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Oviposition deterrent and repellent activity of bio-inputs and insecticides against melon fruit fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) in bitter gourd

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Abstract
The study was conducted to assess the oviposition deterrent and repellent activity of bio-inputs and insecticides against melon fruit fly (*B. cucurbitae*) in laboratory condition (28±2^{0}C, 60% RH). The Indigenous Technical Knowledge (ITK) practices followed by the organic farmers are playing pivotal role in reducing the pest problem in crop protection leading to minimal use of toxic chemicals. The effect of different bio-inputs (ITK concoction) and insecticides were superior over the control in reducing the fruit fly oviposition deterrent and increase repellent activity. The insecticides spinosad 45 SC and chlorantraniliprole 18.5 SC recorded 80.28 and 73.23 per cent in no choice test, 74.13 and 70.68 per cent in free choice test of oviposition deterrent, respectively. Among the bio-inputs agniastram (tobacco mixture) 5 % recorded 49.29 and 55.17 per cent of oviposition deterrent and had 84.94 and 73.31 per cent of repellent activity in free choice and no choice test, respectively followed by karpurakaraisal (camphor extract) 3%, neem seed kernel extract (NSKE) 5%, fish acid (mennamilam) with oviposition deterrency (38.02, 30.98 and 23.94 %) in no choice test and free choice test (46.55, 34.48 and 29.31 %) and repellent activity (69.72, 35.09 and 46.53 %) in free choice test and no choice test (62.70, 47.92 and 41.34 %), respectively. Ten leaf extract (pathilaikasayam) 5% showed minimum ovipositional deterrency and repellent activity compared to other bio-inputs. The present study concluded that application of agniastram and karpurakaraisal were effective next to insecticides for the management of fruit fly on bitter gourd.

Keywords: Bitter gourd, melon fruit fly, Indigenous traditional knowledge (ITK), bio-inputs, insecticides

Introduction
Bitter gourd is the most important vegetable in cucurbitaceous crops which occupies a predominant place in Indian vegetables. The tender fruit is found to have medicinal and nutritional properties like reducing blood glucose, asthma and ulcers (Oishi *et al*., 2007) [13] due to the presence of steroidal compound saponins (charantin) insulin like peptide (Altinterim, 2012) [1]**.** Bitter gourd is being attacked by different pests *viz*. aphids, melon fruit fly, hadda beetle, pumpkin caterpillar and gall fly during different growth stages. Among the pests of bitter gourd, melon fruit fly is more important as it causes yield loss from 30 to 100 per cent in bitter gourd (Dhillon *et al*., 2005) [3]. Generally the female fruit flies prefer young, soft and tender fruits for egg laying at 2 to 4 mm deep inside with its sharp ovipositor (Rabinthranath and Pillai, 1986) [15]**.** Melon fruit fly cause 41 to 95 per cent reduction in fruit yield. To meet this reduction management of fruit fly with like tactis bagging of fruits, food lure attractant, parapheromone trap and insecticides (Saptoka, 2010) [16]. Due to repeated usage of toxic insecticides, fruit fly has gained resistance and resurgence against those toxic substances (Jin *et al*. 2011) [9]. The insecicides are amount to about 25 per cent of cost of cultivation in bitter gourd production (Nasiruddin *et al*., 2004) [12]**.** The synthetic insecticides were found to be carcinogenic, teratogenic in humans and pollutes the environment by upsetting the balance of nature, ground water contamination, reducing the population of natural enemies and non-targeted organism (Jenkins *et al*., 2013) [8]. Therefore, desirable alternative methods of pest control is needed. Botanicals and traditional pest management practices were found to be an alternative to synthetic chemical pesticides for pest management

(Wakeil, 2013) [4]. Botanical extracts *viz*. neem (*Azadiracta indica* A.), lantana (*Lentena camera* L.), garlic (*Alium sativam* L.), turmeric (*Curcuma longa* L.), acacia (*Acacia auriculiformis* L.) (Thakur and Gupta, 2013, Ignacimuthu, 2019) [19, 7] and bio-inputs like, cow urine (Karkar *et al*, 2014) [10], meenamilam, neem oil, ginger-garlic extracts, ten leaf extract, tobacco leaf extract, agniasthiram and neemastram (Priya *et al*, 2018) [14] induce the ovipositional deterrent, repellent and antifeedent activity against melon fruit fly (Rehman *et al*, 2009) [21].

Materials and Methods

Laboratory evaluation of oviposition deterrent and repellent activity of bio-inputs and insecticides against melon fruit fly

The study was carried out in ambient laboratory condition (26 ± 2^0C, 65 ± 5 % RH) during November and December, 2018. The concentration of bio-inputs like karpurakaraisal (camphor mixture) 5%, agniastram (tobacco mixture) 5%, fish acid (mennamilam) 0.5%, ten leaf extract (pathilaikasam) 5%, NSKE 5%, spinosad 45 SC 0.12 ml/l and chlorantraniliprole 18.5 SC 0.4 ml/l were used for efficacy study. The selected fresh young fruit of bitter gourd were cut into 3 cm^2 diameter and dipped into each treatment for 1 min and treated pieces were kept in clean petri dish and allowed to dry for 1hr at room temperature. A test cage (35 × 30 × 35 cm) with wooden board at the bottom and all three sides were covered with white net to view the movement of fruit fly in choice test of repellence and oviposition test. The petri dish was kept into cages and 25 pairs of female fruit fly released for both experiments. No choice test was conducted in a small plastic box (10×10×15 cm) top covered with transparent lid for fruit fly observation and each box in small piece of bitter gourd with three pairs of female fruit flies released.

Mass culturing of melon fruit fly

The melon fruit fly infested bitter gourds were collected from field and kept in 20 × 20 × 8 cm plastic trays on a layer of sieved moist sand to facilitate pupation. Once in 3 – 4 days, sand was sieved and pupa were collected and kept in 10 cm diameter petri dishes on moisture filter paper. The pupae were placed inside the rearing cages of 35 × 30 × 35 cm. Each rearing cage had wire mesh on 3 sides with glass on top and round trap door to facilitate the collection of adult flies for experiment purpose and glucose solution (10 % W/V) was kept in 50 ml beaker and water soaked cotton swab was laid in such a way that half of it was immersed in glucose solution and half stayed above the rim of beaker. Slices of bitter gourd were kept inside each breeding cages for oviposition and there slices were replaced by fresh one daily to avoid decay. The entire culture was maintained at 26 ± 2°C temperature and 65 ± 5 per cent relative humidity. The eggs collected were placed in petri dish on moist filter paper for hatching along with fresh bitter gourd slices for the maggots to feed. After 24 h bitter gourd slices were replaced by fresh once. This procedure was repeated until the female died.

Oviposition deterrent test

The free Choice and no choice test cages of fruit fly with treated and untreated bitter gourd fruit pieces were allowed upto 12 h (7.00 pm to 7.00 am) for egg laying and then fruit pieces were removed from the cages for oviposition observation. Fruit fly eggs were count in each treatment in fruit pieces using magnifying lens. The experiment was conducted with three replications. The oviposition deterrency was calculated using the following formula by Thakur and Gupta, (2013) [19].

$$\text{Oviposition deterrency (\%)} = \frac{\text{No. of eggs in control (C)} - \text{No. of eggs in treatment (T)}}{\text{No. of eggs in control (C)}} \times 100$$

Repellency test

The repellent activity of free choice and no choice test was recorded by counting the number of female flies settled on treated and untreated bitter gourd fruit pieces from 1 h to 48 h of each replication separately. The repellency was calculated using the formula given by Rehman *et al*., (2009) [21].

$$\text{Repellency (\%)} = \frac{\text{Half of the no. of flies settled on both treated and untreated bitter gourd} - \text{No. of flies settled on treated bitter gourd}}{\text{Half of the no. of flies settled on both treated and untreated bitter gourd}} \times 100$$

Preparation of bio-inputs

Meenamilam (Fish acid): Fish waste and jaggery, each at the rate of one kg were taken and mixed well in a plastic bucket. The content was mixed once in five days upto one month and then kept undisturbed for fermentation upto 40 days. The content was filtered using muslin cloth after 45 days and kept in an airtight container for further use.

Agniasthiram (Tobacco extract): The main constituents of 'Agniasthiram' is green chill, ginger, garlic and dry leaves of tobacco. Five hundred gram of each ingredient *viz.* chilli, ginger, garlic and 250 g of dry tobacco leaves crushed and mixed in 10 liters of country cows urine and boiled in a mud pot till the total volume of the extract reached one third of the initial volume. The extract was kept for 24 h and then filtered and stored in an air tight plastic container in room temperature for further use in the experiments.

Pathilaikasayam (Ten leaf extract): The ten leaf extracts includes the leaves of notchi (*Vitex negundo* L.), aristolochia (*Aristolochia indica* L.), papaya (*Carica papaya* L.), heartleaf moonseed (*Tinospora cordifolia* Miers.) and custard apple (*Annona squamosa* L.) as basic five ingredients in addition to leaves from other five plants *viz.*, neem (*Azadirachta indica* A.), calotrophis (*Calotrophis gigantea* L.), waste land weed (*Tephrosia purpurea* L.), physic nut (*Jatropha curcas* L.), pungam (*Pongamia pinnata* L.). The leaves from notchi, aristolochia leaves, papaya, heartleaf moonseed and custard apple (5 kg) and leaves from neem, calotropis, waste land weed, physic nut, pungam (2 kg) were taken in 200 litres of water and 5 litres of country cow urine and 3 kg cow dung and stored in an airtight plastic container for three months for fermentation. The plastic container was kept in a cool shaded place and stirred three times in a day for efficient mixing and uniform fermentation.

NSKE (Neem seed kernel extract): The neem seed kernel (4 kg) was ground gently into powder by using blender. One kg of powdered neem seed kernel was tied in a filter cloth and soaked in one litre of water overnight. Then the extract was filtered twice or thrice and the filtered extract was diluted to 5 per cent for laboratory and field experiments.

Karpurakaraisal (camphor mixture): The camphor mixture was prepared by mixing one litre of neem oil with 50 ml of fresh country cow urine and 5 g of camphor (pachai karpuram), stirred gently and kept in closed containers. The prepared mixture (5 %) was used in field and laboratory experiments. Since, the camphor is insoluble in water, alcohol was used to dissolve the camphor and mixed with neem oil.

Statistical analysis

The collected data was statistically analysed in a Complete Randomized Design of laboratory experiments through AGRES programme. The treatment mean values were compared by Latin Square Design (LSD). Graphical work was done through Microsoft Excel program.

Results and Discussion

The results of no choice test, clearly showed that treatments were superior over the control (Table 1). The treatment of spinosad 45 SC and chlorantraniliprole 18.5 SC recorded minimum number of eggs laid and had high oviposition deterrency (4.67 eggs and 80.28 %) and (6.33 eggs and 73.23 %) of melon fruit fly, respectively. Among bio-inputs agniastram recorded 12.00 eggs and 49.29 per cent oviposition deterrent activity followed by karpurakaraisal which showed 14.67 eggs and 38.02 per cent oviposition deterrent activity, The NSKE had 16.33 eggs and 30.98 per cent oviposition deterrency. The oviposition rate in fish acid and ten leaf extract was 18.00 and 21.00 eggs and showed 23.94 and 11.26 per cent oviposition deterrency against melon fruit fly, respectively. In free choice test, the results showed that treatments were superior over the control (Table 1). The spinosad 45 SC and chlorantraniliprole 18.5 SC treated fruits showed minimum number of eggs (5.00 and 5.67 eggs) and high oviposition deterrent activity (74.13 and 70.68 %) against melon fruit fly, respectively. Among the bio-inputs agniastram recorded minimum number of eggs (8.67) and high oviposition deterrent (55.17) followed by karpurakaraisal (10.33 and 46.55 %), NSKE (12.67 and 34.48%), fish acid (13.67 and 29.31 %), respectively. The minimum number of eggs and repellent activity was recorded in ten leaf extract (15.67 and 18.96%).

Table 1: Evaluation of bio-inputs and insecticides as ovipositional deterrents on melon fruit fly, *B. cucurbitae*

S. No.	Treatment	Dose (ml/l)	No choice test		Free choice test	
			Eggs laid* (no.)	Oviposition deterrency (%)	Eggs laid* (no.)	Oviposition deterrency (%)
1	Karpura Karaisal (Camphor mixture)	30.0	14.67^{c} (3.89)	38.02	10.33^{b} (3.29)	46.55
2	NSKE (Neem Seed Kernel Extract)	50.0	16.33^{cd} (4.10)	30.98	12.67^{c} (3.63)	34.48
3	Ten leaf extract (Pathilaikasayam)	50.0	21.00^{ef} (4.64)	11.26	15.67^{d} (4.02)	18.96
4	Fish acid (Meenamilam)	5.0	18.00^{de} (4.30)	23.94	13.67^{cd} (3.76)	29.31
5	Agniastram (Tobacco mixture)	50.0	12.00^{b} (3.54)	49.29	8.67^{b} (3.03)	55.17
6	Spinosad 45 SC	0.12	4.67^{a} (2.27)	80.28	5.00^{a} (2.35)	74.13
7	Chlorantraniliprole 18.5 SC	0.4	6.33^{a} (2.61)	73.23	5.67^{a} (2.48)	70.68
8	Untreated control		23.67^{f} (4.92)		19.33^{e} (4.45)	
	SEd		0.14		0.14	
	CD (p=0.05)		0.30		0.30	

*Mean of three replications

Figures in parentheses are √x+0.5 transformed values, DAS-Days After Spray

In a column, means followed by different letters are significantly different (p=0.05) as per LSD

The free choice test, repellent activity of bio-inputs is clearly proved theis repellent action against melon fruit fly (Table 2). The result showed that agniastram had maximum repellent activity from 1 h to 48 h (97.22 to 63. 99 %) and overall repellency was 84.94 per cent followed by karpurakaraisal (84.62 to 47.81 %) with pooled mean data 69.72 per cent, NSKE showed 1 h (72.02 %) to 48 h (35.09 %) with overall mean 46.60 per cent and fish acid had 1 h to 48 h (60.92 to 22.07 %) and overall mean 46.53 per cent. The ten leaf extract showed low repellent activity recorded 1 h (40.67) to 48 h (13.04) with pooled value 29.59 per cent, respectively.

Table 2: Evaluation of bio-inputs for the repellent action against melon fruit fly *B. cucurbitae* (Free choice test)

Treatment	Dose (ml/l)	Repellent action of melon fruit fly - hr. after exposure (%)*									Repellency (%)
		1	3	5	7	12	18	24	36	48	
Karpura karaisal (Camphor mixture)	30.0	84.62^{b} (67.06)	79.80^{b} (63.80)	76.85^{b} (61.25)	72.69^{b} (58.66)	70.09^{b} (56.90)	67.71^{b} (55.43)	65.77^{b} (54.22)	62.20^{a} (52.08)	47.81^{b} (43.74)	69.72 (57.01)
NSKE (Neem Seed Kernel Extract)	50.0	72.02^{c} (58.11)	70.33^{bc} (57.17)	68.98^{bc} (56.28)	64.82^{bc} (53.87)	53.12^{c} (46.81)	47.22^{c} (43.40)	46.03^{c} (42.62)	39.68^{b} (38.50)	35.09^{b} (36.20)	46.60 (48.10)
Ten leaf extract (Pathilaikasayam)	50.0	40.67^{d} (39.55)	40.67^{d} (39.45)	37.96^{d} (37.87)	37.96^{d} (37.87)	29.91^{d} (32.71)	26.39^{d} (30.85)	24.40^{d} (29.37)	15.38^{c} (22.93)	13.04^{c} (21.15)	29.59 (32.14)
Fish acid (Meenamilam)	5.0	60.92^{e} (50.44)	60.86^{c} (51.30)	53.24^{cd} (46.90)	53.24^{cd} (46.90)	46.43^{c} (42.94)	44.44^{c} (41.77)	40.57^{c} (39.55)	37.00^{b} (37.41)	22.07^{c} (27.57)	46.53 (42.75)
Agniastram (Tobacco mixture)	50.0	97.22^{a} (85.13)	96.42^{a} (84.45)	95.83^{a} (83.10)	92.13^{a} (76.61)	86.61^{a} (68.54)	79.17^{a} (63.10)	78.37^{a} (63.42)	74.80^{a} (59.89)	63.99^{a} (53.24)	84.94 (70.83)
SEd		3.49	5.20	5.73	5.63	3.86	2.64	3.21	3.95	3.63	
CD (p=0.05)		7.45	11.08	12.22	12.02	3.86	5.63	6.84	8.43	7.74	

*Mean of three replications

Figures in parentheses are arc sine transformed values

In a column, means followed by different letters are significantly different (p=0.05) as per LSD

No choice test findings was similar to free choice test but repellent activity was reduced compared to free choice repellent test (Table 3). The agniastram recorded maximum repellent activity of 1 h to 48 h (85.42 to 56.25 %) with average repellency 73.31 per cent, respectively followed by karpurakaraisal which showed 1 h (79.17 %) to 48 h (50.00 %) and pooled mean 62.70 per cent against melon fruit fly, respectively. The NSKE showed 1 h to 48 h (64.58 to 31.25 %) and average mean 47.92 per cent and fish acid repellent activity showed 1 h (56.25 %) to 48 h (25.00 %) with average repellency of 41.34 per cent respectively. The Ten leaf extract was recorded 1 h (50.00 %) to 48 h (18.75 %) and average 32.44 per cent.

Table 3: Evaluation of bio-inputs for the repellent action against melon fruit fly *B. cucurbitae* (No choice test)

Treatment	Dose (ml/l)	Repellent action of melon fruit fly – hr. after exposure (%)*									Repellency (%)
		1	3	5	7	12	18	24	36	48	
Karpura karaisal (Camphor mixture)	30.0	79.17^{ab} (62.84)	72.92^{b} (58.64)	70.83^{b} (57.31)	64.28^{b} (53.30)	62.50^{b} (52.24)	58.33^{ab} (49.80)	54.16^{b} (47.39)	52.07^{b} (46.19)	50.00^{a} (45.00)	62.70 (52.36)
NSKE (Neem Seed Kernel Extract)	50.0	64.58^{c} (53.48)	58.33^{c} (49.80)	56.25^{c} (48.59)	50.00^{c} (45.00)	47.92^{c} (43.81)	45.83^{bc} (42.61)	39.59^{c} (38.99)	37.50^{c} (37.76)	31.25^{b} (33.99)	47.92 (43.81)
Ten leaf extract (Pathilaikasayam)	50.0	50.00^{d} (45.00)	45.83^{d} (42.61)	41.67^{d} (40.20)	35.71^{e} (36.70)	29.17^{d} (32.69)	27.08^{d} (31.36)	25.00^{d} (30.00)	18.75^{c} (25.66)	18.75^{c} (25.66)	32.44 (34.72)
Fish acid (Meenamilam)	5.0	56.25^{b} (48.59)	52.08^{c} (46.19)	50.00^{c} (45.00)	42.86^{d} (40.89)	41.67^{c} (40.20)	39.58^{cd} (38.99)	33.33^{c} (35.26)	31.25^{c} (33.99)	25.00^{b} (30.00)	41.34 (40.01)
Agniastram (Tobacco mixture)	50.0	85.42^{a} (67.55)	85.42^{a} (67.55)	79.17^{a} (62.84)	78.57^{a} (62.42)	77.08^{a} (61.40)	72.92^{a} (58.64)	64.58^{a} (53.48)	60.42^{a} (51.01)	56.25^{a} (48.59)	73.31 (58.90)
SEd		6.38	4.11	2.65	1.84	1.81	4.90	3.36	3.53	2.19	
CD (p=0.05)		13.91	8.95	5.78	4.00	3.94	10.45	7.32	7.69	4.79	

*Mean of three replications
Figures in parentheses are arc sine transformed values
In a column, means followed by different letters are significantly different (p=0.05) as per LSD

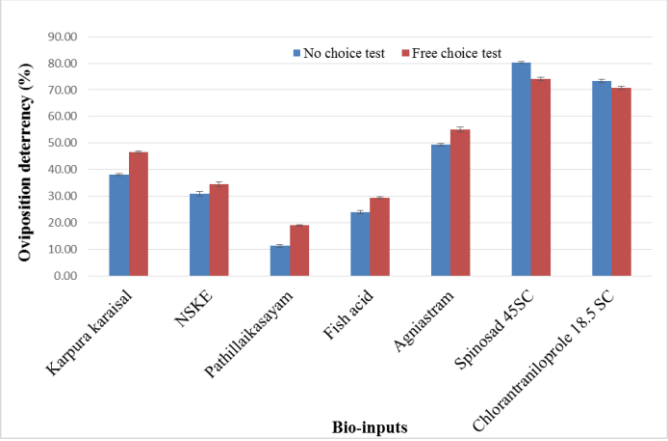

Fig 1: Ovipositional deterrency of bio-inputs and insecticides on melon fruit fly, *B. cucurbitae*

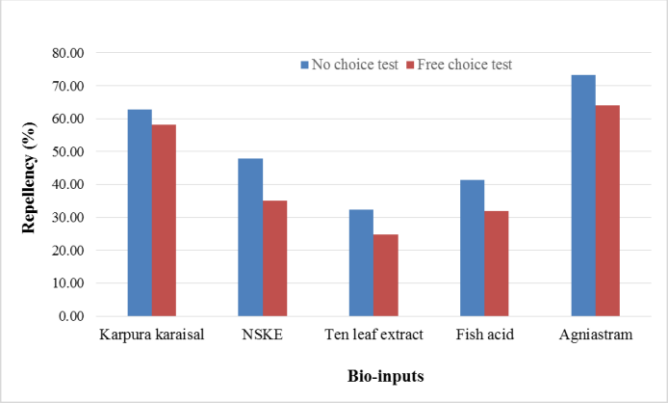

Fig 2: Repellent activity of bio inputs against melon fruit fly *B. cucurbitae*

The results revealed that spinosad 45 SC and chlorantraniliprole 18.5 SC had significantly reduced the oviposition of melon fruit fly in no choice test (80.28 and 73.23 %) and free choice test (74.13 and 70.68 %) (Fig 1 and 2). Shivangi *et al.*, (2017) [17] reported similarity finding that spinosad 45 SC had significantly reduced the fruit oviposition mark (1.5/five plants) of cucumber against melon fruit fly. According to Bharadiya and Bhut, (2017) [2] chlorantraniliprole 18.5 SC had lowest egg and larval population of melon fruit fly on sponge gourd. In bio-inputs, agniastram showed maximum oviposition deterrent (49.29 and 55.17 %) and repellent activity (84.94 and 73.31 %) in no choice and free choice test of bitter gourd. Similarly ginger garlic extract effectively reduced the defoliaters (65.51%) of cabbage (Tuan *et al*., 2014) [20]. Karpurakaraisal (camphor mixture) showed 38.02 and 46.55 per cent in no choice and free choice test of oviposition deterrent and repellent activity (69.72 and 62.70 %). Fu *et al*. (2015) [5] proved toxicity and repellent activity of camphor oil against fire ant, *Solenopsis invicta*. The knock down time of camphor was 10.82 and 14.73 h at two different concentrations (1.62 and 4.28 µg) and 2.55 µg/ml gave maximum mortality and strong repulsion. The NSKE recorded oviposition deterrency (30.98 and 34.48 %) and repellent activity (46.60 and 47.92 %). Singh and Singh, 1998 reported that NSKE causes oviposition deterrency, reduction in fecundity, affects post embryonic development of melon fruit fly. Hossain and Khalequzzaman, (2018) [6] proved *A. indica* with maximum oviposition deterrency (90.1 and 88.7 %) against fruit fly. The fish acid and ten leaf extract minimum oviposition deterrent in no choice test (23.94 and 11.26 %) and free choice test (29.31 and 18.96 %). According to Weinert *et al*., (2014) [22] fish acid supplements nutrients to plants and increased the availability of microorganism and repellent activity against insect pests of crop. Tribal people used various plant extract mixtures (concoction) *viz*., lantana leaf extract, tulsi leaf extract, nerium extract, cow urine, vitex leaf extract and agave mixture were used as pest control agents (Mohapatra *et al*., 2009) [11].

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