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Effect of *Melia* extracts on oviposition, gonad development and field infestation of melon fruit fly (*Bactrocera tau*) in cucumber (*Cucumis sativus*)

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ABSTRACT

Studies on the effect of darek (*Melia azedarach* L.) seed kernel extract (DSKE) in aqueous and bait forms on the oviposition, gonad development and field infestation of melon fruit fly (*Bactrocera tau* walker) in cucumber (*Cucumis sativus* L.) revealed that of the treatments evaluated under laboratory conditions, 2% aqueous DSKE gave maximum oviposition deterrence (79.1%) after 24 hr of treatment over control. The deterrent effect decreasing with time lasted for 168 hr. In bait treatments, mean number of eggs laid at 24 hr intervals after 144 hr was minimum (8.1 eggs/five females) in fruits treated with Bait IV (DSKE 2% + diet) as compared to 11.0 eggs in standard malathion bait and 24.3 eggs in control/24 hr. Upon dissection, it was found that fruit flies fed on Bait IV had significantly under developed ovaries measuring 1.9±0.06 × 1.7±0.03mm in size against 3.4±0.08 × 2.8±0.04mm in those fed on normal proteinaceous diet. Moreover, baits carrying higher concentration of DSKE than in Bait IV caused significant adult mortality. Among the concentrations tested, Bait VIII (DSKE 6.0% + diet) caused maximum mortality (59.1%) 24 hr after treatment. Under field conditions, fruit fly infestation was lowest (3.1%) in plots sprayed with Bait X (DSKE 12.0% + diet) against 11.1 and 64.8% in standard bait sprayed plot and the untreated, respectively. No phyto-toxic effects were observed. The *Melia* bait sprays proved more effective than standard malathion bait in the management of melon fruit fly and increasing the total crop yield.

Key words: *B. tau*, Bait, Cucumber, Extract, Gonads, *Melia azedarach*, Oviposition

Cucurbits such as cucumber, gourds, melons, pumpkin etc. comprise an important group of summer vegetables grown in India and several other countries of the world. According to FAO estimate, India's total fresh vegetable production was 27 480 800 metric tonnes of which cucurbits account for 5.6% covering a ground of 4 290 000 ha (FAO 2006). Thus, these are the crops of great economic value. For cucurbits, fruit fly attack is the major limiting factor in obtaining good quality produce and high yields. In India, *Bactrocera cucurbitae* (Coquillett) is more prominent than the other fruit fly species like *Bactrocera ciliatus* Loew. and *B. tau* (Walker) which have been noticed recently (Kapoor 2004). However in the Western Himalayan state of Himachal Pradesh (India), *B. tau* is the most serious pest than any other fruit fly species on cucurbits.

Since the fruit fly lays eggs inside the fruit and maggots feed internally, it is very difficult to control the pest with direct insecticidal sprays. Previously various methods have been suggested for the management of this pest but successful control is still eluding. Moreover, the future environmental thrusts call for ecologically compatible pest management programme. Therefore, there is need to explore and develop some alternative measures that will supplement the IPM programme for this notorious pest and at the same time, are environmentally safe also. In the light of above facts, present studies were undertaken to investigate the effect of extracts of *Melia azedarach* L. on oviposition and gonad development of the melon fruit fly, (*B. tau*) and their subsequent field evaluation on cucumber (*Cucumis sativus* L.).

MATERIALS AND METHODS

Fruit fly infested cucumber fruits were brought from the field to laboratory and were kept in specially designed rearing cages (90 × 45 × 45cm). The cages were fitted with a removable tray at the base filled with sieved and sterilized sand up to a height of 25cm. The adult flies thus emerged were identified as *B. tau* on the basis of morphological characters and provided with protein food and sugar solution (30%). Food was replaced every third day. Peeled cucumber fruits were provided as substrate for egg laying by the mature females. Legs of the rearing cage were placed in water filled

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cups so as to avoid the menace of predatory ants which may affect the strength of fruit fly culture.

The fruits of *Melia azedarach* L. (Meliacae) commonly referred to as darek or Chinaberry tree, a relative of neem (*Azadirachta indica* A. Juss) collected locally were soaked in water overnight. Peel and pulp were removed and kernels were extracted from the seed manually. The extracted kernels were shade dried and powdered.

The aqueous darek seed kernel extract (DSKE) was prepared. A stock solution of 20% strength was prepared by dissolving 20g darek seed kernel powder in small amount of distilled water and filtered through Whatman filter paper No. 1. Total volume was made 100 ml. Further dilutions were made using distilled water by single dilution method. Different bait combinations containing active ingredient + diet were prepared. These included one standard protein bait containing malathion (0.05%) and others containing aqueous DSKE. The diet was prepared by mixing protinex and gur (jaggery) (1:1) and yeast (5%). To prepare a standard malathion bait, 0.1 ml of malathion (commercial formulation 50 EC) was added to this mixture. Total volume of the final solution was made 100 ml with distilled water. Solution was stirred with a glass rod to dissolve the contents completely. For the preparation of *Melia* bait of concentration 1% aqueous DSKE, 1.0g darek seed kernel powder was soaked in distilled water for 12 hr and was processed further as described before. This extract was added to the diet mixture to make the total volume 100 ml. Similarly, other baits of different DSKE concentrations were prepared in required volumes.

Under laboratory conditions, the immature green cucumber fruits were sprayed finely with the aqueous extracts and *Melia* baits as per the treatment under consideration. One shade-dried treated fruit was offered to five pairs of *B. tau* kept in testing cages. Five such replications per treatment were kept. The fruits were dissected at the sites of oviposition punctures and eggs were counted under the microscope. The per cent oviposition deterrence was calculated as below:

$$\text{Oviposition deterrence (\%)} = \frac{\text{Eggs laid in control-}\ \text{Eggs laid in treatment}}{\text{Eggs laid in treatment}} \times 100$$

In laboratory treatments DSKE in aqueous form was tested @ 1.1, 1.5 and 2.0% concentrations and in bait form as Bait II (DSKE 1.0% + diet), Bait III (DSKE 1.5% + diet) and Bait IV (DSKE 2.0% + diet). Bait I, the standard malathion bait was also tested for comparison. Out of these treatments, the most effective bait treatment (Bait IV as found later) was further subjected for evaluation of its effect on the development of fruit fly gonads. Female fruit flies were fed on Bait IV continuously for one week. Thereafter, the size of gonads was measured by dissecting them under the microscope. Similar experiment was conducted with the fruit flies fed on normal proteinacious diet. The difference in size of the ovaries was recorded. Concentrations higher than Bait IV caused adult mortality therefore Bait V (DSKE 3.0% + diet), Bait VI (DSKE 4.0% + diet), Bait VII (DSKE 5.0% + diet) and Bait VIII (DSKE 6.0% + diet) were tested for their insecticidal effect on similar lines and the mortality was calculated as:

$$\text{Mortality (\%)} = \frac{\text{Number of dead files}}{\text{Total number of flies}} \times 100$$

Under field conditions, the treatments were tested on cucumber cultivar- Khira 90 which is moderately resistant to fruit fly attack (Sharma *et al*. 2010). Higher concentrations of DSKE were used since the plant extracts are susceptible to photo-degradation and also the pest population tends to be more heterogeneous or variable. The field evaluation consisted of sprays of Bait I, Bait VIII, Bait IX (DSKE 8.0% + diet), Bait X (DSKE 12.0% + diet) and aqueous DSKE (5% concentration). Each treatment was evaluated in three randomly selected plots with each plot having equal number of plants. One experiment was kept as control where no spray was done. Sprays were repeated at 10-day intervals and observations were recorded for per cent fruit fly infestation. Simultaneously, their effect on crop yield was also studied. The fruit picking was also done at 10-day intervals. The data recorded were subjected to statistical analyses so as to confirm the significance of results obtained.

RESULTS AND DISCUSSION

Oviposition deterrent effect

Studies on the deterrent effect of aqueous DSKE revealed (Table 1) that after 24 hr of treatment the per cent deterrence over control was 40.6, 53.1 and 79.1% at 1.0, 1.5 and 2.0% concentration respectively. More or less same trend continued up to 72 hr of treatment. At all concentrations deterrent effect of DSKE decreased with time and after 168 hr of treatment no deterrence was found at 1.0% concentration while at 1.5 and 2.0% concentration only 0.3 and 1.3% deterrence was recorded. The mean deterrence after 168 hr was 22.2, 29.1 and 40.5% at 1.0, 1.5 and 2.0% concentrations respectively. All treatments differed significantly from each other, aqueous DSKE @ 2% being most effective.

Data recorded on the effect of *Melia* poison baits on fruit fly oviposition revealed (Table 2) that after 24 hr of treatment minimum egg laying of 12.0 eggs/five females was recorded in Bait IV (DSKE 2.0%), whereas in standard malathion bait (Bait I) 21.0 eggs were recorded. Untreated control recorded an egg laying of 32.0 eggs. Minimum oviposition of 5.0 eggs was observed in Bait IV after 120 hr of treatment against 6.7 in standard malathion bait and 20.0 eggs in control. After 144 hr of treatment, the mean oviposition in different bait treatments was 8.1, 9.0, 11.0 and 16.8 eggs in Bait IV, Bait III, Bait II and Bait I respectively against 24.3 eggs in control. Bait IV proved to be significantly superior to all the treatments tested.

Table 1 Oviposition deterrent effect of aqueous darek seed kernel extract (DSKE) over control against *Bactrocera tau*

	Per cent oviposition deterrence over control after indicated hours of treatment							
DSKE conc. (%)	24	48	72	96	120	144	168	Mean
1.0	40.6	37.9	37.0	36.8	20.0	5.3	0.0	22.2
1.5	53.1	49.3	48.2	41.4	28.3	10.5	0.3	29.1
2.0	79.1	70.7	69.3	59.2	30.0	12.3	1.3	40.5
Mean	57.6	52.6	51.5	45.8	26.1	9.4	0.5	

CD (*P*=0.05)
Concentration 0.66, hours 1.01, concentration × hr 1.24

Table 2 Effect of poison baits containing darek seed kernel extract on egg laying of *Bactrocera tau*

	Mean oviposition after indicated hours of treatment						
Teatment	24	48	72	96	120	144	Mean
Bait I (Malathion 0.05%)	21.0	12.7	12.7	6.7	6.7	6.0	11.0
Bait II (DSKE 1.0%)	22.7	16.3	15.7	13.3	15.3	17.7	16.8
Bait III (DSKE 1.5%)	13.0	12.0	8.7	5.0	5.3	9.7	9.0
Bait IV (DSKE 2.0%)	12.0	7.0	8.3	6.0	5.0	10.3	8.1
Control	32.0	29.0	27.0	19.0	20.0	19.0	24.3
Mean	20.1	15.4	14.5	10.0	10.5	12.5	

CD (*P*=0.05)
Treatment 0.97, hours 1.1, Treatment × hours 1.7

Effect of Melia *poison baits on gonad development*

In fruit flies fed on normal proteinaceous diet, a fully developed set of ovaries was observed upon dissection which measured to be 3.4±0.08mm in length and 2.8 ± .04mm across its breadth. On the other hand, the size of the ovaries was much reduced in fruit flies fed on the poison bait measuring only 1.9 ± 0.06mm in length and 1.7 ± 0.03mm in width (Table 3). Fig 1 well confirms the fully developed ovaries dissected from female fed on normal diet compared to the under developed ovaries dissected from the females fed on Bait IV, thus a very conspicuous effect of *Melia* poison baits on the gonad development of fruit flies was observed.

Fig 1 Normally developed fruit fly gonads (centre) and those (sides) of fed on *Melia* bait (DSKE 2% + diet)

Insecticidal effect of Melia *poison baits*

Concentrations above Bait IV caused adult mortality in fruit flies. An experiment was therefore, performed to determine the insecticidal properties of *Melia* poison baits against *B. tau* which revealed that after 24 hr of treatment, the adult mortality recorded was 16.7, 25.0, 46.7 and 59.1% in treatment consisting of Bait V (DSKE 3%), Bait VI (DSKE 4%), Bait VII (DSKE 5%), and Bait VIII (DSKE 6%), respectively as compared to 7.7% natural mortality in control (Table 4). After 48 hr of treatment, the per cent fruit fly mortality decreased to 13.3, 23.3, 40.0 and 53.3% in treatment consisting of Bait V, Bait VI, Bait VII and Bait VIII respectively. Similar trend continued and adult mortality decreased to 10.0, 23.3, 30.0 and 43.3% in Bait V, Bait VI, Bait VII and Bait VIII respectively after 72 hr of treatment. The mean mortality at 24 hr intervals after 144 hours of treatment was 7.7, 17.8, 27.2 and 39.8% in Bait V, Bait VI, Bait VII and Bait VIII respectively as compared to 2.2% mortality in control. The treatments differed significantly from each other. It was observed that effectiveness of baits was concentration dependent and decreased with time.

Table 3 Effect of poison baits on gonad development of female *Bactrocera tau*

Treatment	Length (mm) ± SE*	Breadth (mm) ± SE*
Bait IV (DSKE 2.0%)	1.9 ± 0.06	1.7 ± 0.03
Control	3.4 ± 0.08	2.8 ± 0.04

SE = Standard error, * each value is mean of seven replications

Effect on field infestation of B. tau

A perusal of data recorded is presented in the Table 5 which states that the infestation was 13.9, 4.9, 3.1, 11.1 and 6.4% in plots sprayed with Bait VIII, Bait IX (DSKE 8%), Bait X (DSKE 12%), Bait I and aqueous DSKE (5%), respectively whereas in control about 64.8% fruits were found infested. Thus, the minimum infestation was recorded in plots sprayed with Bait X, followed by those sprayed with Bait IX. During the course of four sprays of different poison baits, the infestation ranged from literally 0.0% (Bait X) to a maximum of 20.1% (Bait I). In plots sprayed with Bait VIII, the per cent fruit fly infestation ranged between 11.1 and 16.7%. In plots sprayed with Bait IX and Bait X, it varied between 0.0 to 8.3% and 0.0 to 6.7%, respectively. In treatment comprising Bait I, the fruit fly infestation ranged between 11.1 and 20.0% and in case of aqueous DSKE, it ranged between 0.0 to 10.0%. Whereas in control untreated plots, the infestation ranged between 60.0 and 70.1%. All the treatments were found to differ significantly from each other at 0.05% level of significance.

Effect on total crop yield

The total yield in three plots treated with Bait VIII ranged from 12.82 to 13.18 tonnes/ha. In the plots sprayed with Bait IX, the total yield ranged between 13.94 and 14.75 tonnes/ha which significantly increased in plots sprayed with Bait X ranging between 14.62 and 15.42 tonnes/ha. The total yield in plots sprayed with standard malathion Bait (Bait I), was significantly lower and varied between 12.24 to12.55 tonnes/ha. The total yield was again found significantly increased in plots treated with the aqueous darek seed kernel extract (5%) and ranged between 14.86 to15.24 tonnes/ha. On the contrary, in untreated control plots the total yield recorded ranged between 8.01 to 8.34 tonnes/ha (Table 6). The *Melia* baits and aqueous DSKE sprays were significantly better than the standard malathion bait in checking the fruit fly attack and increasing the crop yields.

Such deleterious effect of *Melia* extracts in aqueous as well as bait form is attributed to the presence of metabolically active alkaloids. Being a close relative to neem, DSKE contains many toxic compounds which can be held responsible for oviposition deterrent effect (Schmutterer 1995). Three active compounds structurally related to azadirachtin present in *M. azedarach* namely, 1-cinnamoyl-3-feruloyl-11-hydroxymeliacarpin, 1-cinnamoyl-3-feruloyl-11-hydroxy-22, 23-dihydro-23-methoxymeliacarpin and 1-tigloyl-11-methoxy-20-acetylmeliacarpinine have growth inhibitory effect (Krause *et al*. 1987) which can be credited for the under developed ovaries in *B. tau* when forced to feed on *Melia* baits. Bodhade and Borle (1985) have also reported

Table 4 Insecticidal effect of *Melia* poison baits against *Bactrocera tau*

Treatment	Per cent fruit fly mortality after indicated hours of treatment						Mean
	24	48	72	96	120	144	
Bait V (DSKE 3.0%)	16.7	13.3	10.0	3.3	3.3	0.0	7.7
Bait VI (DSKE 4.0%)	25.0	23.3	23.3	20.0	10.0	6.7	17.8
Bait VII (DSKE 5.0%)	46.7	40.0	30.0	26.7	13.3	6.7	27.2
Bait VIII (DSKE 6.0%)	59.1	53.3	43.3	43.3	23.3	16.7	39.8
Control	7.7	3.3	0.0	6.7	0.0	0.0	2.2
Mean	31.0	26.6	21.3	20.0	10.0	6.0	

CD (P=0.05)

Treatment 3.9, hours 4.4, Treatment × hours 6.9

Table 5 Effect of poison baits and aqueous darek seed kernel extract on field infestation of *Bactrocera tau*

Treatment	Per cent fruit fly infestation at 10-day spray interval				Mean
	Spray I	Spray II	Spray III	Spray IV	
Bait I (malathion 0.05%)	11.1 (19.5)	20.0 (26.6)	16.7 (24.1)	16.7 (24.1)	11.1 (23.6)
Bait VIII (DSKE 6%)	11.1 (19.5)	16.7 (24.1)	14.2 (22.1)	13.9 (21.7)	13.9 (21.9)
Bait IX (DSKE 8%)	4.7 (12.5)	6.7 (15.0)	8.3 (16.7)	0.0 (0.0)	4.9 (11.1)
Bait X (DSKE 12%)	0.0 (0.0)	6.7 (15.0)	0.0 (0.0)	5.6 (13.7)	3.1 (7.2)
DSKE (aqueous 5%)	10.0 (18.4)	8.3 (16.7)	7.1 (15.5)	0.0 (0.0)	6.4 (12.6)
Control	60.0 (50.8)	70.1 (56.9)	66.7 (54.8)	62.5 (52.2)	64.8 (53.7)
Mean	18.7 (20.1)	25.8 (25.7)	18.9 (22.2)	18.6 (22.2)	

Values in parentheses represent arc sine transformations

CD (P = 0.05)

Treatment 1.16, spray interval 0.95, treatment × interval 1.64

Table 6 Effect of poison baits and aqueous darek seed kernel extract on the total crop yield (tonnes/ha) of the crop

Treatment	Yield (tonnes/ha)			
	Plot 1	Plot 2	Plot 3	Mean
Bait I (malathion 0.05%)	12.24	12.55	12.41	12.40
Bait VIII (DSKE 6%)	13.18	12.82	12.85	12.95
Bait IX (DSKE 8%)	13.94	14.09	14.75	14.26
Bait X (DSKE 12%)	14.87	14.62	15.42	14.97
DSKE (aqueous 5%)	15.24	15.08	14.86	15.06
Control	8.01	8.34	8.28	8.21
Mean	12.91	12.92	13.10	12.98

CD(P = 0.05)
Treatment 5.27

petroleum ether and ethanol extracts of drupe-powder of *M. azedarach* to show sterilant activity against *B. cucurbitae*. Exposure of fruit flies to higher concentration of DSKE results in adult mortality which is because of the presence of toxic principal meliacin (1-cinnamoyl melianone) as recorded by Lee et al (1991). Such metabolically active compounds present in DSKE at various concentrations cause deleterious effects such as oviposition deterrence, effect on gonad development and adult mortality in *B. tau* as recorded in present findings.

The bait sprays have been found quite effective in reducing the fruit fly damage. Most of the baits evaluated till date carry a synthetic chemical insecticide, whereas in the present studies, DSKE acts as toxic principal in baits and protinex, gur (jaggery) and yeast as attractants for the fruit flies as previously reported by Satpathy and Rai (2002) and Fabres *et al*. (2003). The use of bait application technique in fruit fly management has been found to reduce the damage upto 95% (Singh *et al.* 2008) and thus increase the yield as already reported by Sookar and Khayatree (2000) and Akhtaruzzaman *et al*. (2001).

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