

TOXIC AND GROWTH REGULATING EFFECT OF ETHANOL EXTRACT AND PETROLEUM ETHER EXTRACT OF *Valariana officianalis* L. AGAINST *Bactrocera zonata* Saunder

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ABSTRACT

Ethanol extracts and Petroleum ether extract of *Valariana officianalis* L. at 1000ppm, 500ppm and 250ppm were evaluated for their toxic and growth inhibiting effects against *Bactrocera zonata* Saunder at Insect Pest Management Program, Institute of Plant and Environmental Protection, National Agriculture Research Center, Islamabad.

Mortality in the flies was 25%, 48.66% and 61.67% when fed on diet containing 1000ppm ethanol extract of *V. officianalis* for 10, 15 and 20 days respectively, which were significantly greater than 8.33%, 12.00%, and 16.67% mortality in the flies fed with normal diet. Toxic effect of the tested material at 500ppm was also apparent, as 23.33, 27.33 and 31.33 mortality after 10, 15 and 20 days feeding respectively, was statistically more than that in the control. Extracts at 250ppm did not affect the mortality of the test insect significantly. Similar results were obtained when flies were fed on diet with different concentrations of petroleum ether extract of *V. officianalis*. Diet with 250ppm petroleum ether extract did not show any significant toxic effect; however, the extract at 500ppm and 1000ppm significantly reduced the population of fruit flies when fed for 10, 15, and 20 days.

When flies were fed on diet with 1000ppm and 500ppm ethanol extract of *V. officianalis*, significantly lower number of pupae were produced. The diet with 250ppm, although, did not effect the pupal production, it significantly reduced the adult emergence as compared to that in control. Petroleum ether extract of *V. officianalis* at 1000ppm, 500ppm and 250ppm in diet significantly prohibited the pupal formation, however, only the 1000ppm and 500ppm extracts in diet reduced the adult emergence significantly.

INTRODUCTION

In Pakistan fruits and vegetables are grown on large area and are used not only for local consumption but are exported as well. Some of our fruits like mango because of their delicious nature and quality have a high demand in different part of the world (Irshad and Jilani, 2003). Fruits and vegetables provide essential nutritional elements of the consumer body. A lot of progress has been made in the production and quality, still there is a scope of improvement of this crop sector.

Besides, climatic conditions, supply of inputs, supply/demand and price structure; insect pests play important role in the regulation of quality and yield production of fruits and vegetables. Among the insect pests of fruits and vegetables, flies are of special importance. Out of 24 different fruit flies species in different part of the world, 11 are widely distributed in Pakistan (White and Harris, 1992). They cause colossal losses to fruits and vegetables in tropical, subtropical and temperate regions of the world (Bateman, 1972). Flies feed on the pulp of fruit and make it unfit for human consumption, therefore, cause 25-50% losses every year (Qureshi *et al.*, 1976). The fruit fly larvae in the pulp of fruit if eaten cause intestinal discomfort and diarrhoea (Hashim, 1994).

In Pakistan fruit flies are mainly controlled by synthetic chemicals for their quick knock down effect. But because of the several ill-effects of these chemicals to man and other non-target organisms, the natural plants compounds are gaining importance in the management of insect pests for their safe and friendly nature. In fruit orchards, the use of synthetic chemicals have also caused out break of population of other insect pests like, scales on mango due to killing of their natural enemies (Mohayuddin 1989).

Plants rich in bioactive chemicals may provide potential alternative to currently used insect controlling agents. There are 34 local plants including harmal, kuth, Balcher and neem, which carry repellent, anti-feedant qualities against stored grain insect pests (Jilani *et al.*, 1989).

Therefore, studies were undertaken to evaluate the toxic and growth inhibitory effects of ethanol and Petroleum ether extracts of Balcher (*Valariana officianalis*) against Peach fruit fly (*Bactrocera zonata*).

METHODS AND MATERIALS

To test the toxic and growth inhibiting effect of ethanol and petroleum ether extracts of *Valariana officianalis* on *Bactrocera zonata*, an experiment was conducted at

Insect Pest Management Program Institute of Plant and Environmental Protection, National Agricultural Research Center, Islamabad.

Raising Fruit flies culture

The laboratory reared fruit flies *Bactrocera zonata* culture was maintained at controlled temperature with $27 \pm 1^{\circ}\text{C}$ temperature, $60 \pm 5\%$ relative humidity and 14:10 light and dark. Fruit flies were reared on artificial diet in fruit fly rearing cage (18 x 16 x 16) having muslin cloth on the two opposite sides. The other two sides top and bottom were made of transparent glass sheet. On alternate day, fresh fruits were offered to adult flies for oviposition for 48 hours. The infested fruits were placed in glass jars having sand at the base for pupation. To allow aeration these jars were covered with muslin cloth. After pupation, these were separated from sand by 20 mesh sieve. Pupae were placed in rearing cages with cotton soaked in water in Petri dishes to avoid desiccation. After emergence of flies, proteinaceous diet was provided on daily basis. In this way fresh and uniform age adult flies were available for experimentation.

Constituents of the artificial diet for adult flies

| <u>Ingredient</u> | <u>quantity</u> |
|--------------------|-----------------|
| Bananas | 2 No |
| Egg yolk | 6 No |
| Honey | 4 table spoon |
| Leaderplex vitamin | |
| B. Complex syrup | 2 table spoon |
| Yeast | 1 table spoon |
| Sugar | 8 table spoon |

The ingredients were mixed in a blender to make a thick syrup solution. The diet was poured in Petri dishes and kept in a refrigerator for further use in the experiment.

Preparation of Plant extracts

Roots of *Valeriana officianalis* were purchased from an ayurvedic shop in Rawalpindi. The plant material was ground in to fine 60 mesh powder. The plant powder were separately extracted with ethanol and Petroleum ether (60-80 $^{\circ}\text{C}$) in Soxhlets extraction apparatus concentrated in rotary evaporator and finally made solvent free in a vacuum desiccator. Since plant extracts are insoluble in water, therefore, a small quantity of detergent (Surfactant) was added in distilled water. To get the required concentration either in percentage or ppm, known amount of plant extract was taken and dissolved in the distilled water with detergent by thoroughly stirring. In this way a homogenous solutions were prepared.

Toxic and growth inhibiting effect of *Valeriana officianalis* extracts on peach fruit fly

Ethanol and Petroleum ether extracts of *V. officianalis* at 1000ppm, 500ppm and 250ppm were mixed separately in the artificial diet. Seventy five pairs of freshly emerged fly adults were released for 20 days in the rearing cages having either 1000ppm, 500ppm or 250ppm mixed artificial diet. The same number of fruit flies were also released in the normal diet to be used as control. Each treatment was replicated three times. Dead flies in each treatment after 5 days, 10 days, 15 days and 20 days were counted and the data recorded. The data were analyzed with analysis of variance (ANOVA) and means separated with Least Significant Difference (LSD). Eight pairs of flies survived after feeding for consecutive 20 days in each treatment were collected and released on separate rearing cages. Fresh loquat (*Eriobotrya japonica*) fruits were offered to these flies for oviposition. Each treatment in the experiment was replicated three times. After 48 hours fruits were removed from the cages and placed in plastic jars having sand at the base for pupation. These jars were properly labeled and covered with muslin cloth and placed in the lab. Water soaked cotton was also placed on the top of jar to prevent pupal desiccation. The number of pupae formed and the adult emerged were counted. The data recorded during these trials were analyzed with analysis of variance (ANOVA) and means were separated with Least Significant Difference (LSD).

RESULTS

Toxic Effect of *Valeriana officianalis* Extracts against *Bactrocera zonata* Ethanol Extract

Mortality caused by 20 days feeding the flies on diet containing ethanol extract of *V. officianalis* at different concentrations is given in Table-1. It revealed that their was only 4.33%, 8.33%, 12.00% mortality in flies when fed on normal diet for 5, 10 and 15 days respectively, which reached to 16.67% after 20 days. In the flies fed on diet containing 1000ppm of *V. officianalis* extract, mortality (6.67%) after 5 days feeding was not significantly different from that in the normal diet; however, mortality increase to 25.33%, 48.67% and 61.33% when flies were fed on the same diet for 10, 15 and 20 days, which were statistically higher than that in the control. When flies were fed on diet containing 500ppm extract of *V. officianalis* for 5, 10, 15 and 20 days respectively, the mortality except in after 5 days feeding was significantly higher than that in the flies fed on normal diet. Diet with 250ppm extract of *V. officianalis* showed no significant toxic effect to the test insect.

Table-1. Mean Percent Mortality of Fruit fly *Bactrocera zonata* after day-wise feeding on diet with various concentrations of ethanol extracts of *Valeriana officianalis* in a cage containing 75 pairs of fruit fly

| Treatment | Percent Mortality of Fruit flies after Feeding for | | | |
|-----------|--|--------|--------|--------|
| | 5days | 10days | 15days | 20days |
| Control | 4.33A | 8.33A | 12.00A | 16.67A |
| 250ppm | 6.67A | 9.33A | 13.33A | 17.33A |
| 500ppm | 7.33A | 23.33B | 27.33B | 31.33B |
| 1000ppm | 6.67A | 25.33B | 48.67C | 61.33C |
| LSD | 3.052 | 5.469 | 3.346 | 6.628 |

Each value is a mean of three replications. Mean values among the columns followed by the same letters are not significantly different at $\alpha = 0.05$

Petroleum Ether Extract

Mortality of the fruit flies caused by feeding diet with and without petroleum ether extract of *V. officianalis* at different concentrations is given in Table-2. There was negligible mortality of fruit flies fed on normal diet for 5 days. This number slightly increased to 6.67% after 10 days, 9.67% after 15 days and 10.00% after 20 days feeding. When flies were exposed to diet with 1000ppm petroleum ether extract of *V. officianalis*, the mortality reached to 12.67% after 5 days, 26.00% after 10 days, 39.33% after 15 days and 54.67% after 20 days. This reduction in the population of the flies was statistically more than that in the control. Diet containing 500ppm petroleum extract of the test plant only showed significant toxic effect when flies were fed for 15 and 20 days. Feeding diet by flies containing 250ppm plant extract resulted into 5.00%, 8.67%, 12.67% and 13.00% mortality after 5, 10, 15 and 20 days which was almost similar to the mortality of flies in the control.

Table-2. Mean Percent Mortality of Fruit fly *Bactrocera zonata* after day-wise feeding on diet with various concentrations of Petroleum Ether extracts of *Valeriana officianalis* in a cage containing 75 pairs of fruit fly

| Treatment | Percent Mortality of Fruit flies after Feeding for | | | |
|-----------|--|---------|---------|---------|
| | 5days | 10days | 15days | 20days |
| Control | 3.00 A | 6.67 A | 9.67 A | 10.00 A |
| 250ppm | 5.00 AB | 8.67 A | 12.67 B | 13.00 A |
| 500ppm | 7.67 B | 17.33 A | 31.33 C | 42.00 B |
| 1000ppm | 12.67 C | 26.00 B | 39.33 D | 54.67 C |
| LSD | 4.583 | 9.206 | 2.484 | 6.136 |

Each value is a mean of three replications. Mean values among the columns followed by the same letters are not significantly different at $\alpha = 0.05$

Growth regulating/inhibition effect of *Valeriana officianalis* extracts against *Bactrocera zonata*

Ethanol Extract

When 8 pairs of a uniform age adults of the surviving peach fruit flies collected from each treatment after 20 days feeding were allowed for oviposition on fresh

loquat, the number of pupae produced were only 32.33 and 44.00 in those fed on diet with 1000ppm and 500ppm ethanol extract of *V. officianalis*, which were significantly lower than the 93.00 pupae in the control (Table-3), percent pupal inhibition was 65.23 and 52.68, respectively. The number of adult emergence at 1000ppm and 500ppm were significantly lower than those in the control. Percent adult inhibition was 76.46, 55.88 and 21.84, respectively. Ethanol extract of *V. officianalis* at 250ppm, although did not effect the pupal production of the test insect, it significantly reduced the adult emergence of the flies as compared to that in the control. Morphological changes were also noticed in the developing immature stages of flies.

Table-3. No. of Fruit fly *Bactrocera zonata* pupae and adults obtained and % Inhibition by feeding various concentrations of Ethanol extract of *Valeriana officianalis* in cage containing 8 pairs of *Bactrocera zonata*

| Treatment | Pupae | % Pupal Inhibition | Adult | % Adult Inhibition |
|-----------|---------|--------------------|---------|--------------------|
| 1000ppm | 32.33 B | 65.23 | 18.67 D | 76.46 |
| 500ppm | 44.00 B | 52.38 | 35.00 C | 55.88 |
| 250ppm | 74.00 A | 20.43 | 62.00 B | 21.84 |
| Control | 93.00 A | | 79.33 A | |
| LSD | 20.91 | | 15.17 | |

Each value is a mean of three replications. Mean in columns followed by the same letters are not significantly different at $\alpha = 0.05$

Petroleum Ether Extract

The number of pupae produced in guava by 8 pairs of a uniform age adult fruit flies survived after 20 on diet containing 1000ppm, 500ppm and 250ppm petroleum ether extract of *V. officianalis* were significantly lower than the pupae in the control (Table-4). The pupal inhibition recorded was 79.00%, 60.1% and 25%, respectively. The adult flies emergence at 1000ppm, 500ppm were 9.00 and 24.67, respectively; which were statistically lower than the 75.00 adult emergence in the control. Unlike the 250ppm ethanol extract of *V. officianalis*, the 250ppm petroleum ether extract, although, did not affect the pupal production of the flies, it significantly reduced the adult emergence as compared to those in the control.

The percent adult emergence inhibition of adult flies at 1000ppm and 500ppm were 88 and 67.11, respectively.

Table-4. No. of Fruit fly *Bactrocera zonata* pupae and adults obtained and % Inhibition by feeding various concentrations of Ethanol extract of *Valeriana officianalis* in cage containing 8 pairs of *Bactrocera zonata*

| Treatment | Pupae | % Puapl Inhibition | Adult | % Adult Inhibition |
|-----------|---------|--------------------|---------|--------------------|
| 1000ppm | 19.67 C | 79.00 | 9.00 B | 88 |
| 500ppm | 37.33 C | 60.1 | 24.67 B | 67.11 |
| 250ppm | 70.00 B | 25 | 55.33 A | 24.89 |
| Control | 93.67 A | | 75.00 A | |
| LSD | 9.80 | | 20.03 | |

Each value is a mean of three replications. Mean in columns followed by the same letters are not significantly different at $\alpha = 0.05$

DISCUSSION

In the management of fruit flies, major emphasis had been on the use of insecticides. Out of the total insecticides used in Pakistan about 10% is applied against this insect pest. Because of the various ill effects of these conventional insecticides, plant derivatives for the management of insect pests are gaining importance due to their friendly nature to the non-target organisms. It is obvious from the present studies that *Valeriana officianalis* has potential to be used for the management of fruit flies. The ethanol and petroleum ether extracts had significant toxic and growth inhibiting effects on Fruit fly at 500ppm and 1000ppm concentrations.

Similar results were shown by Chander (1999), he observed that extracts of sweetflag kuth root, turmeric, and nimbidine were highly effective at 2.5-3.12 mg/25cm² Jute fabric against *T. castaneum*. Van Randen *et al.* (1998b) observed a decrease survival of adult and negative effect on the development of eggs when fruit fly (*Rhagoletis indifferens*) ingested azadirachtin in food. Longevity of *Bactrocera cucurbitae* fed continuously on sugar treated with 0.15% extract of *Acorus calamus* at 1ml/g sugar was 26.6 days than the 119.2 days for untreated flies. Hassan (1998) evaluated neem seed kernel extract and 7% azadirachtin against the developmental stages of Queensland fruit fly on persimmon. Result showed that high proportion of adults emerging from the treated pupae showed wing deformation preventing them from flying. Van Randen and Roitberg (1998a) found that incorporation of neem based insecticides in artificial larval diet resulted in a significant decrease in the formation of pupae and subsequent adult emergence of the Western fruit fly. The significant effect of neem volatiles & eucalyptus on the post-embryonic development & reproduction of *Corcyra cephalonica* has also been noticed by Pathak and Krishna (1991). Shakunthala and Thomas (2001) observed remarkable changes in the size and morphology of reproductive organs of adult flies when *A. calamus* extract were administered to flies through food from the day of emergence. Jilani *et al.* (1990) have shown strong growth inhibiting effect of neem, turmeric and sweetflag oil against some major insect pests of stored grains.

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