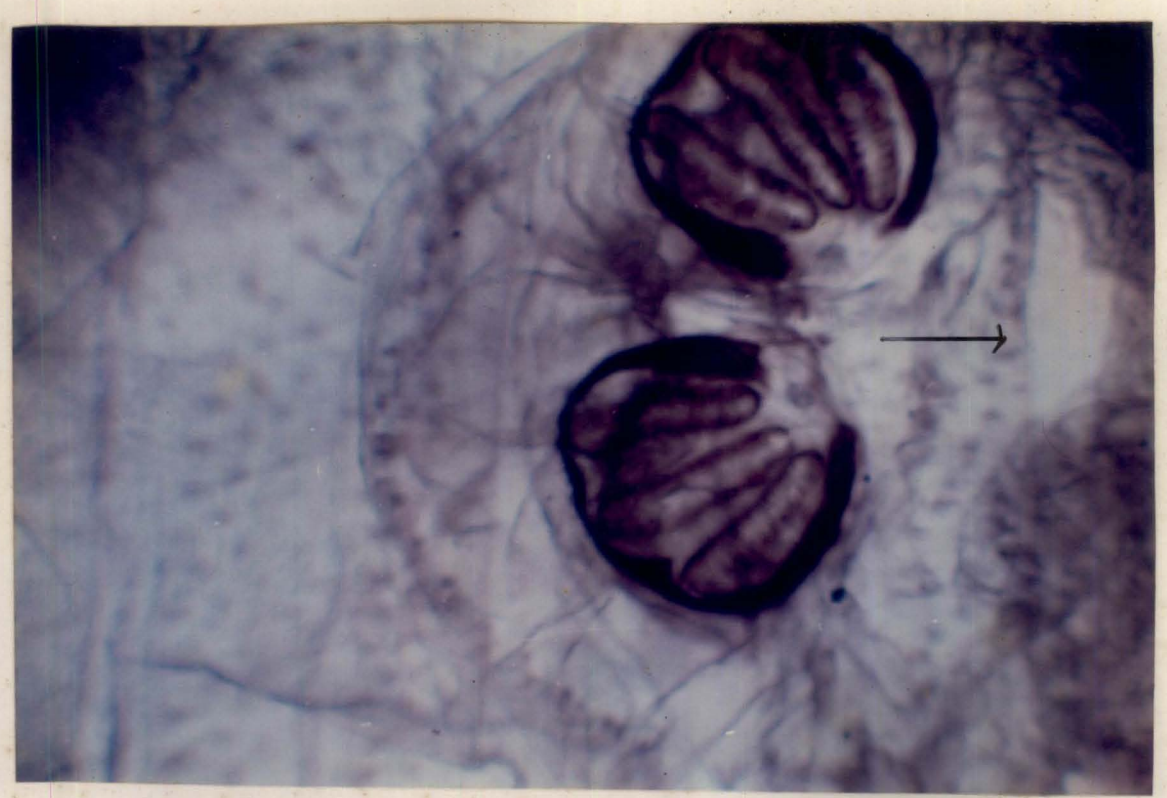


Fig.23. *C. bezziana* - Third stage larva - rupture of cuticle on diflubenzuron treatment - 40x

Fig.24. *C. megacephala* - Second stage larva - rupture of cuticle on diflubenzuron treatment - 40x



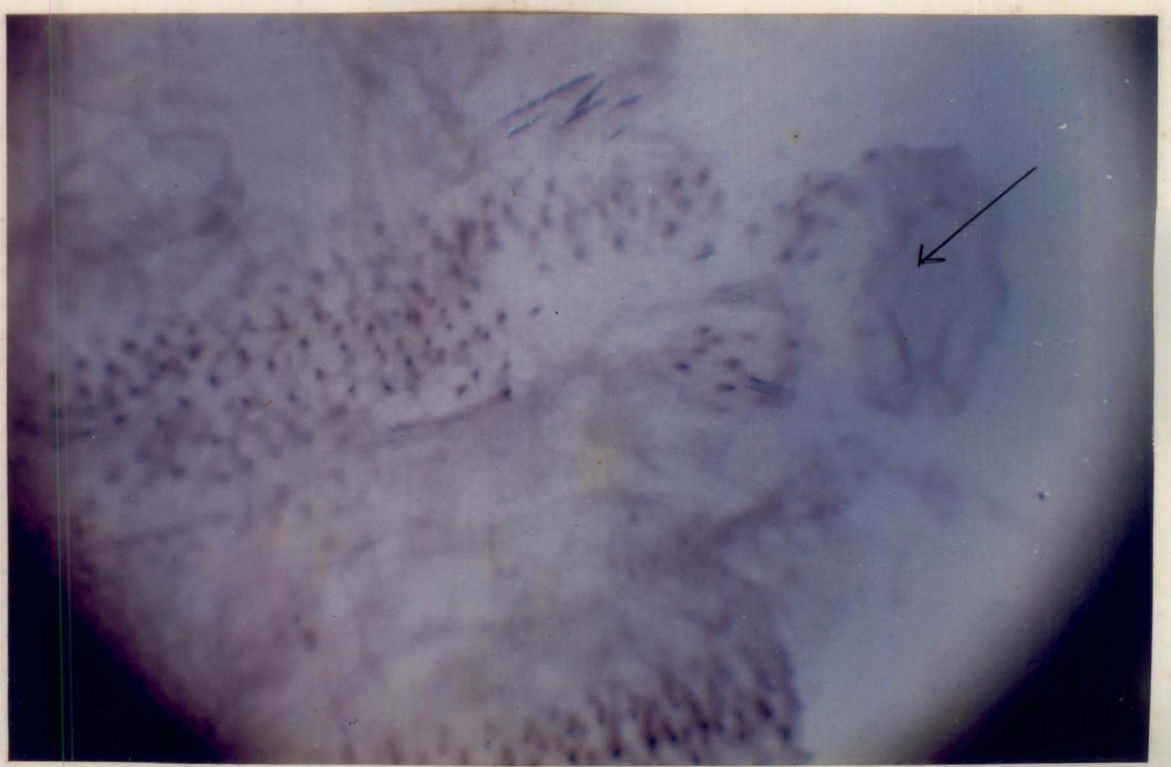
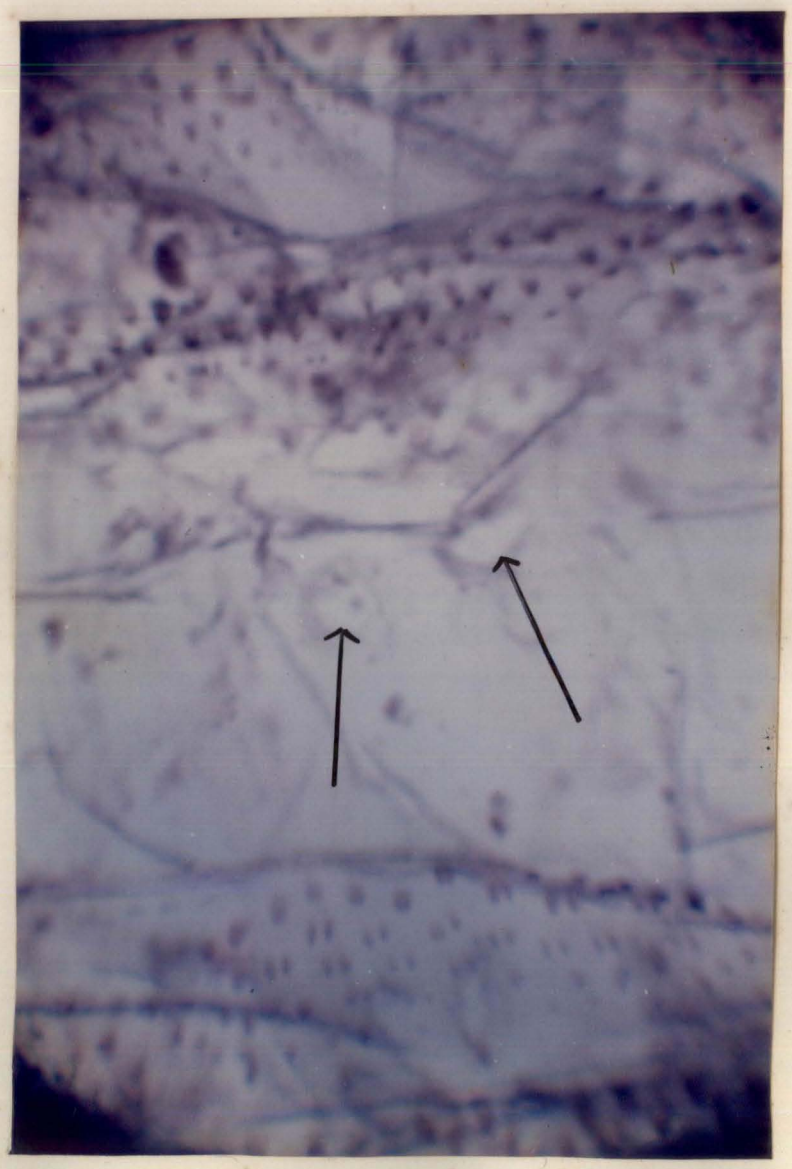


Fig.25. *C. megacephala* - Third stage larva - rupture of cuticle on diflubenzuron treatment - 40x

Fig.26. *C. megacephala* - Third stage larva - rupture of cuticle on diflubenzuron treatment - 40x



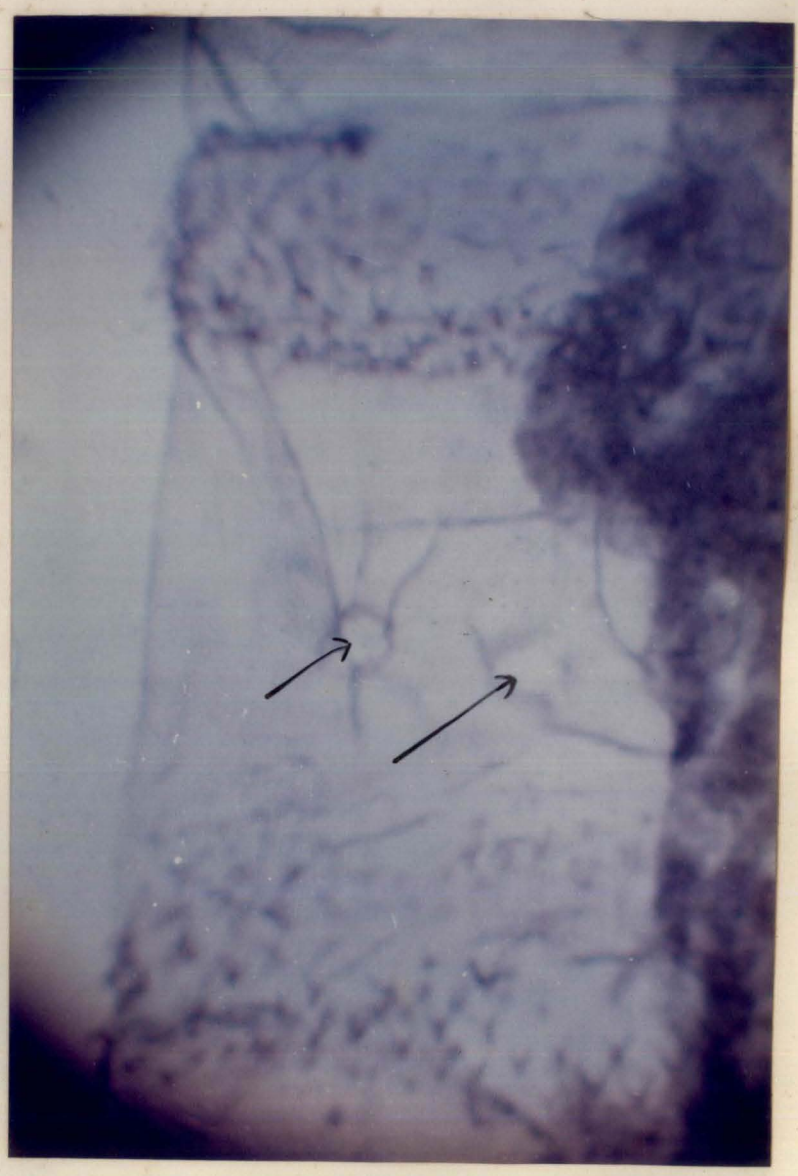
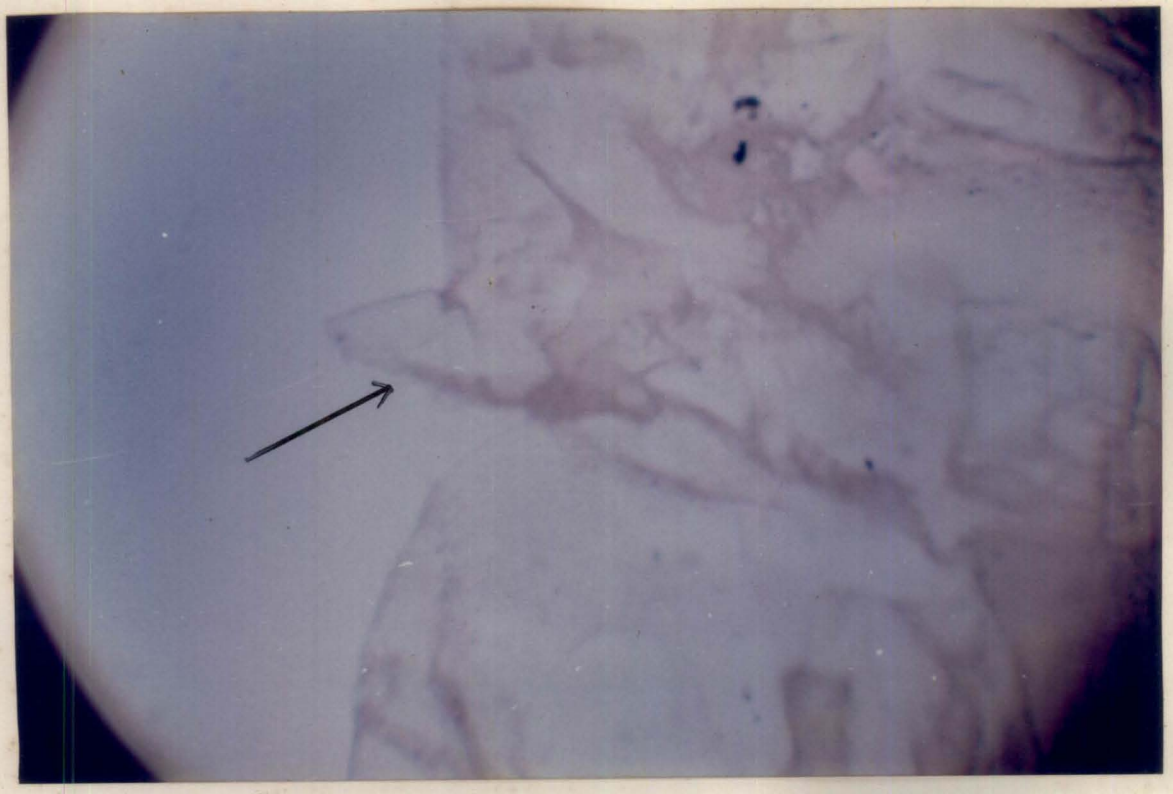


Fig.27. *L. cuprina* - Second stage larva - rupture of cuticle on diflubenzuron treatment - 40x

Fig.28. *L. cuprina* - Third stage larva - rupture of cuticle on diflubenzuron treatment - 40x



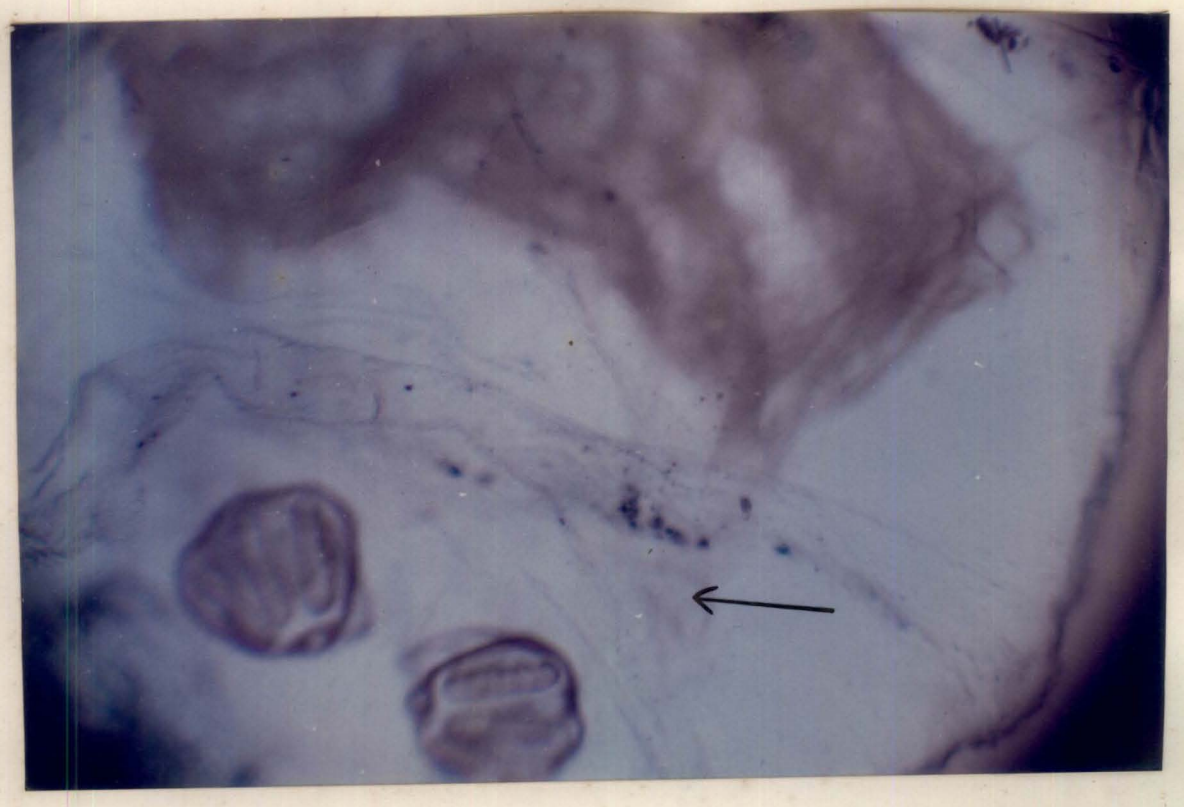


Fig.29. *L. cuprina* - **Abnormally thin shelled pupa developed on diflubenzuron treatment in larval stages**

4.4 EFFECT OF *BACILLUS TURINGIENSIS* (Table 13)

Medium to low mortality effect on eggs and larval stages I, II and III of *C. megacephala*, *C. bezziana* and *L. cuprina* were obtained on application of 160, 320 and 800 ppm concentrations of *Bacillus thuringiensis* suspension. The mortality effect was lower in mature larvae when compared to younger ones irrespective of the species studied. The mortality of eggs and larval stages were highest in *C. bezziana* followed by *L. cuprina* and *C. megacephala*.

C. megacephala

The ovicidal and larvicidal effect ranged in the descending order from 53 to 35 per cent, 57 to 30 per cent and 54 to 30 per cent from eggs to larval stage III at 160, 320 and 800 ppm concentrations respectively. In the controls, 2 per cent mortality of eggs only was noted.

C. bezziana

The mortality effect ranged in the descending order from 71 to 40 per cent, 70 to 42.5 per cent and 74 to 37.5 per cent for the respective stages and concentrations mentioned as in the case of *C. megacephala*. In the controls, 13 per cent mortality on eggs was observed.

L. cuprina

The mortality effect ranged in the descending order from 62 to 42.5 per cent, 60 to 40 per cent and 69 to 42.5 per cent for the respective stages and concentrations mentioned as in the case of *C. megacephala*. In the controls, 6 per cent mortality on eggs was observed.

Table 13. Ovicidal and larvicidal effect of Bacillus thuringiensis (israelensis) on myiasis producing flies (Mean percentage of mortality)

Concentration (ppm)	*C. megacephala*				*C. bezziana*				*L. cuprina*			
	Egg	Larval stage I	Larval stage II	Larval stage III	Egg	Larval stage I	Larval stage II	Larval stage III	Egg	Larval stage I	Larval stage II	Larval stage III
160	53	42.5	37.5	35.0	71	52.5	47.5	40.0	62	57.5	47.5	42.5
320	57	42.5	40	30	70	50	42.5	42.5	60	55	47.5	40
800	54	47.5	40	30	74	50	40	37.5	69	60	45	42.5
Control	2	0	0	0	13	0	0	0	6	0	0	0

4.5 EFFECT OF *AZADIRACHTIN*

4.5.1 Ovicidal and larvicidal effect (Table 14)

The mortality effect on eggs and three larval stages on treatment with 6, 10.5 and 15 ppm concentrations of azadirachtin was significant. The effect reduced gradually as the larvae matured. The highest effect was noted in *L. cuprina* and the lowest in *C. megacephala*.

C. megacephala

The mortality effects on eggs and different larval stages were 94, 87.5, 80 and 75 per cent respectively at 6 ppm concentration. The effects were 100 per cent in eggs and larval stage I and 95 and 87.5 per cent in larval stage II and III respectively at 10.5 ppm. In the higher concentration of 15 ppm, the effect was 100 per cent in all the stages. In the controls the egg mortality was 2 per cent and the larval mortality was nil.

C. bezziana

The mortality effects on eggs and different larval stages were 97, 92.5, 85 and 82.5 per cent respectively at 6 ppm. The effects were 100 per cent in eggs, larval stage-I and II and 95 per cent in larval stage-III at 10.5 ppm. The effect

was 100 per cent in all the stages at higher concentration. In the control low egg mortality of 13 per cent was observed.

L. cuprina

The mortality effects on eggs and different larval stages were 98, 95, 90 and 87.5 per cent respectively at 6 ppm and 100 per cent in all the stages in higher concentrations. In the control mortality effect on eggs was only 6 per cent.

The comparative mortality effect of azadirachtin on larval stage-III of myiasis producing flies is graphically represented in chart 16.

4.5.2 Antifeedant effect (Table 15, Chart 17)

The quantity of meat consumed by the larvae of *C. megacephala*, *C. bezziana* and *L. cuprina* during their growth phase reduced considerably with the application of azadirectin. The reduction in meat consumption was directly proportional to the concentration of azadirachtin used.

C. megacephala

The mean quantity of meat consumed by 20 larvae and the percentage of meat consumed with respect to controls were 1.65 gm (44.6 per cent), 1.35 gm (36.5 per cent) and 1.16 gm (31.1 per cent) at 1.5, 3 and 6 ppm respectively. In the controls, the larvae consumed 3.70 gms of meat.

C. bezziana

The corresponding values with regard to *C. bezziana* were 1.45 gms (41.6 per cent), 1.20 gm (34.5 per cent) and 0.85 gms (24.4 per cent) at 1.5, 3 and 6 ppm respectively, while in the controls, the larvae consumed 3.48 gms of meat.

L. cuprina

The mean quantity of meat consumed by 20 larvae and the percentage of meat consumed with respect to controls were 1.10 gm (35.3 per cent), 0.85 gm (27.2 per cent) 0.75 gm (24 per cent) at 1.5, 3 and 6 ppm respectively, while in the controls, the larvae consumed 3.12 gms of meat.

4.5.3 Ovipositional deterrent effect (Table 16, Chart 18)

Azadirachtin exhibited significant ovipositional deterrent effect on adult flies of *C. megacephala*, *C. bezziana* and *L. cuprina* at 1.5, 3 and 6 ppm concentrations. The effect increased with the increase in concentration of azadirachtin applied. In *C. megacephala* the average number of eggs laid and the percentage of eggs laid with respect to controls were 89 (18.2 per cent), 41 (8.4 per cent) and 38 (7.8 per cent) at the above mentioned concentration respectively, while in the controls, the eggs laid were 490. In *C. bezziana* in the same concentrations the figures were 47 (21.4 per cent), 19 (8.6 per cent) and 0, while in the controls the eggs laid were 220.

In *L. cuprina*, the figures were 67 (19.1 per cent) 18 (5.1 per cent) and 12 (3.4 per cent) at the respective concentrations while in the controls 351 eggs were laid.

4.5.4 Repellant effect (Table 17, Chart 19)

Azadirachtin also exhibited profound repellancy against meat infesting flies at 1.5, 3 and 6 ppm concentrations. The effect increased with the increase in concentration of azadirectin used. The flies took 60, 70 and 120 minutes for being attracted to the bait at the respective concentrations, whereas in the control the flies took only 6 minutes. The time of oviposition or larviposition were 80 and 85 minutes at 1.5 and 3 ppm concentration respectively and 15 minutes in the control. The flies did not lay any eggs or larvae at 6 ppm concentration. The mean number of eggs or larvae laid and their percentage of eggs or larvae laid with respect to controls were 45 (8.9 per cent) and 7 (1.4 per cent) at 1.5 and 3 ppm respectively, whereas in the controls the mean number of eggs or larvae found on the bait was 507.

4.5.5 Effect on larval stage III (Table 18)

Substantial effects were observed on the development of third stage larvae of the three different species of flies on treatment with 1.5, 3 and 6 ppm of azadirachtin.

C. megacephala

The ratio of development descended in the following order; the pupation from 47.5 to 22.5 per cent, average pupal weight 49 to 35 mgs, adult development 40 to 17.5 per cent and adult malformations 5 to 2.5 per cent at 1.5 to 6 ppm concentrations. The longevity of adults decreased from 9 to 6 days in males and 20 to 10 days in females at the above concentrations of azadirachtin. The number of eggs laid decreased from 431 to 212 with a hatchability of 24 to 9 per cent at similar concentrations. The failure of wings to unfold properly was the only morphological deformity noted. Azadirachtin treatment in larval stages also produced small sized flies in *C. megacephala* (Fig.31). The lifecycle was not totally arrested in any of the concentrations studied. In the controls, the values were 95 per cent, 52 mgs, 90 per cent, 0 per cent, 15 and 60 days, 2238 eggs and 98 per cent for the above parameters respectively.

C. bezziana

The pupation decreased from 32.5 to 12.5 per cent, average pupal weight 50 to 37 mgs (Fig.30), adult development 22.5 to 7.5 per cent, and adult malformations with partially folded wings 5 to 2.5 per cent at 1.5 to 6 ppm concentrations. The longevity reduced from 10 to 4 days in males and 22 to 8 days in females at the above concentrations. At 1.5 and

3 ppm concentrations, the flies laid 194 and 42 eggs on an average with a hatchability of 12 and 4.8 per cent respectively. The lifecycle could not be completed at 6 ppm concentration. In the controls, the values were 85 per cent, 56 mgs, 82.5 per cent, 0 per cent, 18 and 52 days, 2046 eggs and 87 per cent for the above parameters respectively.

L. cuprina

The pupation descended from 27.5 to 10 per cent, average pupal weight from 39 to 30 mgs, adult development from 20 to 5 per cent and adult malformations with partially folded wings from 7.5 to 2.5 per cent at 1.5 to 6 ppm concentrations. At the above concentrations longevity reduced from 9 to 2 days in males and 18 to 5 days in females. The flies laid 292 and 84 eggs at 1.5 and 3 ppm concentration, with a hatchability of 9 and 2.3 per cent respectively. The lifecycle was not completed at 6 ppm level. In the control group, values were 87.5 per cent, 41 mgs, 82.5 per cent, 0 per cent, 17 and 58 days, 1390 eggs and 94 per cent for the above parameters respectively.

4.5.6 Effect on adult flies (Table 18)

The effects of azadirachtin were not prominent on the three different species of flies unlike the larval stages. At the highest concentration of 6 ppm the males and female flies of *C. megacephala* lived for 10 and 39 days respectively, laid

1112 eggs with subsequent hatchability of 82 per cent and adult development of 67 per cent. In *C. bezziana*, the corresponding values were 9 and 19 days, 432 eggs, 75 per cent and 71 per cent respectively and for *L. cuprina* the values were 8 and 18 days, 461 eggs, 81 per cent and 70 per cent respectively for the above mentioned parameters. In the controls, except for the adult development of 87, 81 and 80 per cent respectively for the species of flies mentioned above, values of all other parameters were similar to the respective species mentioned in larval stage III.

Table 14. Ovicidal and larvicidal effect of Azadirachtin on myiasis producing flies (Mean percentage of mortality)

Concentration (ppm)	*C. megacephala*				*C. bezziana*				*L. cuprina*			
	Egg	Larval stage I	Larval stage II	Larval stage III	Egg	Larval stage I	Larval stage II	Larval stage III	Egg	Larval stage I	Larval stage II	Larval stage III
6	94	87.5	80	75	97	92.5	85	82.5	98	95	90	87.5
10.5	100	100	95	87.5	100	100	100	95	100	100	100	100
15	100	100	100	100	100	100	100	100	100	100	100	100
Control	2	0	0	0	13	0	0	0	6	0	0	0

Table 15. Effect of Azadirachtin on larval meat consumption in myiasis producing flies (Mean values)

Species	Meat consumed	Concentration (ppm)			Control
		1.5	3	6	
C. megacephala	Quantity (gms)	1.65	1.35	1.16	3.70
	Per cent	44.6	36.5	31.1	100.00
C. bezziana	Quantity (gms)	1.45	1.20	0.85	3.48
	Per cent	41.6	34.5	24.4	100.00
L. cuprina	Quantity (gms)	1.10	0.85	0.75	3.12
	Per cent	35.3	27.2	24.0	100.00

(Values are quantity and percentage meat consumed by 20 larvae in their total larval period)

Table 16. Effect of Azadirachtin on fecundity in myiasis producing flies (Mean values)

Species	Eggs laid (average)	Concentration (ppm)			Control
		1.5	3	6	
C. megacephala	Number	89	41	38	490
	Per cent	18.2	8.4	7.8	100
C. bezziana	Number	47	19	0	220
	Per cent	21.4	8.6	0	100
L. cuprina	Number	67	18	12	351
	Per cent	19.1	5.1	3.4	100

Table 17. Repellant effect of Azadirachtin on meat infesting flies (Mean values)

Parameters	Concentration (ppm)	Repellant effect
Time of fly arrival and sitting on bait	1.5	60 minutes
	3	70 minutes
	6	120 minutes
	Control	6 minutes
Time of oviposition/ larviposition	1.5	80 minutes
	3	85 minutes
	6	Not laid
	Control	15 minutes
Number and percentage of eggs/larvae laid	1.5	45 (8.9)
	3	7 (1.4)
	6	0 (0)
	Control	507 (100)

Table 18. Effect of Azadirachtin on larval stage III and adults of myiasis producing flies (Mean values)

Species	Concentration (ppm)	Larval stage III								Adult flies					
		Pupation per cent	Pupal weight average (mgs)	Adult development per cent	Adult malformation per cent	Longevity of adult flies M	Longevity of adult flies F	Eggs laid average	Eggs hatch per cent	Longevity of adult flies M	Longevity of adult flies F	Eggs laid average	Eggs hatch per cent	Adult development per cent	Adult malformation per cent
C. megacephala	1.5	47.5	49	40	5	9	20	431	24	14	48	1898	88	72	0
	3	30	42	22.5	5	9	16	308	15	10	40	1233	82	73	0
	6	22.5	35	17.5	2.5	6	10	212	9	10	39	1112	82	67	0
	Control	95	52	90	0	15	60	2238	98	15	60	2238	98	87	0
C. bezziana	1.5	32.5	50	22.5	5	10	22	194	12	15	32	1119	80	74	0
	3	25	45	15	2.5	6	12	42	4.8	12	28	876	76	72	0
	6	12.5	37	7.5	2.5	4	8	0	0	9	19	432	75	71	0
	Control	85	56	82.5	0	18	52	2046	87	18	52	2046	87	81	0
L. cuprina	1.5	27.5	39	20	7.5	9	18	292	9	12	24	637	84	72	0
	3	17.5	34	12.5	2.5	6	14	84	2.3	9	20	592	80	70	0
	6	10	30	5	2.5	2	5	0	0	8	18	461	81	70	0
	Control	87.5	41	82.5	0	17	58	1390	94	17	58	1390	94	80	0

M: Male F: Female

Chart.16 COMPARATIVE MORTALITY EFFECT OF AZADIRACHTIN ON LARVAL STAGE-III OF MYIASIS PRODUCING FLIES

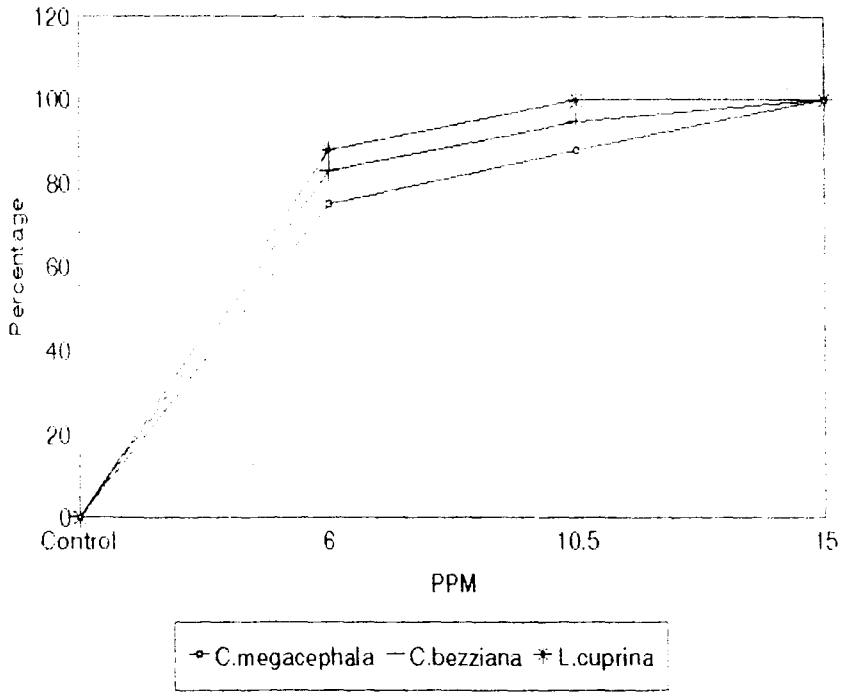


Chart.17 EFFECT OF AZADIRACHTIN ON LARVAL MEAT CONSUMPTION OF MYIASIS PRODUCING FLIES

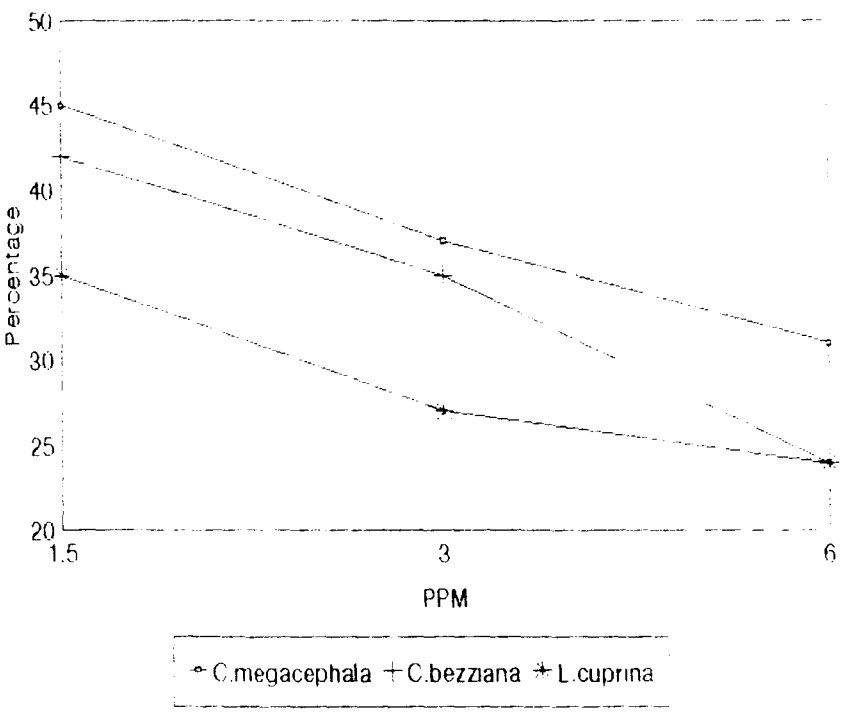


Chart.18 EFFECT OF AZADIRACHTIN ON FECUNDITY OF MYIASIS PRODUCING FLIES

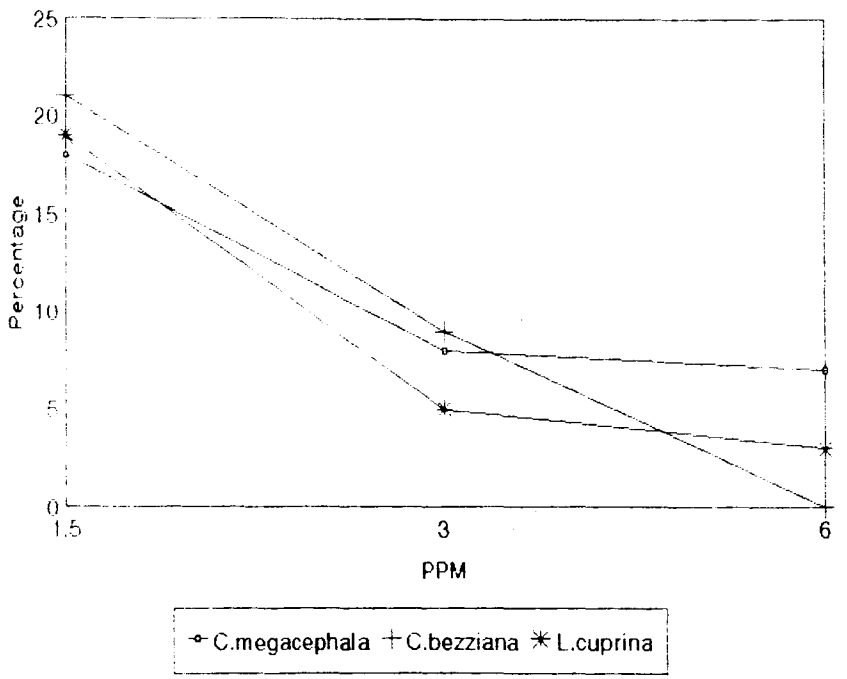


Chart.19 REPELLANT EFFECT OF AZADIRACHTIN ON MEAT INFESTING FLIES

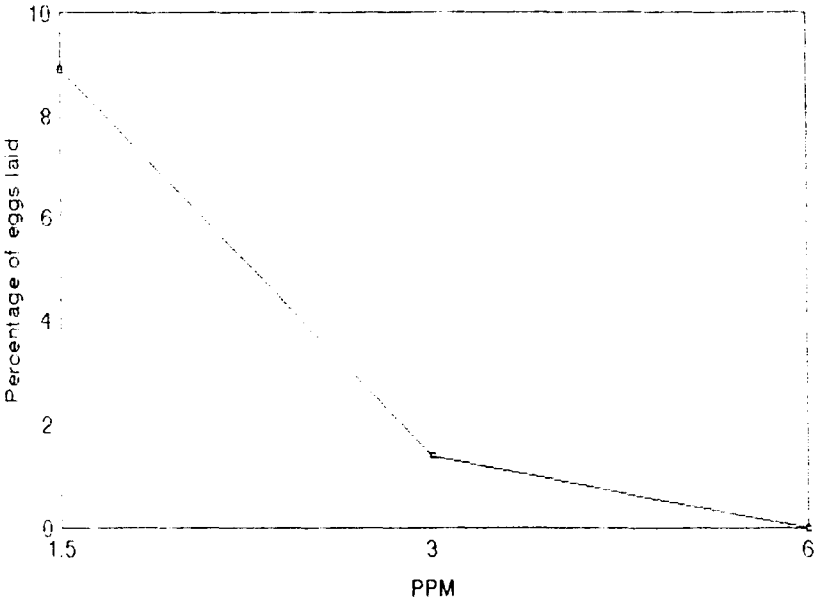


Fig.30. *C. bezziana* - Small sized pupae developed on treatment of Azadirectin in the larval stages compared with normal pupae

Fig.31. *C. megacephala* - Small sized fly developed on Azadirectin treatment in larval stages compared with normal fly

4.6 EFFECT OF EXTRACTS OF ACORUS CALAMUS

4.6.1 Ovicidal and larvicidal effect (Table 19)

The mortality effect on eggs and the 3 respective larval stages of the different species of flies on treatment with 1.5, 2 and 2.5 per cent of Petroleum ether extract (PEE) of *Acorus calamus*, was significant followed by steam distillate (SD) and alcoholic extract (AE) in the order of effect. The effect increased with the increase in concentrations of extracts used. The effect was higher in younger larvae than in older ones irrespective of the species.

C. megacephala

The mortality effect on eggs and 3 respective larval stages were 100, 100, 92.5 and 82.5 per cent respectively at 2.5 per cent concentration of PEE. A mortality rate of 100, 82.5, 62.5 and 55 per cent were observed at 2.5 per cent concentration of SD whereas, lower mortality effect of 84, 45, 35 and 32.5 per cent were noted at 2.5 per cent concentration of AE. The mortality effect was lesser when lower concentrations of the three extracts were applied. In the controls 4 per cent mortality of eggs and no mortality of the larval stages were noted.

C. bezziana

The mortality effect on eggs and 3 respective larval stages at 2.5 per cent concentration were 100, 100, 87.5 and 85 per cent with PEE, 100, 82.5, 65 and 62.5 per cent with SD and 90, 47.5, 37.5 and 37.5 with AE. The mortality effect was lesser at lower concentrations of the three different extracts. In the controls 11 per cent mortality of eggs alone could be noted.

L. cuprina

The mortality effect on eggs and the 3 respective larval stages at 2.5 per cent concentration were 100, 100, 95 and 90 per cent with PEE, 100, 87.5, 70 and 72.5 per cent with SD and 96, 92.5, 67.5 and 47.5 per cent with AE respectively. The mortality effect was lesser at lower concentrations of the three different extracts. In the controls 7 per cent mortality effect on eggs with no causality in the larval stages were noted.

The comparative mortality effect of 2.5 per cent extracts of *Acorus calamus* on larval stage-III in myiasis producing flies is graphically represented in Chart 20.

4.6.2 **Antifeedant effect** (Table 20)

A moderate reduction in the quantity of meat consumed by the larvae of *C. megacephala*, *C. bezziana* and *L. cuprina* was observed with the application of different extracts of *Acorus calamus* on meat. Petroleum ether extract proved to be superior in antifeedant activity followed by steam distillate and alcoholic extract.

C. megacephala

The mean quantity and percentage of meat consumed by 20 larvae compared with controls at 0.1, 0.5 and 1 per cent concentrations were 2.35 g (63.5 per cent), 1.85 g (50 per cent) and 1.75 g (47.3 per cent) with PEE, 3.05 g (82.4 per cent), 2.50 g (67.6 per cent) and 2.35 g (63.5 per cent) with SD and 3.45 g (92.3 per cent), 3.30 g (89.2 per cent) and 3.25 g (87.8 per cent) with AE respectively while in the control group larvae consumed 3.70 g of meat.

C. bezziana

At the above concentrations, the larvae consumed 2.10 g (60.3 per cent), 1.55 g (44.5 per cent) and 1.50 g (43.1 per cent) of meat with PEE, 2.30 g (66.1 per cent), 2.10 g (60.3 per cent) and 1.95 g (56 per cent) with SD and 3.20 g (92 per cent), 2.80 g (80.5 per cent) and 2.70 g (77.5 per cent) with

AE respectively whereas control larvae consumed 3.48 g of meat.

L. cuprina

At the same concentrations, *L. cuprina* larvae consumed 1.45 g (46.5 per cent), 0.95 g (30.4 per cent) and 1.00 g (32.1 per cent) of meat with PEE, 2.40 g (76.9 per cent), 1.75 g (56.1 per cent) and 1.65 g (52.9 per cent) with SD and 2.55 g (81.7 per cent), 2.45 g (78.5 per cent) and 2.45 g (78.5 per cent) with AE respectively. In the controls, the larvae consumed 3.12 g of meat.

The comparative effect of 1 per cent extracts of *Acorus calamus* on larval meat consumption in myiasis producing flies is graphically represented in Chart 21.

4.6.3 Ovipositional deterrent effect (Table 21, Chart 22)

The extracts of *Acorus calamus* exhibited moderate ovipositional deterrent effect on the 3 different species of flies. The effect increased moderately with the increase in concentration of the extracts used. PEE gave better ovipositional deterrent effect than SD and AE.

The number and percentage of eggs laid compared with controls at the highest concentration of 2.5 per cent of PEE, SD and AE were 90 (17 per cent), 174 (33 per cent) and 258

(48.9 per cent) respectively in *C. megacephala*, 44 (18.8 per cent), 83 (35.5 per cent) and 132 (56.4 per cent) in *C. bezziana* where as in *L. cuprina* the values were 68 (20.4 per cent), 90 (26.9 per cent) and 184 (55 per cent). In the controls, the flies of *C. megacephala*, *C. bezziana* and *L. cuprina* laid 528, 234 and 334 eggs respectively.

4.6.4 Reppellant effect (Table 22, Chart 23)

The extracts of Acorus calamus showed good repellant effect against the meat infesting flies. The effect increased with the increase in concentration of the extracts. PEE gave better results than SD and AE. At the highest concentration of 2.5 per cent of the above extracts tested, the time of attraction to the bait were 49, 32 and 25 minutes respectively. In the control kept separately, the flies took only 9, 10 and 10 minutes respectively. The time of oviposition or larviposition were 125, 69 and 35 minutes respectively and in the controls, the flies took 15, 12 and 17 minutes for the above function. The number of eggs laid and the percentage of eggs laid with respect to controls were 80 (17.8 per cent), 135 (28.4 per cent) and 156 (39.2 per cent). In the controls kept separately for the extracts, the flies laid 449, 476 and 398 eggs or larvae respectively.

4.6.5 Effect of Acorus calamus extracts on larval stage III and adult flies

The application of different extracts of *Acorus calamus* at 0.1, 0.5 and 1 per cent concentration affected all the subsequent developmental stages of larval stage III of the three different flies, while in the controls though the longevity was not reduced, the eggs laid were fewer with lesser development due to significant sterility effect of *Acorus calamus*. PEE gave better results followed by SD and AE. The effect increased with the increase in concentration of extracts used.

C. megacephala (Table 23)

Larval stage III

At the highest concentration of 1 per cent of PEE, the pupation was 32.5 per cent, adult emergence 17.5 per cent, longevity 6 days in males and 12 days in females, average number of eggs laid, 436 with an egg hatch of 7 per cent. In 1 per cent SD the values for the respective parameters were 47.5 per cent, 27.5 per cent, 6 and 15 days, 644 eggs and 19 per cent whereas in AE 1 per cent the values were 65 per cent, 47.5 per cent, 10 and 29 days, 1008 eggs and 32 per cent respectively. Lower effects were noted on application of the different extracts at lesser concentrations. In the controls,

the values for the respective parameters were 95 per cent, 90 per cent, 15 and 62 days, 2310 eggs and 97 per cent.

Adult flies

With PEE application at 0.1 and 0.5 per cent concentrations, the flies laid 290 and 48 eggs with an egg hatch of 13 and 2.1 per cent and subsequent development of 4 and 0 per cent respectively. The eggs were not laid at 1 per cent and the life cycle was arrested at 0.5 per cent concentration. In SD application, 310, 92 and 12 eggs were laid in the respective concentrations. At 0.1 and 0.5 per cent, 18 and 7.6 per cent of eggs hatched respectively with subsequent 9 per cent adult development in 0.1 per cent concentration only. The egg hatching was totally absent in 1 per cent concentration. The life cycle was arrested at 0.5 per cent level. In AE application the flies laid 899, 196 and 47 eggs with a hatchability of 46, 21 and 4.3 per cent and subsequent development of 18, 3 and 0 per cent to adults at the respective concentrations. Studies made on mounted specimens of the ovary of treated flies irrespective of species and extracts used, revealed partial to complete arrest of the development of ovarian follicles. In the controls, the values were 2310 eggs, 97 per cent and 87.5 per cent for the respective parameters mentioned above.

C. bezziana (Table 24)

Larval stage-III

At 1 per cent concentration, the pupation was 17.5 per cent, adult emergence 7.5 per cent, longevity 7 days in males and 20 days in females and average number of eggs laid 372 with 3 per cent egg hatch in PEE. In SD 1 per cent application, the values for the respective parameters were 25 per cent, 20 per cent, 7 and 26 days, 398 eggs and 17 per cent whereas in AE 1 per cent application, the values were 62.5 per cent, 27.5 per cent, 9 and 26 days, 639 eggs and 23 per cent respectively. Lower effects were noticed on application of different extracts at lesser concentrations. In the controls, the values for the respective parameters were 85 per cent 82.5 per cent, 18 and 50 days, 2098 eggs and 88 per cent respectively.

Adult flies

In PEE application at 0.1 and 0.5 per cent concentrations, the flies laid an average 149 and 23 eggs. At 0.1 per cent concentration 9 per cent egg hatch and 2 per cent adult development were noticed. No eggs were laid at 1 per cent and life cycle was arrested at 0.5 per cent concentration. In S.D. application 344, 103 and 14 eggs were laid in the respective concentrations. At 0.1 and 0.5 per cent 11 and 6 per cent of eggs hatched respectively with

subsequent adult development of 6 per cent in 0.1 per cent concentration only. The life cycle was arrested at 0.5 per cent concentration. In AE application, the flies laid 677, 299 and 92 eggs with an egg hatch of 29, 8 and 1.8 per cent and subsequent development of 11, 3 and 0 per cent to adults at the respective concentrations. The life cycle was arrested at 1 per cent level only. Mounted specimen of ovary revealed morphological features similar to that of *C. megacephala* (Fig.32-35). In the controls the values were 2098 eggs, 87 per cent and 80 per cent for the respective parameters mentioned above.

L. cuprina (Table 25)

Larval stage-III

The effect on the larvae were very pronounced at 1 per cent concentration of PEE. Only 5 per cent of the larvae pupated and no development was observed thereafter. The life cycle was arrested at 0.5 per cent concentration. In SD 1 per cent application, the pupation was 12.5 per cent, adult development 7.5 per cent and longevity of 2 days in males and 4 days in females. The flies did not lay any eggs. In AE 1 per cent application, the pupation was 57.5 per cent, adult development 37.5 per cent, longevity of 8 days in males and 17 days in females and the flies laid 210 eggs on an average with 9 per cent fertility. Lower effects were noticed on

application of different extracts at lesser concentrations. In the control group, the values for the above parameters were 87.5 per cent, 82.5 per cent, 17 and 60 days, 1494 eggs and 95 per cent respectively.

Adult flies

In PEE application, the flies did not lay eggs in any of the concentrations used showing complete sterility. In SD, the flies laid 32 eggs at 0.1 per cent concentration only without any further development. In AE the flies laid 186, 77 and 12 eggs in the respective concentrations with 17 and 2.3 per cent egg hatch at 0.1 and 0.5 per cent concentration and subsequent development of 4 per cent in 0.1 per cent concentration only. The life cycle was arrested at 0.5 per cent concentration of AE. Mounted specimens of ovary revealed morphological features similar to that of the treated *C. megacephala*. In the controls, the values for the above parameters were 1494 eggs, 95 per cent and 80 per cent respectively.

Table 19. Ovicidal and larvicidal effect of Acorus calamus on myiasis producing flies (Mean percentage of morality)

Extracts	Concentration (%)	Chrysomyia megacephala				Chrysomyia bezziana				Lucilia cuprina			
		Egg	L1	L2	L3	Egg	L1	L2	L3	Egg	L1	L2	L3
Petroleum ether extract	1.5	88	72.5	60	55	91	85	80	72.5	94	92.5	87.5	80
	2	100	95	90	82.5	100	95	87.5	82.5	100	100	95	87.5
	2.5	100	100	92.5	82.5	100	100	87.5	85	100	100	95	90
Alcoholic extract	1.5	58	42.5	32.5	30	50	37.5	30	65	71	65	52.5	42.5
	2	80	47.5	30	30	86	50	25	70	83	82.5	50	40
	2.5	84	45	35	32.5	90	47.5	37.5	37.5	96	92.5	67.5	47.5
Steam distillate	1.5	74	70	52.5	42.5	86	65	62.5	65	87	80	72.5	62.5
	2	92	67.5	55	52.5	100	72.5	60	57.5	100	87.5	70	67.5
	2.5	100	82.5	62.5	55	100	82.5	65	62.5	100	87.5	70	72.5
Control		4	0	0	0	11	0	0	0	7	0	0	0

Table 20. Effect of extracts of Acorus calamus on larval meat consumption in myiasis producing flies (Mean values)

	Meat consumed	Petroleum ether extract (concentration per cent)			Alcoholic extract (concentration per cent)			Steam distillate (concentration per cent)			Control
		0.1	0.5	1	0.1	0.5	1	0.1	0.5	1	
Chrysomyia megacephala	Qty. gms	2.35	1.85	1.75	3.45	3.30	3.25	3.05	2.50	2.35	3.70
	Per cent	63.5	50.0	47.3	92.3	89.2	87.8	82.4	67.6	63.5	100.0
Chrysomyia bezziana	Qty. gms	2.10	1.55	1.50	3.20	2.80	2.70	2.30	2.10	1.95	3.48
	Per cent	60.3	44.5	43.1	92.0	80.5	77.5	66.1	60.3	56.0	100.0
Lucilia cuprina	Qty. gms	1.45	0.95	1.00	2.55	2.45	2.45	2.40	1.75	1.65	3.12
	Per cent	46.5	30.4	32.1	81.7	78.5	78.5	76.9	56.1	52.9	100.0

Values are quantity and percentage of meat consumed by 20 larvae in their total larval period

Table 21. Effect of extracts of Acorus calamus on fecundity in myiasis producing flies (Mean values)

Species	Eggs laid (average)	Petroleum ether extract (concentration per cent)			Alcoholic extract (concentration per cent)			Steam distillate (concentration per cent)			Control
		1.5	2	2.5	1.5	2	2.5	1.5	2	2.5	
C. megacephala	Number	96	88	90	302	284	258	206	186	174	528
	Per cent	18.2	16.7	17	57.2	53.8	48.9	39	35.2	33	100
C. bezziana	Number	64	47	44	146	160	132	100	75	83	234
	Per cent	27.4	20.1	18.8	62.4	68.4	56.4	42.7	32.1	35.5	100
L. cuprina	Number	75	76	68	196	180	184	94	98	90	334
	Per cent	22.5	22.8	20.4	58.7	53.9	55	28.1	29.3	26.9	100

Table 22. Repellant effect of extracts of Acorus calamus on meat infesting flies (Mean values)

Parameters	Concentration (per cent)	Petroleum ether extract	Alcoholic extract	Steam distillate
Time of fly arrival and sitting on bait	1.5	38 minutes	15 minutes	20 minutes
	2	45 minutes	25 minutes	30 minutes
	2.5	49 minutes	25 minutes	32 minutes
	Control	9 minutes	10 minutes	10 minutes
Time of oviposition/ larviposition	1.5	80 minutes	20 minutes	45 minutes
	2	120 minutes	38 minutes	60 minutes
	2.5	125 minutes	35 minutes	69 minutes
	Control	15 minutes	17 minutes	12 minutes
Number and percentage of eggs/larvae laid	1.5	128 (28.5)	251 (63.1)	187 (39.3)
	2	90 (20.0)	151 (38.0)	140 (29.4)
	2.5	80 (17.8)	156 (39.2)	135 (28.4)
	Control	449 (100)	398 (100)	476 (100)

Table 23. Effect of extracts of Acorus calamus on larval stage III and adult flies of *Chrysomyia megacephala* (Mean values)

Extracts	Concentration (per cent)	Larval stage-3						Adult flies				
		Pupation per cent	Adult emergence (per cent)	Adult life span (days) M	F	Eggs laid (average)	Egg hatch (per cent)	Adult life span (days) M	F	Eggs laid (average)	Egg hatch (per cent)	Adult development (per cent)
Petroleum ether extract	0.1	72.5	60	12	41	1238	81	14	58	290	13	4
	0.5	37.5	27.5	9	26	899	32	14	50	48	2.1	0
	1	32.5	17.5	6	12	436	7	14	52	0	0	0
Alcoholic extract	0.1	82.5	72.5	12	51	1639	85	15	56	899	46	18
	0.5	70	62.5	12	38	1201	53	15	57	196	21	3
	1	65	47.5	10	29	1008	32	14	60	47	4.3	0
Steam distillate	0.1	70	62.5	11	40	1310	80	14	52	310	18	9
	0.5	52.5	35	8	29	928	41	15	56	92	7.6	0
	1	47.5	27.5	6	15	644	19	14	56	12	0	0
Control		95	90	15	62	2310	97	15	62	2310	97	87.5

M: Male F: Female

Table 24. Effect of extracts of Acorus calamus on larval stage III and adult flies of *Chrysomyia bezziana* (Mean values)

Extracts	Concentration (per cent)	Larval stage-3						Adult flies				
		Pupation per cent	Adult emergence (per cent)	Adult life span (days) M	F	Eggs laid (average)	Egg hatch (per cent)	Adult life span (days) M	F	Eggs laid (average)	Egg hatch (per cent)	Adult development (per cent)
Petroleum ether extract	0.1	67.5	47.5	10	31	710	52	16	40	149	9	2
	0.5	25	15	8	24	428	21	16	42	23	0	0
	1	17.5	7.5	7	20	372	3	16	43	0	0	0
Alcoholic extract	0.1	82.5	60	12	40	1016	72	17	47	677	29	11
	0.5	62.5	45	9	31	712	48	16	40	299	8	3
	1	62.5	27.5	9	26	639	23	16	42	92	1.8	0
Steam distillate	0.1	65	50	11	36	890	68	18	44	344	11	6
	0.5	45	25	7	25	588	34	16	41	103	6	0
	1	25	20	7	26	398	17	16	42	14	0	0
Control		85	82.5	18	50	2098	88	18	50	2098	87	80

M: Male F: Female

Table 25. Effect of extract of Acorus calamus on larval stage III and adult flies of *Lucilia cuprina* (Mean values)

Extracts	Concentration (per cent)	Larval stage-3						Adult flies				
		Pupation per cent	Adult emergence (per cent)	Adult life span (days) M	F	Eggs laid (average)	Egg hatch (per cent)	Adult life span (days) M	F	Eggs laid (average)	Egg hatch (per cent)	Adult development (per cent)
Petroleum ether extract	0.1	52.5	40	7	11	92	2.8	15	49	0	0	0
	0.5	17.5	7.5	1	1	0	0	15	50	0	0	0
	1	5	0	0	0	0	0	14	47	0	0	0
Alcoholic extract	0.1	50	35	12	38	739	49	16	56	186	17	4
	0.5	60	42.5	9	23	392	23	15	49	77	2.3	0
	1	57.5	37.5	8	17	210	9	15	46	12	0	0
Stean distilled	0.1	45	32.5	9	13	174	8	15	48	32	0	0
	0.5	25	20	6	10	54	3.2	15	52	0	0	0
	1	12.5	7.5	2	4	0	0	15	49	0	0	0
Control		87.5	82.5	17	60	1494	95	18	61	1494	95	80

M: Male F: Female

Chart.20 COMPARATIVE MORTALITY EFFECT OF 2.5% EXTRACTS OF ACORUS CALAMUS ON LARVAL STAGE-III IN MYIASIS PRODUCING FLIES

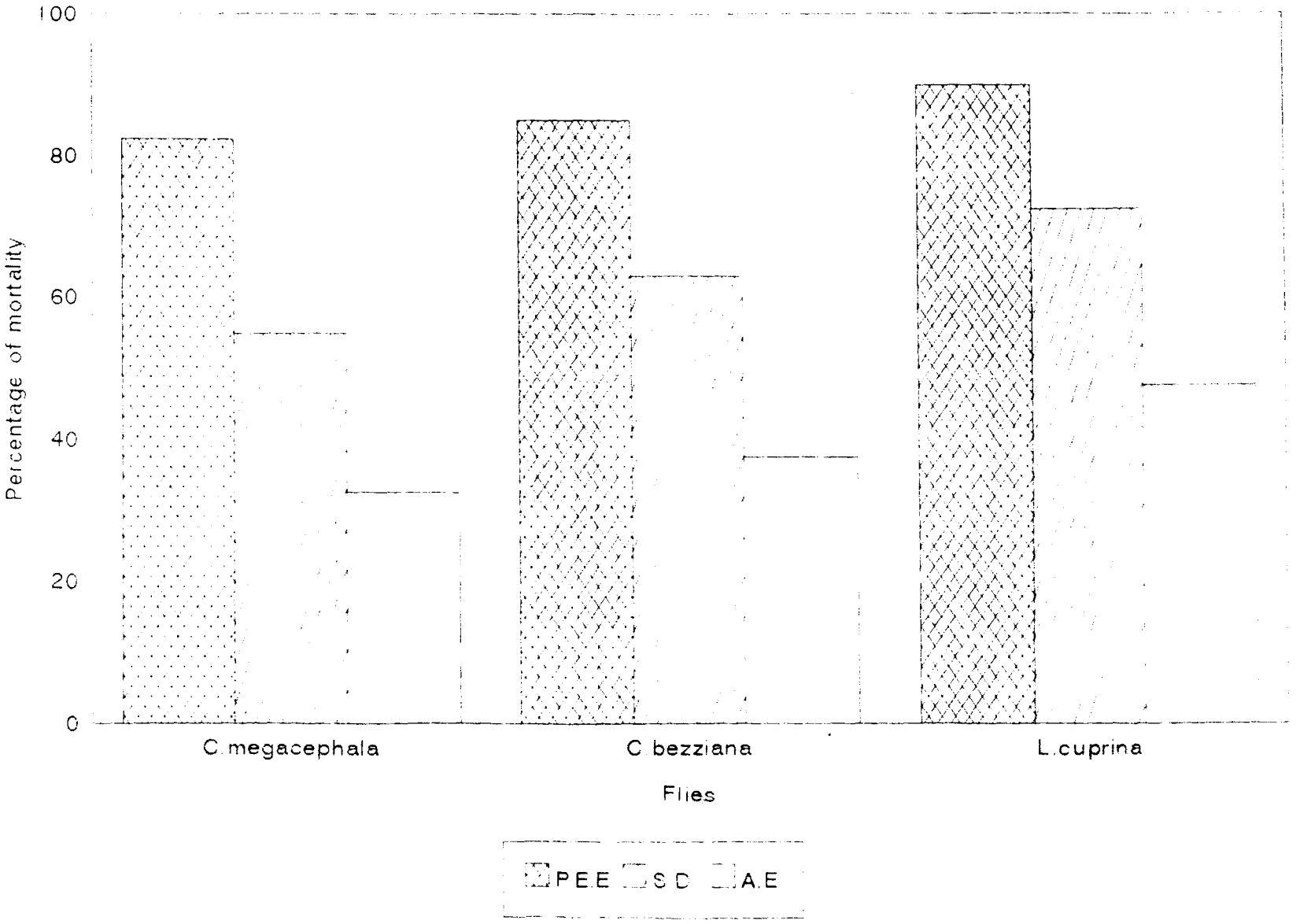


Chart.21 COMPARATIVE EFFECT OF 1% EXTRACTS OF ACORUS CALAMUS ON LARVAL MEAT CONSUMPTION OF MYIASIS PRODUCING FLIES

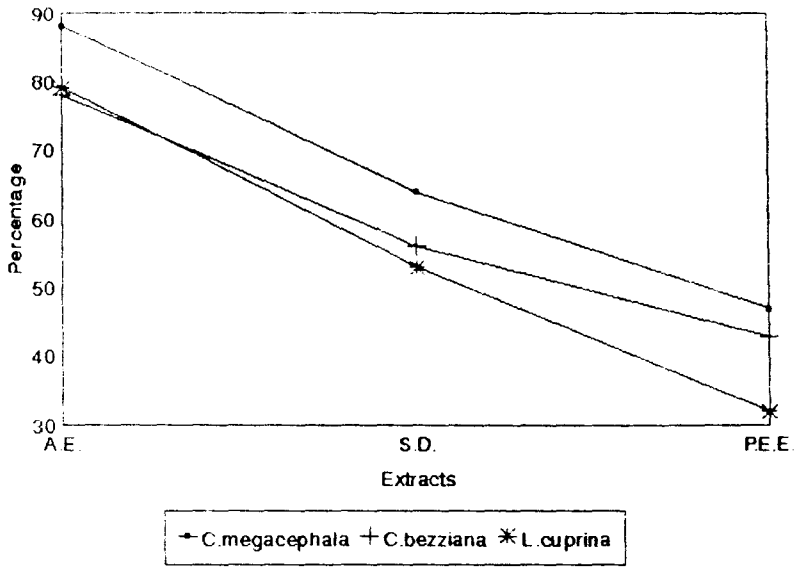


Chart.22 COMPARATIVE EFFECT OF 2.5% EXTRACTS OF ACORUS CALAMUS ON FECUNDITY OF MYIASIS PRODUCING FLIES

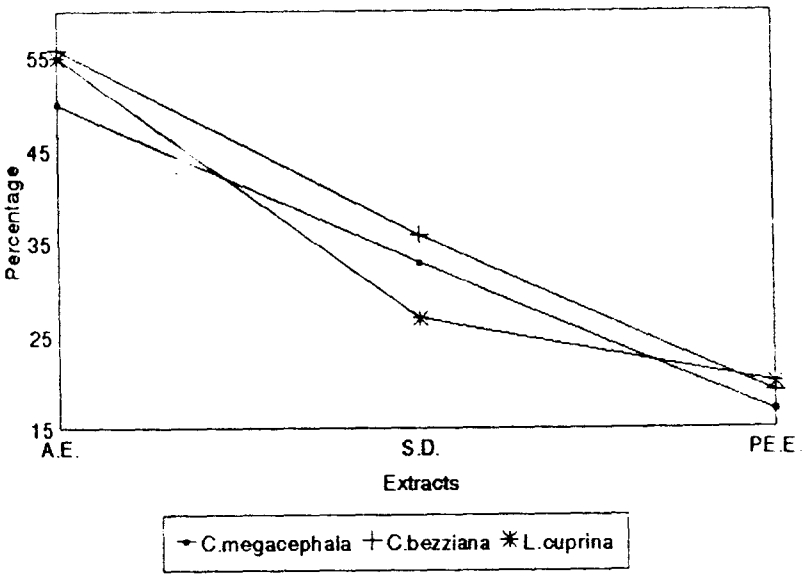


Chart.23 COMPARATIVE REPELLANT EFFECT OF EXTRACTS OF ACORUS CALAMUS ON MEAT INFESTING FLIES

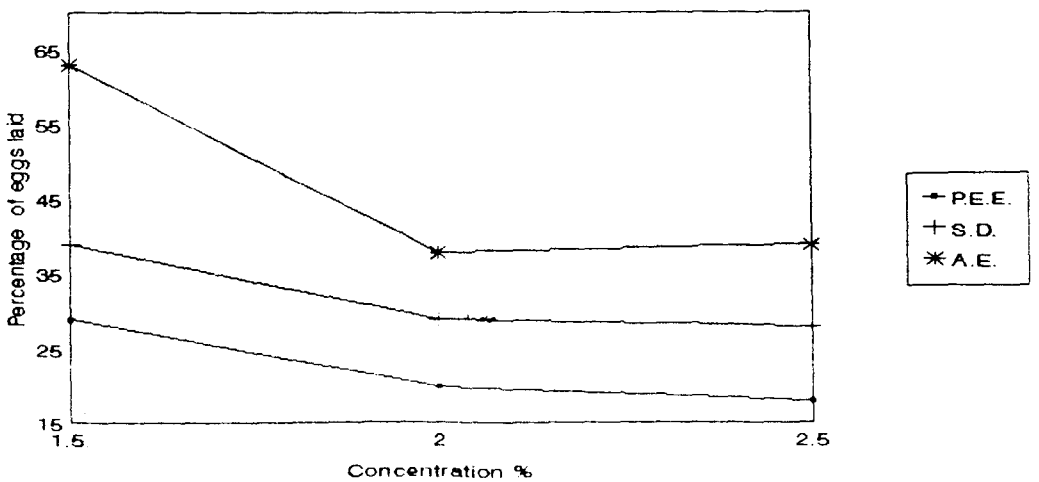


Fig.32. *C. bezziana* - Mature ovarian follicles - 40x

Fig.33. *C. bezziana* - Totally regressed ovarian follicle development on treatment of PEE of Acorus calamus - 40x

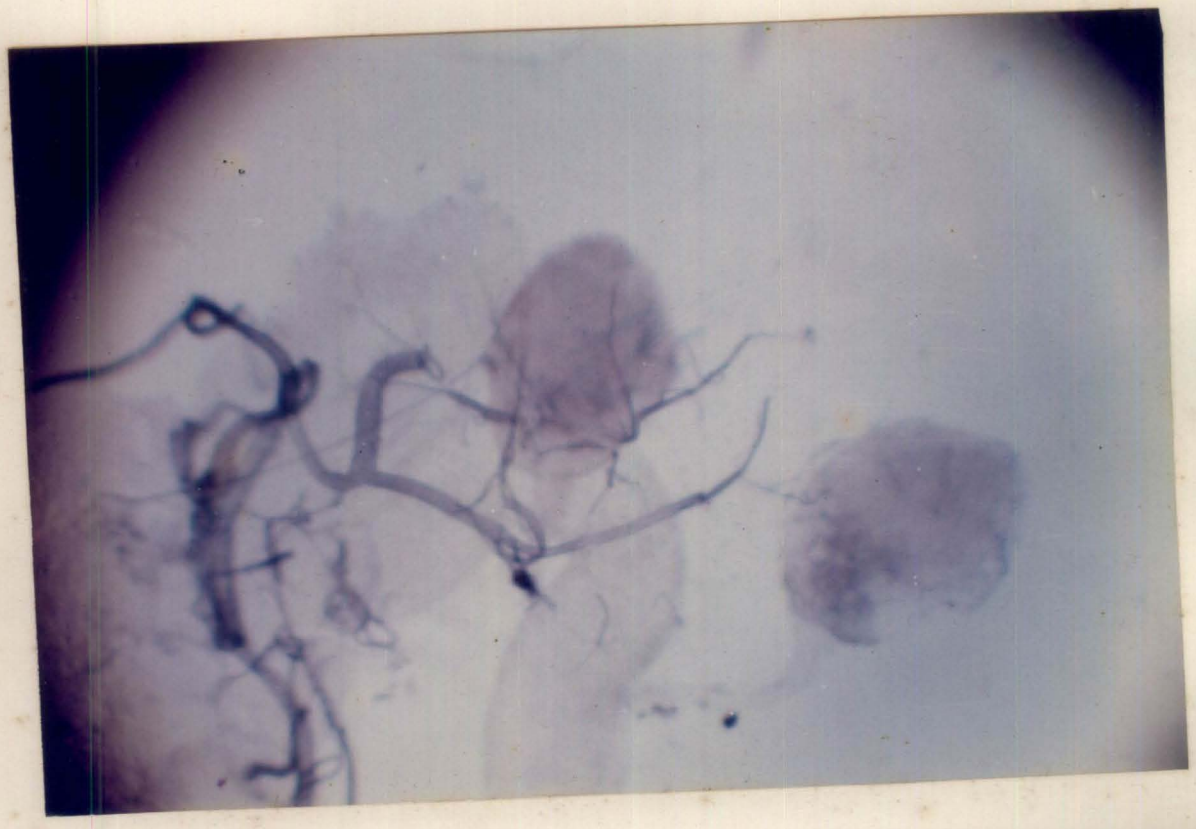
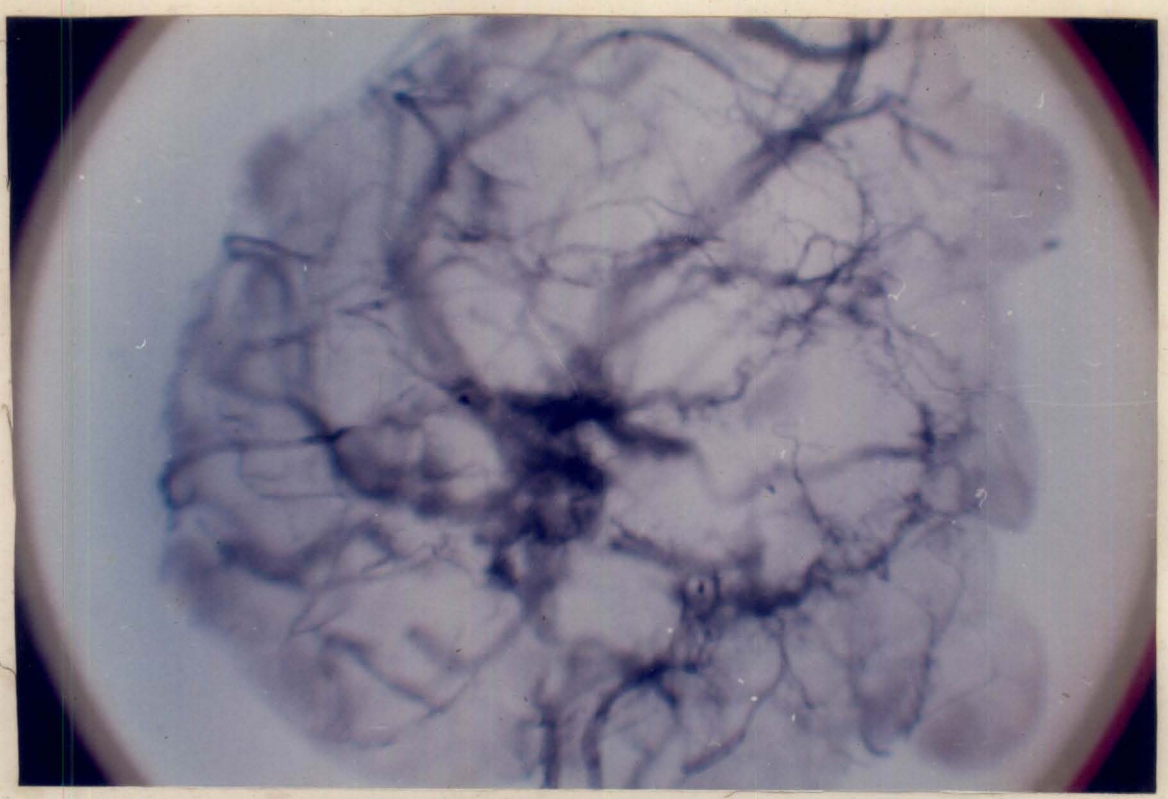
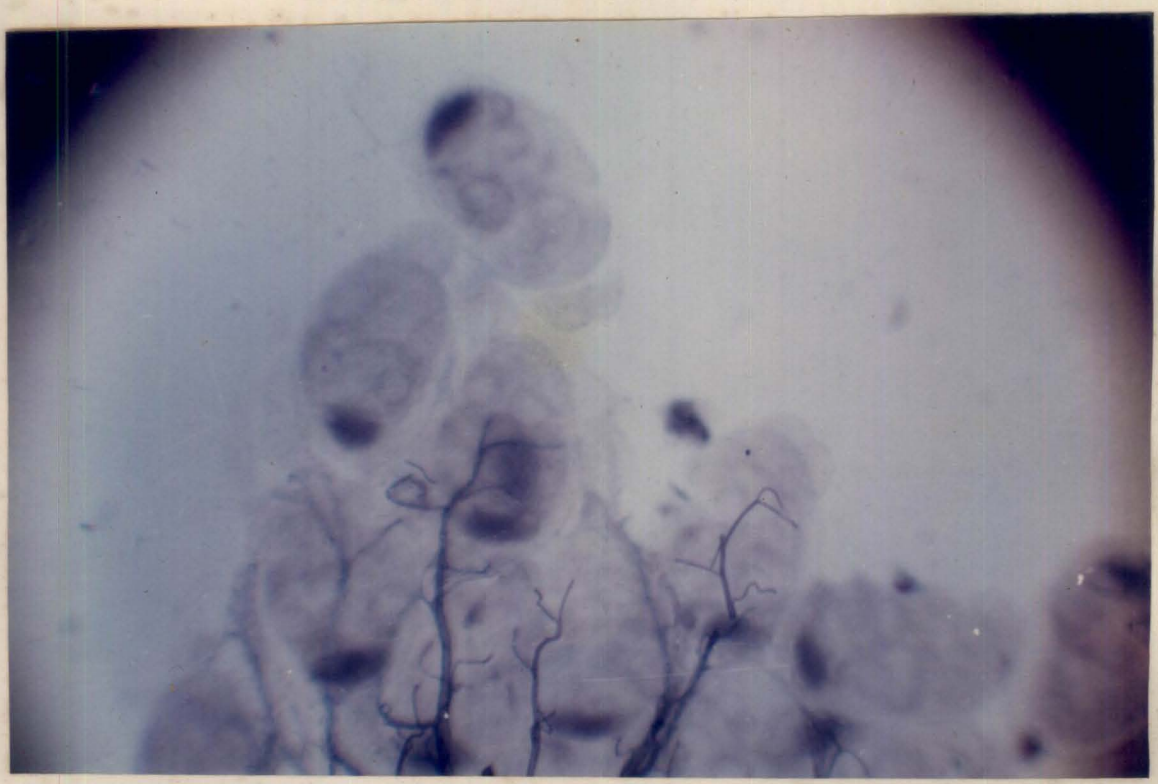


Fig.34. *C. bezziana* - Partially developed ovarian follicle on treatment of PEE of Acorus calamus - 40x

Fig.35. *C. bezziana* - Partly developed ovarian follicle on treatment of PEE of Acorus calamus - 40x





4.7 EFFECT OF GAMMA IRRADIATION

On exposure to cobalt-60 radiation the ovicidal effect was very pronounced even at the minimum dose of 500 rads tested. The detrimental effects on other developmental stages increased with the increase in exposure dose. Younger larvae were more affected than the older ones. Maximum sterility effect in flies of the three different species were obtained when 3 day old pupae were irradiated in which the flies showed sufficient longevity with substantial decrease in egg production.

4.7.1 *C. megacephala* (Table 26)

Eggs

On exposure to 500 rads, only 8 per cent of the eggs hatched. Hatching was prevented at higher exposure doses. In the control group 98 per cent of the eggs hatched.

Larval stage I

At the exposure doses of 500, 1000 and 2000 rads, the pupation were 72, 48 and 9 per cent respectively and 0 per cent at higher exposure doses. Adult emergence was 61 and 39 per cent, longevity 10 and 7 days in males and 48 and 28 days in females and an average number of 1349 and 464 eggs were laid at 500 and 1000 rads exposure respectively. In the control group, the values were 94 per cent, 89 per cent, 15

and 66 days and 2438 eggs respectively for the above mentioned parameters.

Larval stage II

At 500, 1000 and 2000 rads exposure, the pupation rates were 74, 49 and 14 per cent, adult emergence 65, 41 and 2 per cent, longevity 12, 9 and 0 days in males and 50, 36 and 5 days in females. The average number of eggs laid were 1545, 899 and 0 respectively. In the control group, the values were similar to that mentioned in larval stage I of *C. megacephala*.

Larval stage III

At the above mentioned exposures the pupation rates were 75, 51 and 15 per cent, adult emergence 66, 46 and 5 per cent, longevity 12, 9 and 6 days in males and 50, 41 and 14 days in females and the average number of eggs laid were 1669, 1060 and 48 respectively. In the control group, the values were similar to that of larval stage I of *C. megacephala*.

Pupa day I

At the exposure ranging from 500 to 6000 rads, the adult emergence ranged in the descending order from 88 to 42 per cent, and longevity 14 to 5 days in males and 60 to 18 days in females. At 500 to 2000 rads, egg laying decreased from 2301 to 210 with resultant hatch of 92 to 12 per cent. Subsequent adult development of 85 and 32 per cent were noted at 500 and

Larval stage I

At 500, 1000 and 2000 rads exposure the pupation was 67, 40 and 8 per cent respectively. At the above doses adult emergence of 59, 31 and 2 per cent, longevity of 12, 8 and 6 days in males and 40, 17, and 12 days in females, and an average egg out put of 982, 528 and zero were noted. There were no pupation at the exposure of 3000 rads and above and the life cycle was arrested at 2000 rads. In the control group, the values were 85 per cent, 84 per cent, 18 and 49 days and 2018 eggs for the above mentioned parameters respectively.

Larval stage II

At 500, 1000 and 2000 rads exposure, the pupation were 70, 45 and 12 per cent respectively. At the above doses adult emergence were 65, 35 and 6 per cent, adult longevity 12, 7 and 6 days in males and 41, 20 and 14 days in females and average egg output 901, 540 and 68. There were no pupation at the exposure of 3000 rads and above. In the control group, the values were similar to those of the larval stage I of *C. bezziana*.

Larval stage III

At the exposure rate ranging from 500 to 3000 rads, the pupation ranged in the descending order from 69 to 8 per cent,

adult emergence 64 to 1 per cent, longevity 11 to 3 days in males and 38 to 10 days in females and average egg output was 1319 to zero. Pupation was not observed at 4000 rads and above and the life cycle was arrested at 3000 rads. In the control group, the values were similar to that of larval stage I of *C. bezziana*.

Pupa day I

At the exposure range of 500 to 6000 rads, the adult emergence descended from 83 to 7 per cent and longevity from 15 to 3 days in males and 37 to 6 days in females. At 500-2000 rads exposure, the flies laid 1509 to 419 eggs with a hatchability of 55 to 7 per cent and subsequent development of 40 to 1 per cent. The flies did not lay any eggs at 3000 rads and above exposures. In the control group, the values were similar to that of larval stage I except that 81 per cent of adults developed in the second generation cycle.

Pupa day III

At the rate of 500 to 6000 rads exposure, the adult emergence ranged in the descending order from 79 to 72 per cent, longevity from 18 to 12 days in males and 45 to 30 days in females. At 500 to 3000 rads, the flies laid 1219 to 25 eggs with a hatchability of 59 to 1 per cent and subsequent development of 50 to zero per cent. The flies did not lay any eggs at 4000 rads and above exposures. In the control group,

the values were similar to that of one day old pupa of *C. bezziana*.

The longevity and fecundity of female *C. bezziana* on radiation exposure to different developmental stages are graphically represented in Charts 26 and 27.

4.7.3 *L. cuprina* (Table 28)

Eggs

The egg hatchability was only 3 per cent at 500 rads exposure and nil at higher exposure doses, compared to 94 per cent hatch in the control group.

Larval stage I

At 500 and 1000 rads exposure the pupation was 61 and 28 per cent, adult emergence 54 and 19 per cent, longevity 11 and 6 days in males and 39 and 18 days in females, with an egg out of 639 and 111 eggs respectively. There were no pupation at the exposure of 2000 rads and above. In the control group, the values for the above parameters were 88 per cent, 83 per cent, 17 and 60 days and 1474 eggs respectively.

Larval stage II

At 500, 1000 and 2000 rads exposure the pupation was 65, 38 and 3 per cent respectively. At 500 and 1000 rads the

adult emergence was 58 and 20 per cent, longevity 10 and 6 days in males and 35 and 17 days in females with an egg out put of 843 and 179 respectively. There were no adult emergence at 2000 rads and the pupation was totally inhibited at 3000 rads exposure. In the control group, the values were similar to that of larval stage I of *L. cuprina*.

Larval stage III

At 500, 1000 and 2000 rads exposure the rates of pupation were 66, 44 and 5 per cent, adult emergence 51, 28 and 1 per cent, longevity 12, 8 and 0 days in males and 41, 16 and 2 days in females. An average of 885, 231 and 0 eggs were laid by the 3 treated groups of resultant females. The pupation was totally inhibited at 3000 rads exposure. In the control group, the values were similar to that of larval stage I of *L. cuprina*.

Pupa day I

At the range of 500 to 6000 rads exposure the adult emergence ranged in the descending order from 78 to 14 per cent, and longevity 17 to 4 days in males and 51 to 10 days in females. An average of 1201 to 118 eggs were laid with an egg hatch of 69 to 5 per cent at 500 to 2000 rads exposure. The subsequent adult development was 48 and 16 per cent at 500 and 1000 rads respectively. The egg laying was totally inhibited

at 3000 rads and subsequent adult development at 2000 rads exposure. In the control group the values were similar to that of larval stage I except that 80 per cent of adults developed in the second generation cycle.

Pupa day III

At the range of 500 to 6000 rads exposure, the adult emergence ranged in the descending order from 82 to 46 per cent, longivity 16 to 5 days in males and 61 to 20 days in females. At 500 to 2000 rads exposure 1160 to 206 eggs were laid with a hatch of 86 to 9 per cent. The subsequent adult development of 56 and 16 per cent was noted at 500 and 1000 rads exposure respectively. Eggs were not laid at 3000 rads, while subsequent adult development was prevented at 2000 rads exposure. In the control group, the values were similar to that of one day old pupa of *L. cuprina*.

The longevity and fecundity of female *L. cuprina* on radiation treatment to different developmental stages are given in Charts 28 and 29.

Table 26. Effect of Gamma radiation on *Chrysomyia megacephala* (Mean values)

Exposure Rads	Eggs	Larval stage I				Larval stage II					Larval stage III					Pupa day I					Pupa day III				
	Egg hatch per cent	Pupation per cent	Adult emergence per cent	Adult life span (days) M	F	Eggs laid (average)	Pupation per cent	Adult emergence per cent	Adult life span (days) M	F	Eggs laid (average)	Pupation per cent	Adult emergence per cent	Adult life span (days) M	F	Eggs laid (average)	Adult emergence per cent	Adult life span (days) M	F	Eggs laid (average)	Egg hatch per cent	Adult development per cent	Adult emergence per cent	Adult life span (days) M	F	Eggs laid (average)	Egg hatch per cent	Adult development per cent
0	98	94	89	15	66	2438	94	89	15	66	2438	94	89	15	66	2438	89	15	66	2438	98	87	89	15	66	2438	98	87
500	8	72	61	10	48	1349	74	65	12	50	1545	75	66	12	50	1669	88	14	60	2301	92	85	88	15	62	2360	90	86
1000	0	48	39	7	28	464	49	41	9	36	899	51	46	9	41	1060	70	13	55	1008	49	32	87	15	63	1215	68	37
2000	0	9	0	0	0	0	14	2	0	5	0	15	5	6	14	48	66	13	40	210	12	0	87	14	65	342	42	9
3000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	67	10	38	0	0	0	87	14	62	61	1	0
4000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	59	8	42	0	0	0	87	14	63	0	0	0
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	51	6	30	0	0	0	85	13	58	0	0	0
6000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	42	5	18	0	0	0	86	12	50	0	0	0

M: Male F: Female

Table 27. Effect of Gamma radiation on *Chrysomyia bezziana* (Mean values)

Exposure Rads	Eggs	Larval stage I					Larval stage II					Larval stage III					Pupa day I						Pupa day III					
	Egg hatch per cent	Pupation per cent	Adult emergence per cent	Adult life span (days) M	Adult life span (days) F	Eggs laid (average)	Pupation per cent	Adult emergence per cent	Adult life span (days) M	Adult life span (days) F	Eggs laid (average)	Pupation per cent	Adult emergence per cent	Adult life span (days) M	Adult life span (days) F	Eggs laid (average)	Adult emergence per cent	Adult life span (days) M	Adult life span (days) F	Eggs laid (average)	Egg hatch per cent	Adult development per cent	Adult emergence per cent	Adult life span (days) M	Adult life span (days) F	Eggs laid (average)	Egg hatch per cent	Adult development per cent
0	87	85	84	18	49	2018	85	84	18	49	2018	85	84	18	49	2018	84	18	49	2018	87	81	84	18	49	2018	87	81
500	7	67	59	12	40	982	70	65	12	41	901	69	64	11	38	1319	83	15	37	1509	55	40	79	18	45	1219	59	50
1000	0	40	31	8	17	528	45	35	7	20	540	52	40	9	43	801	80	11	38	958	25	18	81	16	46	659	19	21
2000	0	8	2	6	12	0	12	6	6	14	68	20	9	8	27	214	69	9	18	419	7	1	83	17	44	401	11	2
3000	0	0	0	0	0	0	0	0	0	0	0	8	1	3	10	0	47	6	20	0	0	0	82	17	43	25	1	0
4000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	5	12	0	0	0	82	17	45	0	0	0
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18	3	9	0	0	0	69	14	40	0	0	0
6000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7	3	6	0	0	0	72	12	30	0	0	0

M: Male F: Female

Table 28. Effect of Gamma radiation on *Lucilia cuprina* (Mean values)

Exposure Rads	Eggs	Larval stage I					Larval stage II					Larval stage III					Pupa day I						Pupa day III					
	Egg hatch per cent	Pupation per cent	Adult emergence per cent	Adult life span (days) M	Adult life span (days) F	Eggs laid (average)	Pupation per cent	Adult emergence per cent	Adult life span (days) M	Adult life span (days) F	Eggs laid (average)	Pupation per cent	Adult emergence per cent	Adult life span (days) M	Adult life span (days) F	Eggs laid (average)	Adult emergence per cent	Adult life span (days) M	Adult life span (days) F	Eggs laid (average)	Egg hatch per cent	Adult development per cent	Adult emergence per cent	Adult life span (days) M	Adult life span (days) F	Eggs laid (average)	Egg hatch per cent	Adult development per cent
0	94	88	83	17	60	1474	88	83	17	60	1474	88	83	17	60	1474	83	17	60	1474	94	80	83	17	60	1474	94	80
500	3	61	54	11	39	639	65	58	10	35	843	66	51	12	41	885	78	17	51	1201	69	48	82	16	61	1160	86	56
1000	0	28	19	6	18	111	36	20	6	17	179	44	28	8	16	231	69	12	42	846	32	16	82	17	57	949	49	16
2000	0	0	0	0	0	0	3	0	0	0	0	5	1	0	2	0	40	9	39	118	5	0	82	16	56	206	9	0
3000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	29	7	31	0	0	0	81	16	58	0	0	0
4000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	26	7	30	0	0	0	74	14	52	0	0	0
5000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	15	6	16	0	0	0	70	7	29	0	0	0
6000	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14	4	10	0	0	0	46	5	20	0	0	0

M: Male F: Female

Chart.24 LONGIVITY OF FEMALE FLIES ON EXPOSURE TO GAMMA RADIATION IN DEVELOPMENTAL STAGES OF C.megacephala

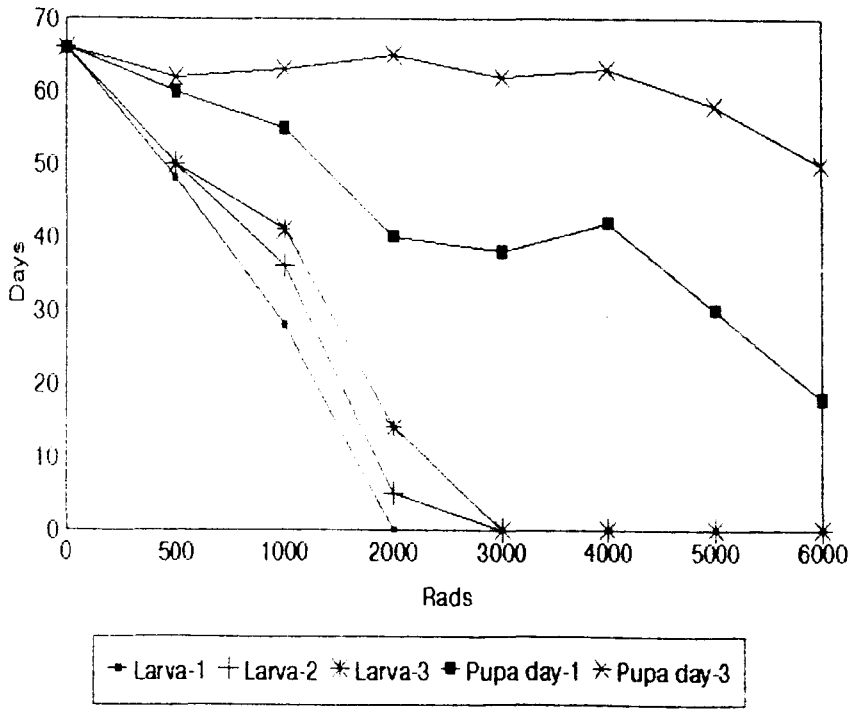


Chart.25 FECUNDITY OF C.megacephala ON EXPOSURE TO GAMMA RADIATION IN DEVELOPMENTAL STAGES

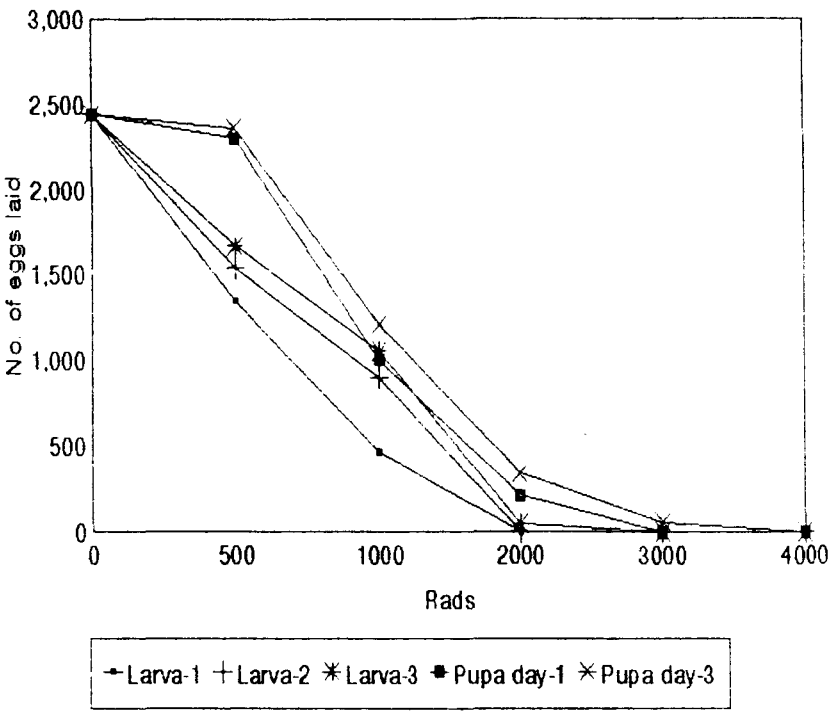


Chart.26 LONGIVITY OF FEMALE FLIES ON EXPOSURE TO GAMMA RADIATION IN DEVELOPMENTAL STAGES OF C.bezziana

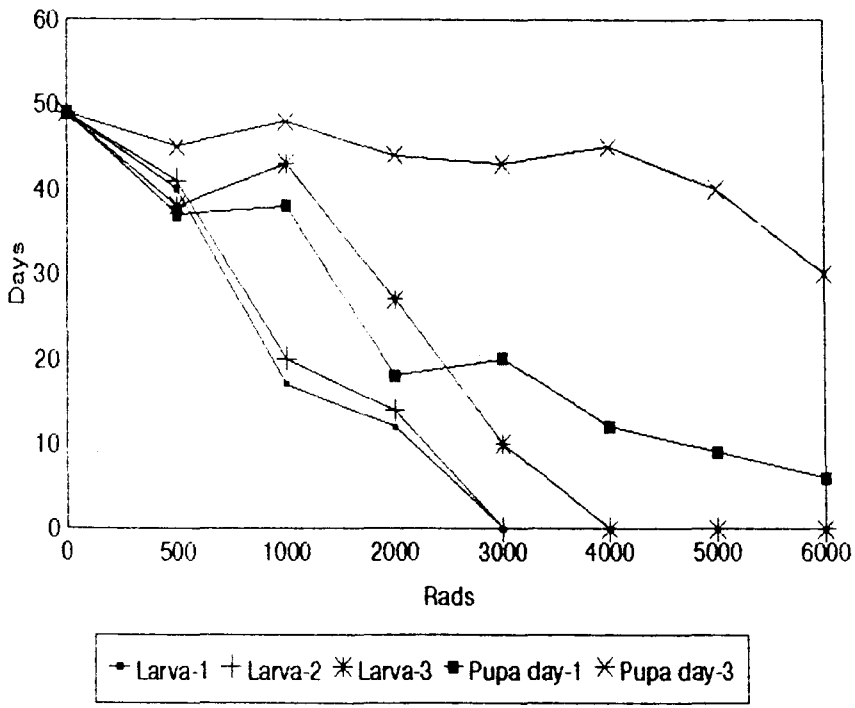


Chart.27 FECUNDITY OF C.bezziana ON EXPOSURE TO GAMMA RADIATION IN DEVELOPMENTAL STAGES

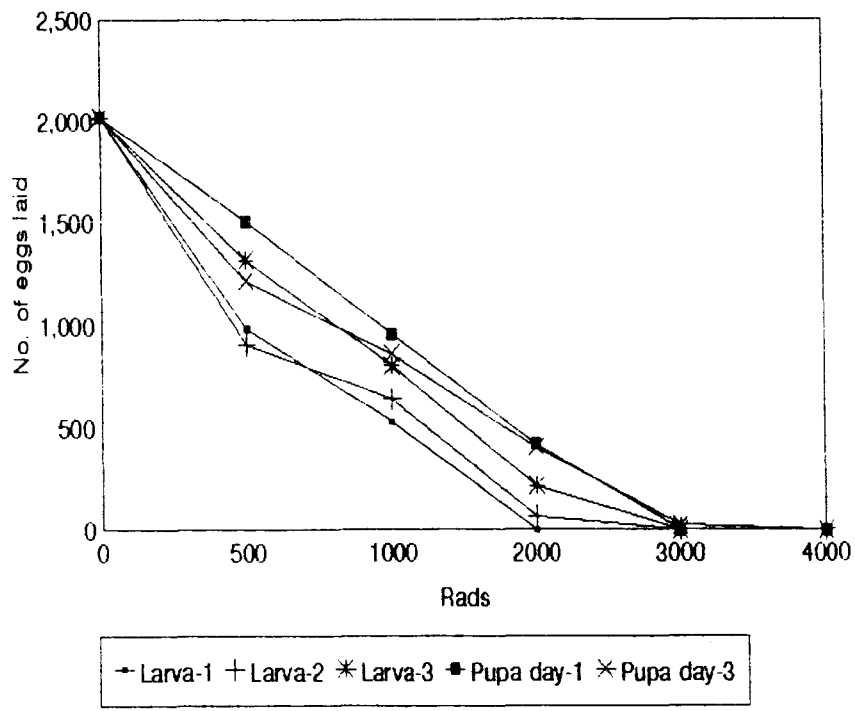


Chart.28 LONGIVITY OF FEMALE FLIES ON EXPOSURE TO GAMMA RADIATION IN DEVELOPMENTAL STAGES OF L.cuprina

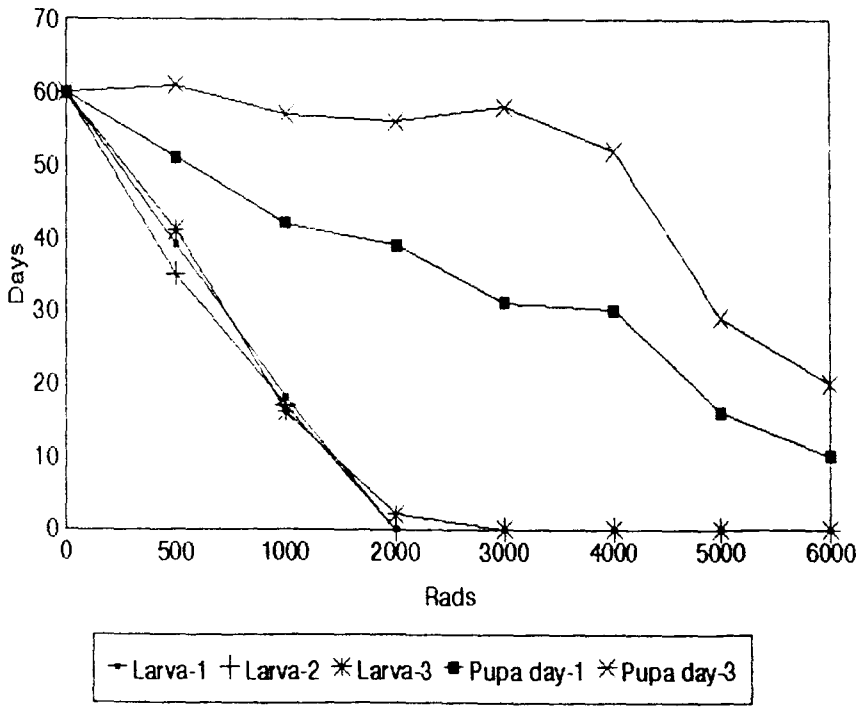
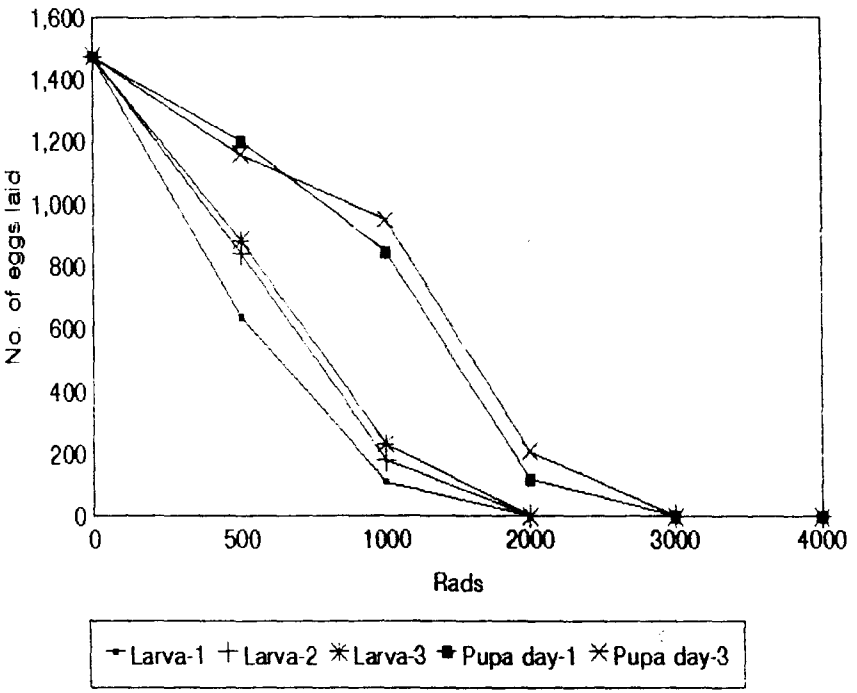


Chart.29 FECUNDITY OF L.cuprina ON EXPOSURE TO GAMMA RADIATION IN DEVELOPMENTAL STAGES



4.8 TREATMENT TRIALS WITH BIOPESTICIDES (Table 29)

In the study to ascertain the comparative larvicidal efficacy of diflubenzuron (5 ppm), azadirachtin (15 ppm) and PEE of *Acorus calamus* (2.5 per cent) in natural cases of cutaneous myiasis in cattle, goats and dogs, diflubenzuron gave the maximum efficacy followed by azadirachtin. PEE of *Acorus calamus* exhibited only moderate efficacy.

Diflubenzuron

Out of the total number of 349 larvae recovered from cattle after treatment, 301 larvae were found dead, giving an efficacy rate of 86.25 per cent. In goats, 88 of 92 larvae recovered were found dead with an efficacy rate of 95.65 per cent while in dogs 104 of 116 larvae were dead with 89.66 per cent efficacy. In the overall efficacy, 493 of 557 larvae were found dead with an average efficacy rate of 88.51 per cent.

Azadirachtin

A total number of 297 larvae were recovered from cattle after treatment, of which 235 were dead with an efficacy rate of 79.12 per cent. In goats 84 of 111 larvae recovered were dead with an efficacy rate of 75.68 per cent while in dogs 78 of 98 larvae were dead with 79.59 per cent efficacy. In the

overall efficacy 397 of 506 larvae were dead with an average 78.46 per cent efficacy.

Acorus calamus

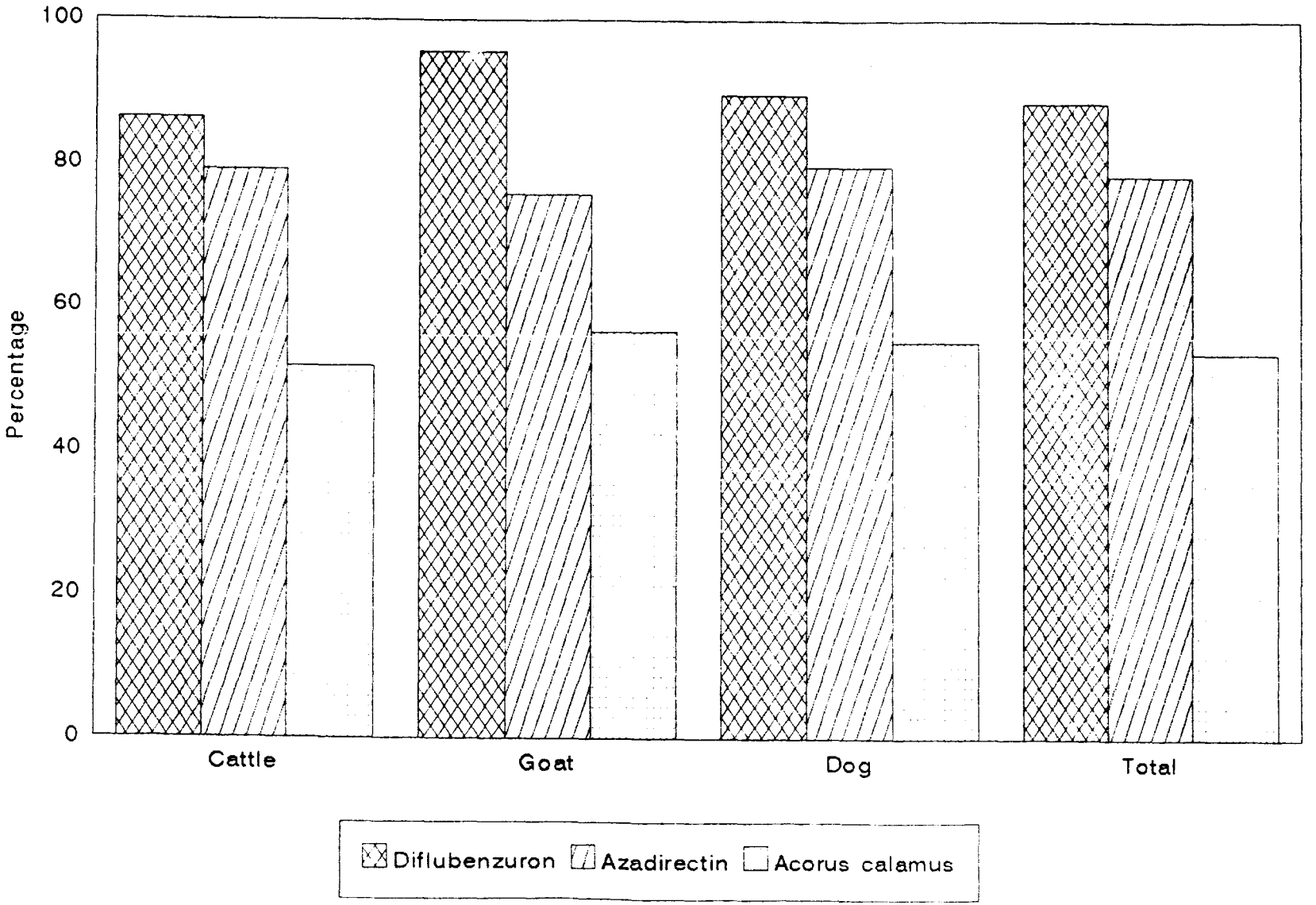
A total number of 308 larvae were recovered in cattle after treatment, of which 159 were dead giving an efficacy rate of 51.62 per cent. In goats 69 of 122 larvae were dead with an efficacy rate of 56.56 per cent while in dogs 74 of 134 larvae were dead with 55.22 per cent efficacy. In the overall efficacy 302 of 564 larvae were dead with 53.55 per cent efficacy.

The efficacy of each biopesticide was statistically compared with the other 2 biopesticides in cattle, goats and dogs separately. As the table values in all the cases were above 1.96, the difference in efficacy of each biopesticide was statistically significant with the other 2 biopesticides.

Table 29. Comparative larvicidal efficacy of Diflubenzuron, Azadirachtin and Acorus calamus (Petroleum ether extract) in natural cases of cutaneous myiasis

Biopesticide	Concentration	Cattle				Goat				Dog				Total			
		No. of cases treated	Total number of larvae	No. of dead larvae	Percentage of efficacy	No. of cases treated	Total number of larvae	No. of dead larvae	Percentage of efficacy	No. of cases treated	Total number of larvae	No. of dead larvae	Percentage of efficacy	No. of cases treated	Total number of larvae	No. of dead larvae	Percentage of efficacy
Diflubenzuron	5 ppm	10	349	301	86.25	5	92	88	95.65	5	116	104	89.66	20	557	493	88.51
Azadirectin	15 ppm	10	297	235	79.12	5	111	84	75.68	5	98	78	79.59	20	506	397	78.46
Acorus calamus (PEE)	2.5%	10	308	159	51.62	5	134	74	56.56	5	1346	744	55.22	20	564	302	53.55

Chart.30 COMPARATIVE EFFICACY OF DIFLUBENZURON, AZADIRECTIN AND PETROLEUM ETHER EXTRACT OF ACORUS CALAMUS IN NATURAL CASES OF CUTANEOUS MYIASIS



Discussion

DISCUSSION

5.1 Prevalence of cutaneous myiasis

The details on the prevalence of cutaneous myiasis in domestic animals were studied by many scientists in India. In the present investigation, among the 9861 animals screened for cutaneous myiasis (from those brought to University Veterinary Hospitals at Kokkalai and Mannuthy, 205 (2.08 per cent) were found positive for the infestation. The prevalence extended from the month of October to April with the peak infestation in January. The higher prevalence of the infestation during the above period associated well with post monsoon fly breeding season of Kerala in high humid climatic condition. The prevalence sharply reduced with the onset of summer. This finding is in conformity with that of Sen Gupta *et al.* (1951), Subramanian and Raja Mohanan (1980) and Thilager *et al.* (1989) who observed the prevalence of cutaneous myiasis in the post monsoon and pre summer seasons, with the difference that they found peak infestation in February or March which may be due to the climatic variations relating to temperature and humidity in the particular month of that year.

The incidence of cutaneous myiasis was significantly higher in cattle (63.41 per cent) compared to dogs (24.88 per cent) and goats (11.71 per cent) probably due to the

unhygienic conditions under which cattle are reared, which is more suitable for the development of myasis producing flies. The higher incidence of the condition in cattle was recorded by Rao and Pillay (1936) Subramanian and Rajamohanan (1980) and Thilager *et al* (1991)

In the present study among the species of larvae involved in causing the condition, *C. bezziana* (90.73 per cent) was observed to be the main myiasis producer followed by *L. cuprina* (7.32 per cent) and *C. megacephala* (1.95 per cent). This finding is in confirmity with that of Patton (1920a) and (1922e), Nachiappan (1971), Thilager *et al.* (1989). Subramanian and Rajamohanan (1980) also found *C. bezziana* as the chief myiasis producer eventhough they could record *C. rufifacies* also in 0.65 per cent cases.

5.2 Effect of methoprene

The pesticidal effect of the juvenile hormone analogue methoprene was tested by many workers against *Musca* and *Stomoxys*. In the present study methoprene was tested against the eggs and three larval stages of *C. megacephala*, *C. bezziana* and *L. cuprina* at 1 to 50 ppm concentrations. At any particular concentration, the effect was highest against *L. cuprina* followed by *C. bezziana* and *C. megacephala*, which

proved that the lifecycle stages of *L. cuprina* are more sensitive to methoprene.

It was observed that methoprene caused severe mortality effect on eggs irrespective of the species of myiasis producing flies. The egg hatching was completely prevented at 30 ppm in *C. megacephala* and *C. bezziana* whereas such effect was noted at 10 ppm in *L. cuprina*. This proved that methoprene has potential contact toxicity as observed by Buci *et al.* (1979) who recorded 95 per cent mortality in eggs of *M. domestica* at 5 ppm concentration of methoprene.

The mortality effect in larval stage I, II and III increased with the increase in concentration of methoprene but was significantly lower in mature larvae than younger ones. Though the mortality ranged from 52.5 to 100 per cent in larval stage I and 27.5 to 45 per cent in larval stage II, the effect was considerably lower (0-17.5 per cent) in larval stage III. This finding is contrary to that of Mohiuddin and Qureshi (1982) who found that methoprene affects newly formed pupae than younger stages in *M. domestica*. The observations of complete larval mortality by Morgan *et al.* (1975) in *M. domestica* and no mortality in horn flies by Miller *et al.* (1976) on methoprene treatment, are different from the present observation on myiasis producing larvae, where the synthetic

analogue produced partial to complete mortality with respect to the different stages of larvae.

The present finding revealed that methoprene at the lowest concentration of 1 ppm increased the duration of larval phase by 2 to 4 hours in the second stage and 1 to 2 hours in the third stage, while at the highest concentration of 50 ppm, it prolonged the period from 8 to 10 hours and 12 to 16 hours in the second and third stage respectively. This clearly denotes that methoprene at higher concentrations delays the moulting, maturation and pupation preserving the juvenile status of the larvae considerably. This is in agreement with Taen *et al.* (1977) who reported that methoprene even at the lowest concentration of 1 ppm prolongs the period of larval instars. But the present findings differed with the report of the above authors on the period of prolongation of larval stage by 10 hours in Musca and stomoxys species of flies.

The finding that methoprene at the various tested concentrations produced 5 to 55 per cent and 10 to 60 per cent larval pupal intermediaries when methoprene was treated to second and third stage larvae respectively suggests that the analogue also affects the larval to pupal transformation by interferring in the process of metamorphosis. This is in agreement with Sehnal and Zadarak (1976) who observed larval pupal intermediaries upto 30 per cent in *M. domestica*,

Sarcophaga crassipalpis and *Calliphora vomitoria* at 1 to 5 ppm concentration, though the percentage of intermediaries varied with regard to myiasis producing flies at higher concentrations of methoprene. It was also observed that the length of pupa increased at a range of 0 to 1.5 millimetres compared to the normal length, though there was no increase in the breadth. This could be probably due to the increase in length of the larvae due to the prolonged larval phase.

Another noteworthy finding was that treatment of methoprene against eggs and larval stages at increasing concentrations significantly affected adversely the adult emergence of myiasis producing flies from the pupae. The adult emergence was totally arrested at 10 and 30 ppm when applied against eggs and all larval stages. This can be attributed to incomplete metamorphosis from pupal to adult stage resulting in the death of the developing fly inside the puparium due to methoprene treatment. This finding is in confirmity with Cerf and Georghiou (1972), Jakob (1973b), Adams *et al.* (1976), Miller *et al.* (1976), Nosec *et al.* (1977), Palaniswamy and Sivasubramanian (1977), Paysinger and Adkins (1977), Buci *et al.* (1979), Matsumara (1979), Styczynska (1979), Lineva and Chunina (1980), Styczynska *et al.* (1980), Asano *et al.* (1984), El-Ela *et al.* (1990), and Fincher (1991) who all observed 40 to 100 per cent inhibition of adult

emergence at a range of 0.13 to 50 ppm of methoprene treatment against *M. domestica* and other non-myiasis producing flies.

Another observation was that a large number of adult flies which developed after treatment of methoprene on eggs and larval stages had morphological deformities, the percentage of which was proportionately increased with the increase in concentration of methoprene. The morphological deformities noted were, failure of the fly to crawl out and emerge fully from puparium, incomplete regression of ptilinal sac, failure to present the metallic colouration on the body, permanently folded wings, incomplete unfolding of one or both the wings and abnormally flexed legs. The morphological aberrations noted can probably be due to the metamorphosis from pupal to adult stage. This finding is in agreement to the observations of Lineva and Chunina (1980) and Styezynska *et al.* (1980) who recorded significant morphological defects in adult *M. domestica* flies on methoprene treatment. The other deformities like the under development of antennae, compound eyes and mouth parts mentioned by Morgan *et al.* (1975), non pigmented eyes and reduced number of bristles by Sehnal and Zdarak (1976), irregular orientation of bristles and partial under development of the abdomen by Palaniswamy and Sivasubramanian (1977) and adults with smaller wings and bodies by Matsumara (1979) with respect to *M. domestica* were

not observed in myiasis producing flies subjected to methoprene treatment.

It has also been observed that methoprene treatment at 1 and 5 ppm concentrations against eggs and larval stages reduced the longevity of adult flies, number of eggs laid, percentage of egg hatch and the percentage of subsequent adult development moderately, while at 10 ppm and above it considerably reduced the longevity of flies with total arrest on laying of eggs. This is in agreement with Qureshi *et al.* (1983), who reported that methoprene reduces the lifespan of adult *M. domestica*, eventhough reports on lifespan of adult flies, effects on fecundity, egg hatch and subsequent development with regard to myiasis producing this are totally lacking in literature.

In as much as the review reveals that the previous workers have undertaken studies only on certain aspects on the effects of methoprene on particular stages of *M. domestica* and other non myiasis producing flies and thus left out the effects of the hormone analogue on the various developmental stages of the flies studied, the present work constitutes the first comprehensive study on the effects of methoprene on the holistic aspects of the development of myiasis producing flies.

5.3 Effect of diflubenzuron

The effect of diflubenzuron on the lifecycle stages of blow flies are meagre in literature but its pronounced larvicidal and antichitin effects were studied by many workers in *M. domestica* and other non myiasis producing flies. In the present work the insect growth regulator diflubenzuron was tested against the eggs and three larval stages of *C. megacephala*, *C. bezziana* and *L. cuprina* at the range of 0.5 to 10 ppm concentrations. The developmental stages of *L. cuprina* were found to be more sensitive to diflubenzuron followed by *C. bezziana* and *C. megacephala*.

The present study revealed that diflubenzuron at the above mentioned concentrations produced low mortality effect on eggs (4-27 per cent) on the three flies studied, which shows that diflubenzuron has little contact toxicity on eggs. This finding concurs with that of Albes *et al.* (1975) who observed 0 to 12 per cent mortality in *M. domestica* eggs on topical application of 5 to 100 ppm diflubenzuron, but is contrary to that of Grosscurt (1976) who recorded complete hatching of the eggs in house flies. The observation of Kunz and Harris (1978) that topical application of diflubenzuron at 5 ppm concentration prevented the egg hatching completely cannot be taken as a comparison with present study due to the difference of species of flies involved.

The most important finding is that diflubenzuron caused significant larval mortality on myiasis producing flies. Though the ovicidal effect was not so prominent, the larvicidal effect of 84 to 100 per cent was obtained on treatment of eggs at 0.5 to 5 ppm of diflubenzuron. Cent per cent mortality of larvae were obtained at 1 to 2.5, 2.5 and 5 ppm concentrations respectively with regard to first, second and third stage larvae. The above findings are comparable with the observations of Jakob (1973), Miller (1974), Albes *et al.* (1975), Barker and Jones (1976), Hayakawa (1976), Buci and Okabe (1977), Grosscurt (1978), El-Khodary *et al.* (1979), Grosscurt (1980), Webb and Wildey (1986), Kandasamy (1987) and Miller *et al.* (1990) who all noted that diflubenzuron in the range between 0.1 to 10 ppm concentration caused 70 to 100 per cent mortality in *M. domestica* and other non-myiasis producing flies.

In the present study, it was also noted that younger larvae of myiasis producing flies were more susceptible to diflubenzuron than mature larvae. This was evident from the observation that 1 to 2.5 ppm diflubenzuron was sufficient to kill first stage larvae totally, while complete larvicidal effect was obtained at 5 ppm only in the third stage larvae. The higher susceptibility of the younger larvae to diflubenzuron in *M. domestica* was also observed by Rupes *et al.* (1977) and Grosscurt (1978).

The mechanism of action of diflubenzuron in causing larval mortality is due to the inhibition of chitin production in the treated larvae. In the present study the chitin content of myiasis producing larvae at 0.5 to 10 ppm concentration of diflubenzuron treatment compared to the non treated larvae were estimated and found reduced considerably from 52.11 to 18.42 per cent. Ishaya and Casida (1974) in a similar finding estimated 55.5 to 26.4 per cent chitin content at increasing concentration of 0.4 to 2.5 ppm diflubenzuron in *M. domestica* larvae. Turnbull *et al.* (1980) also estimated 50 to 28 per cent chitin content at 1 to 5 ppm of diflubenzuron, but in the present study, the chitin content reduced further upto 18.42 per cent in blow flies. Rupes *et al.* (1977), Wright (1978) and Kandasamy (1987) also attributed the high mortality of diflubenzuron treated larvae to chitin deficient cuticle.

Moreover the chitin deficiency in the cuticle caused severe damage and rupture of the cuticle wall of the larvae of myiasis producing flies, though there were no changes observed in the formation of cephalopharyngeal skeleton, body spines and posterior spiracle. This finding is well supported by Bijloo (1975) who attributed the high larvicidal effect to the cuticle rupture due to chitin deficiency.

It was also observed in the present study that diflubenzuron treatment produced thin shelled pupae with a significant average reduction of 9 to 19 mgs in pupal weight. This can be directly attributed to the reduction in chitin production during larval phase on treatment with diflubenzuron. Demeney (1989) also observed the formation of abnormally thin shelled pupa in *M. domestica* on diflubenzuron treatment.

Diflubenzuron totally arrested the lifecycle of myiasis producing flies at 0.5 to 2.5 ppm concentrations when applied to different larval stages. The few number of flies which emerged from mature larvae after pupation showed substantial reduction in lifespan, fecundity, egg hatch and subsequent adult emergence.

From the present study, it is proved that diflubenzuron exerts powerful antichitin effect on the lifecycle stages of blow flies, as in the case of Musca and other non-myiasis producer flies.

5.4 Effect of Bacillus turingiensis

The significant larvicidal effect of the toxin released by *Bacillus thuringiensis* on the lifecycle stages of mosquitoes were worked out by many scientists. But literature on its effect on blow flies are meagre.

The present study revealed that *Bacillus thuringiensis* suspension at 160 to 800 pm concentrations produced moderate effect of mortality on eggs and larvae of myiasis producing flies. Even at the highest concentration, the mortality of eggs ranged from 54 to 74 per cent while 30 to 60 per cent mortality only was obtained in the larval stages in the three different species of flies. The low effect obtained compared to that on mosquitoes, can be attributed to the non-specificity of toxin of *Bacillus thuringiensis* towards the larvae of the targetted species in the present work. This finding is in accordance with Arellano (1990) who gave evidence of moderate toxicity of 50 per cent in the larval stages of *L. cuprina*, on application of *Bacillus thuringiensis* suspension at 600 ppm in the larval culture medium. But this finding is contrary to Korz *et al.* (1977) who observed total mortality in first stage larvae of *M. domestica* and *Stomoxys calcitrans* and absence of any effect noticed by Larget and Barjac (1981) in *M. domestica* larvae.

5.5 Effect of Azadirachtin

Many scientists have reported that the neem ingredient azadirachtin posses significant larvicidal antifeedant and repellant properties in non myiasis producing flies. But its effect on myiasis producing flies is lacking in literature. The present study revealed that azadirectin possessed potent

ovicidal and larvicidal effect at 6 to 15 ppm concentrations in myiasis producing flies. The complete ovicidal effect was obtained at 10.5 ppm concentration irrespective of the species of myiasis producing flies. Total larvicidal effect was obtained at 10.5 to 15 ppm concentrations of azadirachtin. Though Gaaboub and Hayes (1984a) and Miller and Chamberlain (1989) obtained 20 to 100 per cent and 90 per cent mortality in *M. autumnalis* and *M. domestica* larvae at 1 to 100 ppm and 20.2 ppm concentration respectively, complete mortality effect was noted in myiasis producing flies at a lower concentration of 15 ppm. The present finding can also be similated to the observations of Mong Ting Tan and Sudderuddin (1978), Rambold *et al.* (1980), Schmutterer and Rambold (1980), Subrahmanyam (1990), Govindachari (1992) and Mishra (1994) who all noted strong growth disruptive effect of azadirachtin in the larvae of agricultural and livestock infesting pests.

Azadirachtin at 1.5 to 6 ppm concentration significantly reduced the larval meat consumption. The quantity of meat consumed by the larvae reduced with the increase in concentration of azadirachtin irrespective of the species. The reduced consumption of treated meat by the larvae with respect to non treated meat was observed in the decending range of 44.6 to 24 per cent. This can be attributed to the profound antifeedant property of azadirachtin exhibited towards the larval stage of myiasis producing flies. The above

observation is in conformity with the reports of Schmutterer (1976), Warthen (1979), Govindachari (1992) and Mishra (1994) who observed similar predominant effects in agricultural pests. Schmutterer (1988) has also observed 70 per cent reduction in food consumption in *M. domestica* larvae at 3 ppm concentration of azadirachtin.

Azadirachtin also prevented myiasis producing flies from laying eggs. The fecundity of flies reduced considerably with the increase in concentration of azadirachtin. At the concentrations of 1.5 to 6 ppm of the neem ingredient in meat, the percentage of eggs laid by the adult flies with respect to non treated meat descended from 21.4 to 0 per cent. At the highest concentration alone the fecundity ranged from 0 to 7.8 per cent with respect to different myiasis producing flies studied. This denotes that azadirachtin has potent ovipositional deterrent effect in the controlled *in vitro* experiments, due to its significant repellant property. The present finding is in accordance with Schmulterer (1990) who observed 100 per cent ovipositional deterrent effect in *L. serricata* at 5 ppm concentration of azadirachtin. The slight variation in the percentage of the effect observed in the present study may be due to the difference of the species of flies. Though reports on the above mentioned effect in *C. megacephala* and *C. bezziana* flies were lacking in literature, the observation of Rice *et al.* (1985) that

azadirachtin showed mild deterrent effect at 20 ppm and complete effect only at 200 ppm in *L. cuprina* is contrary to the present finding. It was observed that even at 6 ppm concentration of azadir tin. *L. cuprina* flies laid fewer eggs with 96.6 per cent ovipositional deterrence.

A detailed study of the effect of azadirachtin on other parameters relating to meat infesting flies were also undertaken in the present experiment. Though at the lower concentrations of 1.5 and 3 ppm there were slight delay in fly arrival and oviposition with lesser fecundity, at the highest concentration of 6 ppm the fly arival was considerably delayed upto 2 hours without any oviposition or larviposition. Hence it is evident that azadirachtin possessed significant repellant property against meat infesting flies. This finding is supported by Schmutterer (1990) who observed 100 per cent repellant effect in *L. serricata* at 5 ppm concentration of azadirachtin. The present finding can also be similated with that of Govindachari (1992) and Mishra (1994) who observed potent repellant effect of azadirachtin in livestock infesting pests.

Azadirachtin at 1.5 to 6 ppm concentration interfered with the process of pupation also. This was evident from the fact that only 10 to 22.5 per cent of the larvae pupated at the highest concentration. This finding can be compared with that

of Mong Ting Tan and Sudderuddin (1978) and Rambold *et al.* (1980) who observed significant interferrence in pupal formation and further development in agricultural pests.

There was considerable decrease in pupal weight (2 to 19 mgs) and adult fly size by the treatment of azadirachtin in the larval stage. This can be attributed to the antifeedant effect of azadirachtin in the larval phase with resultant formation of small sized pupae and adult flies. Though similar findings were also noted by Gaaboub and Hayes (1984a) who reported significant reduction in pupal weight and adult size in *M. autumnalis* at 0.1 ppm, substantial reduction was obtained only at 6 ppm level in myiasis producing flies in the present study.

Adult development, longevity, fecundity and egg hatch were reduced considerably with increase in concentration of azadirachtin on application to the third stage larvae of myiasis producing flies. The improperly unfolded wings were the only adult deformity noted in all the treatment concentrations. This finding can also be similated to the reports of Gaaboub and Hayes (1984b) who observed 52 per cent inhibition of adult emergence, 85 per cent reduction in fecundity, and 60 per cent reduction in egg hatch at 0.39 ppm of azadir tin in third stage larvae of *M. domestica* though

the concentration of azadirachtin used and the percentage of observation varied with the myiasis producing flies.

Azadirachtin when fed to adult myiasis producing flies caused moderate reduction in longevity and fecundity. Lesser effect was noticed with regard to the percentage of egg hatch and adult development from the eggs laid. The reduction in the total number of eggs laid by myiasis producing flies can be attributed to the reduced longevity of flies. The reports on the effect of azadirachtin on adult myiasis producing flies were not available in literature.

Barring two reports on the antifeedant and ovipositional deterrence on developmental and adult stages of *Lucilia* species, the present work constitutes the first comprehensive study on the potent ovicidal, larvicidal, antifeedant, ovipositional deterrent and repellent effects of azadirachtin in myiasis producing flies.

5.6 Effect of Acorus calamus

The larvicidal and sterility effects of *Acorus calamus* has been demonstrated by many scientists on agricultural pests. But literature is not available on its effect on myiasis producing flies. In the present study, the petroleum ether extract, alcoholic extract and steam distillate of sweet flag *Acorus calamus* at 1.5, 2 and 2.5 per cent concentration

were used to study the ovicidal and larvicidal effects on myiasis producing flies. PEE gave the best results followed by SD and AE. PEE at 2 per cent concentration gave 100 per cent ovicidal effect while 2 to 2.5 per cent concentration were required to produce similar effect in SD. Complete mortality was not obtained with AE. The larvicidal effect on all the stages increased with increase in concentration but lesser susceptibility were noticed in mature larvae than younger ones. PEE at the highest concentration of 2.5 per cent gave 82.5 to 100 per cent mortality with respect to different stages of larvae of myiasis producing flies. Pandey *et al.* (1977) and Deshmukh *et al.* (1982) observed 91 per cent and 100 per cent mortality respectively in *M. domestica* larvae at 0.5 per cent concentration of PEE, while Ahmed *et al.* (1981) found 90 per cent mortality with 1 per cent PEE. Similarly, high mortality effect on larvae of agricultural and livestock infesting pests were also obtained by Chavan *et al.* (1976) and (1979), Sudhakar *et al.* (1978), and Theotia and Pandey (1979). The present finding which concurred with the above reports suggests that petroleum ether extract of Acorus calamus is a potent larvicidal agent though specific information on larvicidal potency in myiasis producing flies is lacking in literature.

The reports on antifeedant effect of extracts of *Acorus calamus* on larvae of any of the livestock infesting flies are

experiments in any of the agricultural and livestock infesting pests has not been recorded in literature.

The repellant property of extracts of *Acorus calamus* against meat infesting flies was studied. The PEE of *Acorus calamus* showed better repellancy than SD and AE and the effect improved with increase in concentration of the extract. At the highest level of 2.5 per cent, the time of fly arrival and oviposition/larviposition delayed by 49 and 125 mts compared to 9 and 15 mts in untreated meat respectively. The flies laid only 17.8 per cent of eggs/larvae on treated meat with respect to untreated meat. Reports by Pandey *et al.* (1977) and Khan and Borle (1985) shows that PEE of *Acorus calamus* at 0.5 per cent concentration produced 82 and 100 per cent repellancy against mustard saw fly and pulse beetle respectively. From the present studies it is confirmed that extracts of *Acorus calamus* and particularly PEE, posses significant repellant property against myiasis producing flies though specific supportive reports on livestock infesting pests are not available in literature.

The three different extracts of *Acorus calamus* on treatment to third stage larvae of myiasis producing flies at 0.1 to 1 per cent concentration reduced the percentage of pupation, adult emergence, adult longevity and fecundity considerably. Though significantly higher effects were

totally laking in literature. In the present study it is observed that extracts of *Acorus calamus* at 0.1 to 1 per cent considerably reduced the meat consumption of larvae. The effect of PEE was more prominent than SD and AE. *Lucilia cuprina* larvae consumed less meat (32.1 per cent) than *C. bezziana* (43.1 per cent) and *C. megacephala* (47.3 per cent) at the highest concentration of PEE. This reveals that PEE of *Acorus calamus* has significant antifeedant activity on myiasis producing flies. The above finding can be similated with reports of Pandey *et al.* (1977) and Sudhakar *et al.* (1978) who observed 72 and 69 per cent antifeedant effect respectively at 0.5 per cent PEE on the larvae of agricultural pest *Athalia proxima*. Banerji *et al.* (1982) also obtained 100 per cent antifeedant effect at 1 to 2.5 per cent concentration in the larvae of above mentioned fly.

Extracts of *Acorus calamus* reduced the oviposition of myiasis producing flies on treated meat. The PEE of *Acorus calamus* gave better result followed by SD and AE. The *Chrysomyia megacephala* flies were found more susceptible than *C. bezziana* and *L. cuprina*. At the highest concentration of 2.5 per cent of PEE, the myiasis producing flies laid 17 to 20.4 per cent eggs when compared to the number of eggs laid by flies in untreated meat. The ovipositional deterrent effect of extracts of *Acorus calamus* in controlled *in vitro*

experiments in any of the agricultural and livestock infesting pests has not been recorded in literature.

The repellant property of extracts of *Acorus calamus* against meat infesting flies was studied. The PEE of *Acorus calamus* showed better repellancy than SD and AE and the effect improved with increase in concentration of the extract. At the highest level of 2.5 per cent, the time of fly arrival and oviposition/larviposition delayed by 49 and 125 mts compared to 9 and 15 mts in untreated meat respectively. The flies laid only 17.8 per cent of eggs/larvae on treated meat with respect to untreated meat. Reports by Pandey *et al.* (1977) and Khan and Borle (1985) shows that PEE of *Acorus calamus* at 0.5 per cent concentration produced 82 and 100 per cent repellancy against mustard saw fly and pulse beetle respectively. From the present studies it is confirmed that extracts of *Acorus calamus* and particularly PEE, posses significant repellant property against myiasis producing flies though specific supportive reports on livestock infesting pests are not available in literature.

The three different extracts of *Acorus calamus* on treatment to third stage larvae of myiasis producing flies at 0.1 to 1 per cent concentration reduced the percentage of pupation, adult emergence, adult longevity and fecundity considerably. Though significantly higher effects were

noticed with application of 1 per cent PEE compared to other extracts in *C. megacephala* and *C. bezziana*, the maximum effect was noted in *L. cuprina* wherein only 5 per cent of the larvae pupated without any subsequent fly emergence. This suggests that PEE of *Acorus calamus* also interferes with the development and performance of myiasis producing flies significantly, when applied to mature larval stages. Reports on the above mentioned effects of extracts of *Acorus calamus* in agricultural and livestock infesting pests are totally lacking in literature.

The finding that extracts of *Acorus calamus*, particularly the PEE produced significant sterility when fed to adult of myiasis producing flies with little effect on their longevity, is the most important observation relating to adult flies. Though PEE at the lowest tested concentration of 0.1 per cent produced total sterility in *L. cuprina*, 0.5 per cent concentration totally inhibitted egg hatching and adult development in *C. bezziana* and *C. megacephala* respectively. Morphological changes were not detected in dissected male reproductive organs but the ovary of the female flies showed partial to complete inhibition in follicle formation on treatment with extracts of *Acorus calamus*. Hence it can be assessed that partial to complete regression of ovarian development is the cause for significant sterility noted in myiasis producing flies. The above findings can be similated

with reports of Mathur and Saxena (1975) who observed 98 to 100 per cent sterility on feeding with 1 per cent oil of *Acorus calamus* in *M. domestica*. Tikku *et al.* (1978) has also observed complete failure of ovarian development with 1 per cent oil of *Acorus calamus* in *Athalia proxima* flies.

The better effect obtained in PEE compared to SD and AE may be due to the increased solubility of the oil present in sweet flag, *Acorus calamus* in petroleum ether with resultant high oil content in the extract. Though few reports were available on the effects of *Acorus calamus* in livestock infesting pests, specific reports on myiasis producing flies are lacking in literature. As such, the present work constitutes the first comprehensive study on the ovicidal, larvicidal, antifeedant, ovipositional deterrant repellant and sterility effects of *Acorus calamus* on myiasis producing flies.

5.7 Effect of gamma irradiation

A comprehensive study was conducted to evaluate the effect of irradiation on eggs, larvae and pupae of myiasis producing flies. Though complete mortality of eggs was obtained at 1000 rads in all the flies, the total mortality of the larvae was noted only at 3000 rads exposure. The pupation, adult emergence, adult longevity, and fecundity were

higher on exposure to mature larvae revealing that younger larvae were more susceptible to irradiation than older larvae. Higher effects of irradiation were noticed in *L. cuprina* than in *C. bezziana* and *C. megacephala*. One day old pupae were found more susceptible to radiation treatment with lesser adult emergence, adult longevity, egg hatch and subsequent adult development than 3 day old pupae.

The best results were obtained on irradiation of 3 day old pupae of myiasis producing flies. Though at 4000 rads exposure a slight reduction of 2 per cent in adult emergence and 1 and 3 to 4 days respectively in the longevity of adult males and females in *C. megacephala* and *C. bezziana* were noted, the flies did not lay any eggs at all. At a lesser dose of 3000 rads, the rate of egg hatch was considerably reduced and adult development was totally arrested. In *L. cuprina*, egg hatch and adult development were completely prevented at the reduced exposure dose of 3000 and 2000 rads respectively suggesting that *L. cuprina* is more susceptible to gamma radiation than *C. megacephala* and *C. bezziana*.

Most of the above findings are well supported by many scientists who studied the effect of radiation in myiasis producing flies. Abdu and Rasik (1975) reported that younger larvae were more susceptible to radiation than older larvae in as they got 62 per cent adult emergence compared to higher

emergence of 78 per cent at 400 rads exposure to 1 and 6 day old larvae respectively in *M. domestica*. Similar observation of higher mortality in younger larvae was also recorded by Srinivasan and Kesavan (1979). Ganeidy *et al.* (1975) also noted higher adult emergence of 85 per cent in 6 day old pupa of *M. domestica* at 5000 rads exposure. Whitfield *et al.* (1978) and Crystal (1979) obtained complete sterility without any other deleterious effect in flies developed from 6 and 3 day old pupae of *Stomoxys calcitrans* and *Cochliomyia hominivorax* after exposure to 2000 and 2500 rads respectively.

The present finding of 91 to 100 per cent reduction in adult development at 2000 rads exposure of 3 day old pupae of myiasis producing flies is comparable to the observation of Abdu *et al.* (1982) who obtained 96.6 per cent reduction at 1500 rads exposure of 2 day old pupae of *M. domestica*. Total sterility obtained in *C. bezziana* flies when 3 day old pupae was exposed to 4000 rads in the present study is in accordance with the findings of Spradberry *et al.* (1983) who also observed total sterility at the similar exposure dose. The findings of Huda *et al.* (1983) that 100 per cent in fecundity observed in *L. cuprina* flies when mature pupae were exposed to 3000 rads is fully in accordance with the present observation.

Altogether it can be assessed from the present study that irradiation of 3 day old pupae at 2000 to 4000 rads does not

affect the adult emergence and longevity compared to younger stages but produced total sterility or arrested development of the hatched eggs completely. This suggests that the above mentioned stage and exposure dose is optimum for irradiation in myiasis producing flies.

5.8 Treatment trials

Diflubenzuron at 5 ppm, azadirachtin at 15 ppm and PEE of *Acorus calamus* at 2.5 per cent concentration, which gave good larvicidal effects in the in vitro studies, were chosen to study their comparative efficacy in natural cases of cutaneous myiasis. Diflubenzuron gave the overall higher maggocide effect of 88.51 per cent, while lower efficacy of only 72.12 per cent in azadirachtin and 51.62 per cent in PEE of *Acorus calamus* were obtained. As reports on treatment trials using the above biopesticide materials are lacking in literature, the finding that diflubenzuron can be employed successfully as a maggocide is the first practical useful finding in the treatment study on cutaneous myiasis.

Summary

SUMMARY

1. Among the 9861 domestic animals screened for cutaneous myiasis in Thrissur, 205 (2.08 per cent) were found positive for the infestation. Though the prevalence extended from October to April, the peak of infestation was noticed in January. Highest incidence was noted in cattle (63.41 per cent) followed by dogs (24.88 per cent) and goats (11.71 per cent). *Chrysomya bezziana* larvae were observed in majority of cutaneous myiasis cases (90.73 per cent) compared to the occasional infestation with the larvae of *Lucilia cuprina* (7.32 per cent) and *C. megacephala* (1.95 per cent).

2. The effect of methoprene on eggs and the respective larval stages of myiasis producing flies at 1 to 50 ppm concentrations was studied. Methoprene at 10 to 30 ppm caused 100 per cent mortality on eggs, but lesser mortality was noticed in the larval stages. This hormone analogue at the highest concentration of 50 ppm prolonged the second and third larval phase by 10 and 16 hours respectively. It also affected the larval pupal transformation by forming 5 to 55 per cent and 10 to 60 per cent larval pupal intermediaries when applied to second and third stage larvae. There was also an increase upto 1.5 mm in the length of pupae at the highest

concentration. The most important observation was that methoprene at 30 ppm concentration totally prevented the adult emergence from the pupae, indicating that the analogue significantly interfered with pupal metamorphosis. Methoprene also produced high percentage of morphological deformities like failure of the fly to crawl out of the puparium, incomplete regression of ptilinal sac, absence of metallic colouration on the body, partial or completely folded wings and abnormally flexed legs. It also considerably reduced the longevity, fecundity, egg hatch and subsequent adult development in myiasis producing flies.

3. The effect of diflubenzuron on eggs and 3 larval stages of myiasis producing flies at 0.5 to 10 ppm concentrations was studied. Though the material caused little ovicidal effect, significant larvicidal effects of 55 to 100 per cent were noted at 0.5 to 5 ppm concentrations. Moreover the chitin content of treated larvae was reduced from 52.11 to 18.42 per cent with increase in concentration of diflubenzuron. The chitin deficiency also caused severe damage and rupture of larval cuticle. In lower concentration, abnormally thin shelled pupa, reduction in longevity, fecundity, egg hatch and subsequent adult development were also noted.

4. *Bacillus thuringiensis* var *israelensis* at 160 to 800 ppm concentration exhibited a moderate effect of 54 to 74 per cent mortality on eggs and 30 to 60 per cent mortality on different stages of the larvae of myiasis producing flies.

5. The ovicidal and larvicidal effect of azadirachtin (neem ingredient) were studied at 6 to 15 ppm concentrations in myiasis producing flies. The total ovicidal and larvicidal effects were noted at 10.5 and 10.5 to 15 ppm concentrations respectively. Significant antifeedant effect in the larvae observed was at 1.5 to 6 ppm concentrations of azadirachtin, wherein the consumption of treated meat was reduced from 44.6 to 24 per cent. Considerable reduction in fecundity (0 to 7.8 per cent) in myiasis producing flies and total repellancy against meat infesting flies were also noticed at 6 ppm concentration of azadirachtin. The treatment with azadirectin also reduced pupal weight, adult longevity and egg hatching considerably.

6. Petroleum ether extract of *Acorus calamus* produced significant deleterious effects on the developmental stages of myiasis producing flies compared to that of steam distillate and alcoholic extract. PEE of Acorus calamus at the highest concentration of 2.5 per cent

produced 82.5 to 100 per cent mortality of the larvae, reduced the oviposition by 79.6 to 83 per cent, and also reduced the deposition of eggs and larvae (82.2 per cent) by the meat infesting flies on treated meat. The most important effect was that PEE at 0.1 to 1 per cent concentrations produced 100 per cent sterility in myiasis producing flies by interfering in the development of ovarian follicles.

7. A comprehensive study to evaluate the effect of radiation on eggs, larvae and pupae of myiasis producing flies was conducted. The complete mortality of eggs and larvae was obtained at 1000 and 3000 rads exposure respectively. Three day old pupae of myiasis producing flies at 2000 to 4000 rads exposure gave total sterility without any deleterious effect on adult emergence or life span.

8. In the practical study to choose the most effective biopesticide in natural cases of cutaneous myiasis, diflubenzuron at 5 ppm concentration caused 88.5 per cent mortality of the larvae whereas azadirachtin and petroleum ether extract of *Acorus calamus* produced 72.12 and 51.62 per cent effect respectively proving that diflubenzuron is the compound of choice for obtaining better results.

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THE EFFECT OF CERTAIN BIOPESTICIDES AND IRRADIATION ON THE DEVELOPMENTAL STAGES OF MYIASIS PRODUCING FLIES

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ABSTRACT OF A THESIS

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ABSTRACT

A study was undertaken on the prevalence of cutaneous myiasis in domestic animals and its control using bio-pesticides and gamma irradiation.

The prevalence of cutaneous myiasis in domestic animals was found to be 205 (2.08 per cent) among the 9861 animals screened. The peak of infestation was noted in the month of January. In host-wise and parasite-wise the highest incidence was noted in cattle (63.41 per cent) and the majority of infestation was produced by *Chrysomya bezziana* larvae (90.73 per cent). Methoprene at 1 to 50 ppm concentration caused only moderate mortality on larvae but significantly increased the mortality rate on eggs, prolonged the larval phase, increased the formation of larval pupal intermediaries and adult deformities and reduced the adult emergence. Diflubenzuron at 0.5 to 5 ppm caused 55 to 100 per cent larvicidal effect due to lowered chitin content of 18.42 to 52.11 per cent in larval cuticle. *Bacillus thuringiensis* var *israelensis* produced only moderate larval mortality at 160 to 800 ppm in myiasis producing flies. Azadirachtin at 10.5 to 15 ppm produced 100 per cent mortality in eggs and larvae. Significant antifeedant, ovipositional deterrent and repellant effects were also produced by Azadirachtin. Among the extracts of *Acorus calamus*, studied, petroleum ether extract at 2.5

per cent concentration gave 82.5 to 100 per cent mortality of the larvae. Moderate antifeedant, ovipositional deterrent and repellant effects were also noticed. The petroleum ether extract produced 100 per cent sterility at 0.1 to 1 per cent concentration by preventing the development of ovarian follicles. Three day old pupae of myiasis producing flies exposed to Γ-rays gave excellent sterility effect at 2000 to 4000 rads radiation exposure without any other deleterious effect in the flies. Diflubenzuron at 5 ppm concentration showed the highest larvicidal effect (88.5 per cent) in natural cases of cutaneous myiasis.