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ENHANCEMENT OF MATING PERFORMANCE OF STERILE MALES OF MELON FLY *ZEUGODACUS CUCURBITAE* (COQUILLETT) THROUGH METHOPRENE AND CUE LURE

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ABSTRACT

The male pupae of Melon fly, *Zeugodacus cucurbitae* were irradiated at 30 Gy of gamma radiation using Cobalt-60 source. Freshly emerged sterile males (30 Gy) were exposed to different regimes of methoprene and yeast hydrolysate as protein source. Sterile males of 10, 12 and 14 days old were exposed to different regimes of cue lure and yeast hydrolysate. Mating parameters such as lek initiation, lek participation, male calling, mating success and female acceptance index (FAI) were recorded. Sterile males of exposed to methoprene and fed with yeast hydrolysate (M+P+) become sexually matured at three days earlier when compare to protein deprived males (M+P-). High percentage of M+P+ males were engaged in lekking and calling behavior. Sterile males treated with methoprene and fed on protein diet had high percentage of matings with female acceptance index (FAI) of 0.8. Sterile males fed on yeast hydrolysate up to 12-days of age and then accessed to cue lure (P+CL+) had greater mating propensity with frequent mating (43.0%) and FAI of 0.84.

Key words: *Zeugodacus cucurbitae*, methoprene, cue lure, yeast hydrolysate, mating parameters, lek intiation, lek participation, male calling, female acceptance index

Melon fly, *Zeugodacus cucurbitae* (Coquillett) (Tephritidae:Diptera) is an economically most important insect pest of horticultural crops inflicting heavy losses to the horticultural industry in India (Kapoor, 2005). It attacks 61 plant species belonging to 19 different families (De Meyer et al., 2005). Cucurbits are subjected to damage by melon fly right from the primordial stages of the crop up to harvest (Viraktamath et al., 2003). In addition to the direct losses, its economic impacts results in the loss of export markets as well as costly requirements of quarantine restrictions and eradication measures (Badii et al., 2015).

The sterile insect technique (SIT) is a species-specific and environmentally non-polluting method of insect control that relies on the radiation-sterilization of mass reared insects and systematic release of potentiated sterile males into a target area to induce sterility (Knipling, 1979). Factors that can undermine the effectiveness of an SIT programme are poor mating performance of mass reared males as compared to wild males. Improvements in insect quality influence the efficacy of sterile insect technique, because released sterile males have to successfully transfer their sperm carrying dominant lethal mutations to females in the target populations (Hendrichs, 2002). For the success of any SIT programme, males must be able to locate a lekking site, perform a courtship, attract wild females and copulate (Jang et al., 1998). Juvenile hormone (JH) is a pivotal hormone coordinating the development of sexual signaling by enhancing pheromone production and accelerating reproductive maturity in tephritid males (Teal et al., 2000). Methoprene is a juvenile hormone analogue widely used as insect growth regulator and their impact on sexual maturation has been explored in number of tephritid species; such as, Mexican fruit fly *Anastrepha ludens* (Loew), West Indian fruit fly *A. oblique* (Macquart) and Queensland fruit fly *Bactrocera tryoni* (Froggatt) giving young males a mating advantage over non-treated males of the same age (Collins et al., 2015). Cue lure is a parapheromone and its feeding increase mating success in fruit flies (Shelly and Villalobos, 1995). Yeast hydrolysate is the main source of amino nitrogen, minerals and vitamins contributes to male gonadal and accessory glands development and known to influence sexual success in Oriental fruit fly *B. dorsalis* (Hendel), *B. tryoni*, Mediterranean fruit fly *Ceratits capitata* (Wiedemann) (Yuval et al., 2002). Release of enhanced sterile males in target area offers a considerable potential and has been used with great success as a part of an area-wide integrated pest management approach (AW-IPM) against major pests of agricultural and horticultural importance (Vreysen et al., 2006). Keeping these observations in view, the present

investigation was conducted to study the influence of methoprene and cue lure along with yeast hydrolysate on mating behavior of sterile males of *Z. cucurbitae* to identify potential pre-release adult holding diet for enhanced mating performance.

MATERIALS AND METHODS

The experiment was conducted at Division of Entomology, ICAR-Indian Agricultural Research Institute (IARI). New Delhi during 2018-19. The initial culture of melon fly was obtained from infested cucurbit fruits such as bitter gourd, ridge gourd and bottle gourd were collected from the fields of Division of Vegetables Science, ICAR-IARI, New Delhi. Infested fruits were brought to the laboratory and were kept at controlled conditions (25-27°C, 65-75% RH) in 8"×6" glass jars provided with 5 cm thickness of sterilized sand for pupation. The fully-grown larvae pop out from the fruit into soil for pupation. Pupa were collected and transferred to adult rearing cages (30×30×30cm) provided with a mixture of sugar and yeast hydrolysate (3:1) as adult food and water-soaked cotton swabs in 100 ml conical flask as source of water. Cages were cleaned and serviced with adult food and water twice a week. After pre-oviposition period of 10-12 days, equal number of males and females of same age were confined to mating cages. Petri dishes containing semi solid pumpkin fruit substrate covered by a thin parafilm were provided as ovipositional devices to the females to collect the eggs required for the experiment.

The insects required for sterilization were raised by rearing maggots on artificial liquid larval diet 17 (Panduranga et al., 2018). The male pupae of 48 h before adult eclosion were exposed to gamma radiation (cobalt-60 source) dose of 30 Gy at discharge rate of 1.147 kGy/hr using gamma irradiator (GC-5000, BRIT, Mumbai) installed at the Nuclear Research Laboratory, ICAR-IARI, New Delhi. Sterile males were treated topically on the day of emergence with methoprene. Male flies were immobilized by chilling at 5ºC for 5-10 min. One microleter of acetone containing 5 µg of methoprene was applied on dorsal surface of thorax of each male with the help of micropipette. For control males, only 1 µl of acetone was applied. These males were accessed to the diet with or without yeast hydrolysate and treatments as follows: M+ P+: Topical application of methoprene (M), sugar and yeast hydrolysate (P) as adult food; M+ P-: Topical application of Methoprene and only sugar as adult food; M- P+: No methoprene but yeast hydrolysate and sugar as adult food; and M- P-: No methoprene and only sugar as adult food.

Sterile males of 10, 12 and 14 days old fed on adult diet with and without protein (yeast hydrolysate) were exposed to cue lure applied on cotton wick for 2-h before release for mating. The four treatments studied were: P+ CL+: Sugar and protein hydrolysate (P) as adult food followed by cue lure (CL) feeding; P- CL+: Only sugars as adult food followed by cue lure feeding; P+ CL-: Sugar and protein hydrolysate as adult food and no cue lure feeding; and P- CL-: Only sugar as adult food and no cue lure feeding. Flies from each treatment were maintained in a separate adult rearing cage at 25-27 ºC and 65-70% RH with photoperiod of 12:12 h (L:D). Virgin untreated females used in the experiments were maintained in the absence of males in separate cage provided with mixture of sugar and yeast hydrolysate (3:1) and water at ad libitum.

Lek initiation, lek participation, and male calling behaviors of sterile males in all the treatments were recorded up to 12 days of age. Observations on these parameters were recorded at every 10 min interval during 5.00-8.00 pm under semi-dark conditions. Lek initiation is the first male started to emit pheromone (wing vibration and anus beating) in a certain area of cage, resulting in initiation of aggregations (Kuba and Sokei, 1988). Lek participation is the number of males that joined the first male for calling (Iwahashi and Majima, 1986). Male calling is the number of males calling in a lek (expansion of pleural regions of the abdomen and eversion of glistening rectal glands to emit pheromones). Mating success is the number of males that achieved copulation. Female acceptance index (FAI) is number of successful matings divided by total number of males attempted to mate. For recording mating success and FAI, untreated virgin females of same age (12-days old) were released to each treatment. The number of matings was recorded at every 10 min interval and mating pairs were collected in a glass vial. FAI was calculated by dividing the number of successful matings by total number of males attempted to mate. These observations were recorded up to 20 days of age. In case of cue lure experiment, mating parameters were recorded for 5-days following cue lure exposure. Data obtained were calculated as mean± standard error (SE). Mean values were obtained from three replications. Data were subjected to analysis ANOVA with the honestly significant difference (HSD) value calculated as Tukey's statistic at α=0.05 using SAS version 9.4 available at ICAR-IASRI, New Delhi, India.

RESULTS AND DISCUSSION

Mating parameters such as mating success female acceptance lek initiation, lek participation and male calling behavior of sterile males of melon fly exposed to different regimes of methoprene and yeast are presented in Table 1. Sterile males that were exposed to methoprene and allowed to fed on protein diet (M+P+) formed leks early and participated in male calling in comparatively shorter time. At 5 DAT, 12% of M+P+ males initiated leks, 16% joined the leks and nearly 11% of males were engaged in male calling and on next day (i.e. 6 DAT), 26.7, 25.3 and 21.3% of males were involved in lek initiation, lek participation and male calling, respectively. Whereas the male flies from the remaining treatments have initiated leks on 8th day onwards except M-P+. The sterile males from M-P+ reached sexual maturity at 7 DAT as 9.3, 12.0 and 8.0% of males were involved in lek initiation, lek participation and male calling behavior, respectively. However, significantly high percentage of M+P+ males initiated leks (40.0) and participated in leks (32.0) and nearly 31.0% of flies were engaged in male calling at 7 DAT. Involvement and participation of M+P+ males in lek intiaiton, lek participation and male calling behaviours increased with increase in age of the flies. It was also observed that the sterile males from M+P- and M-P- exhibited mating behaviour from the age of 8-days onwards and slightly increased their performance up to the age of 10-days. While on 11 and 12-days of age, least number of males was involved in mating behavior due to lack of protein in their diet. More than 50% of M+P+ males initiated and participated in leks and more than 45% of males were involved in male calling at 8 DAT. Male files that were not treated with methoprene (M-P+) showed quite low activity on 8th day as 22.7%, 18.7%, and 12.0% of males were involved in lek initiation, lek participation and male calling behavior, respectively. The percentage of M-P+ sterile males involved in lek initiation, lek participation and male calling was increased (ranged between 30.07-48.7, 28.0-30.7, 21.3-36.0%, respectively) at 11-days of age. Whereas in M-P- and M+P-; the percentage of males involved in lek initiation (16.0 and 10.7%), lek participation (14.7 and 12.0%) and male calling behaviors (10.7 and 9.3%) were not significantly differ, respectively (Table 1).

The sterile males subjected to methoprene application and then access to adult diet consist of yeast hydrolysate and sugar (1:3 parts) showed increasing trend in performance of mating behaviour up to 11-days of age. The percentage of male involvement in lek initiation, participation and male calling (at 9-11 DAT) was ranged between 62.7-72.0%, 54.7-60.0% and 50.7-61.3%, respectively. The next best treatment was M-P+, in which the sterile males involved in lek initiation, lek participation and male calling was increased (ranged between 30.07-48.7, 28.0-30.7, 21.3-36.0%, respectively) at 11-days of age. Whereas at 12-days of age, mating performance of M+P+ and M-P+ males was reduced. The males that were exposed to methoprene and then access to the diet containing yeast hydrolysate and sugar (M+P+) achieved maximum matings throughout the experiment, compared to the remaining treatments. The mating success by M+P+ males was in the increasing trend up to 18 DAT ranging from 26.7 (13 DAT) to 44.0% (18 DAT), Whereas at 19 and 20 DAT; the mating success decreased to 34.7 and 30.7%, respectively. It was followed by M-P+ with mating success ranging from 17.3 (13 DAT) to 26.7% (18 DAT). Whereas in the treatments; M+P- and M-P, matings were significantly lower than M+P+ males. However, the mating success increased from 6.7 to 14.7% and 10.7 to 21.3%, respectively from 13 to 16 DAT and were on par with each other. In the treatments; M+P- and M-P-, the mating gradually decreased at 17 DAT onwards. Where as in M-P+, mating success started declining at 19 DAT. The maximum mating (41.3 and 44%) was achieved by M+P+ males at 17 and 18 DAT, respectively with FAI of 0.88. But at 19 and 20 DAT, the mating success of M+P+ males with untreated females showed decreasing trend i.e. 34.7 (FAI:0.64) and 30.7% (FAI:0.51), respectively (Table 1).

The males treated with methoprene and fed with protein diet (M+P+) had FAI of >0.8, which indicates that males that were engaged in calling behavior are highly potent and readily accepted by the females for mating. The FAI for M-P+ males was ranged from 0.59-0.77 at 13 to 16 DAT, respectively. Where as in M-P- and M+P-, the males harboured FAI of 0.48-0.65 and 0.39-0.43, respectively and were at par with each other (Table 1).

Effect of protein feeding and cue lure exposure on mating behavior of 10, 12 and 14 days old sterile males of *B. cucurbitae* are presented in Table 2. Significantly misl sterile males from P+CL+ were involved in lek initiation (54.7), lek participation (43.3) and male calling (42.7) on 1-day after exposure to cue lure compared to the other treatments. In P+CL-; 32.0% of sterile males initiated leks and 26.7% of sterile males had participated in leks, where as 24.0% of males called for mates. In P-CL+, very low percentage of

Table 1. Influence of methoprene and adult dietary protein on *B. cucurbitae* sterile males

Treatment	Lek initiation (%)	Lek participation (%)	Male calling (%)	Mating success (%)	Female acceptance index (FAI)
	5 DAT			13 DAT	
M+P+	12.0a (6.89± 1.33)	16.0a (9.21± 1.34)	10.7a (6.12± 0.76)	26.7a (15.46± 0.79)	0.87a (0.49± 0.04)
M+P-	0.0b	0.0b	0.0b	6.7b (3.82± 0.76)	0.39b (0.22± 0.03)
M-P+	0.0b	0.0b	0.0b	17.3ab (9.98± 1.54)	0.59ab (0.33± 0.04)
M-P-	0.0b	0.0b	0.0b	10.7b (6.12± 2.03)	0.48b (0.27± 0.04)
	6 DAT			14 DAT	
M+P+	26.7a (15.47± 1.59)	25.3a (14.67± 0.79)	21.3a (12.32± 0.78)	30.07a (17.87± 2.11)	0.87a (0.49± 0.03)
M+P-	0.0b	0.0b	0.0b	8.0b (4.58± 1.32)	0.47b (0.26± 0.08)
M-P+	0.0b	0.0b	0.0b	18.7ab (10.78± 2.81)	0.68ab (0.38± 0.02)
M-P-	0.0b	0.0b	0.0b	13.3b (7.66± 1.53)	0.50ab (0.28± 0.02)
	7 DAT			15 DAT	
M+P+	40.0a (23.58± 1.44)	32.0a (18.67± 1.39)	30.7a (17.88± 2.11)	33.3a (19.53± 3.27)	0.86a (0.49± 0.04)
M+P-	0.0c	0.0c	0.0c	10.7b (6.12± 2.03)	0.42b (0.24± 0.06)
M-P+	9.3b (5.35± 0.76)	12.0b (6.89± 1.33)	8.0b (4.59±1.32)	20.0ab (11.53± 1.35)	0.68ab (0.38± 0.03)
M-P-	0.0c	0.0c	0.0c	13.3b (7.66± 0.77)	0.57ab (0.32± 0.01)
	8 DAT			16 DAT	
M+P+	59.3a (36.42± 1.72)	53.3a (32.40± 3.98)	45.3a (27.08± 3.77)	38.7a (22.77± 2.18)	0.83a (0.47± 0.02)
M+P-	10.7c (6.13± 2.03)	12.0b (6.89± 1.33)	9.3b (5.36± 1.53)	14.7b (8.44± 2.04)	0.43b (0.24± 0.03)
M-P+	22.7b (13.11± 2.07)	18.7b (10.77± 2.05)	12.0b (6.89± 1.33)	25.3ab (14.72± 3.45)	0.77a (0.44± 0.03)
M-P-	16.0bc (9.21± 1.34)	14.7b (8.44± 1.55)	10.7b (6.12± 0.77)	21.3ab (12.35± 3.15)	0.65ab (0.37± 0.04)
	9 DAT			17 DAT	
M+P+	62.7a (38.85± 1.99)	54.7a (33.19± 2.39)	50.7a (30.49± 2.33)	41.3a (24.42± 1.66)	0.88a (0.50± 0.01)
M+P-	12.0c (6.89± 1.33)	14.7c (8.44± 2.04)	13.3b (7.66± 0.77)	12.0c (6.89± 1.33)	0.36c (0.20± 0.02)
M-P+	30.7b (17.87± 1.61)	28.0b (16.26± 1.37)	21.3b (12.32± 0.78)	26.7b (15.48± 2.09)	0.72ab (0.41± 0.01)
M-P-	24.0b (13.88± 1.36)	20.0bc (11.54± 1.35)	14.7b (8.44± 2.04)	20.0bc (11.53± 2.31)	0.54bc (0.30± 0.03)
	10 DAT			18DAT	
M+P+	67.3a (42.46± 3.13)	57.3a (35.18± 4.12)	53.3a (32.33± 3.22)	44.0a (26.21± 3.85)	0.80a (0.45± 0.01)
M+P-	18.7c (10.76± 0.77)	20.0b (11.54± 1.35)	16.0b (9.21± 1.34)	12.0b (6.90± 4.62)	0.28c (0.16± 0.03)
M-P+	34.7b (20.30± 2.14)	26.7b (15.47± 1.59)	28.0b (16.26± 1.37)	26.7ab (15.46± 1.33)	0.47b (0.26± 0.02)
M-P-	29.3bc (17.05± 0.80)	21.3b (12.34± 2.81)	24.0b (13.91± 2.73)	17.3b (9.99± 3.53)	0.34bc (0.19± 0.01)
	11 DAT			19DAT	
M+P+	72.0a (46.10± 1.91)	60.0a (36.96± 2.81)	61.3a (37.97± 3.44)	34.7a (20.35± 3.54)	0.64a (0.36± 0.01)

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(*contd...*)

M+P-	13.3d (7.66± 0.77)	14.7c (8.44± 2.04)	10.7c (6.12± 0.76)	9.3b (6.90± 1.33)	0.13c (0.07± 0.02)
M-P+	48.7b (29.12± 1.16)	30.7b (17.88± 2.11)	36.0b (21.10± 1.41)	22.7b (13.10± 2.67)	0.31b (0.17± 0.02)
M-P-	26.7c (15.48± 2.09)	18.7bc (10.78± 2.82)	22.7c (13.11± 2.07)	13.3b (7.66± 2.67)	0.22bc (0.12± 0.00)
	12 DAT			20DAT	
M+P+	66.7a (41.91± 2.69)	52.0a (31.35± 1.99)	56.0a (34.07± 2.31)	30.7a (17.87± 2.11)	0.51a (0.29± 0.01)
M+P-	10.7c (6.12± 0.76)	12.0c (6.89± 1.33)	9.3c (5.35± 0.76)	9.3b (5.35± 0.76)	0.09b (0.05± 0.00)
M-P+	30.7b (17.90± 2.91)	22.0b (12.72± 1.78)	20.0b (11.56± 2.70)	17.3b (9.98± 0.77)	0.19b (0.10± 0.00)
M-P-	16.0c (9.21± 1.34)	13.3c (7.66± 0.77)	10.7bc (6.12± 0.77)	12.7b (7.66± 1.53)	0.13b (0.07± 0.01)

Within a column, means followed by the same letter are not significantly different (α=0.05; Tukey test, PROC ANOVA).
Figures in parentheses are arc sine transformed values ± standard error, DAT: Days after treatment.

sterile males i.e. 14.7, 10.7, and 8.0 were involved in lek initiation, lek participation and male calling, respectively. The sterile males from P-CL- performed better in terms of lekking and calling behaviour compare to P-CL+ males.

The successful matings (38.7) was significantly high in P+CL+ with FAI of 0.80. While in P+CL, P-CL- and P-CL+, the mating success were 10.7, 8.0 and 5.3 with FAI of 0.38, 0.33 and 0.31, respectively and these three treatments were on par with each other. At 2-day after exposure to cue lure, sexual performance of P+CL+ sterile males gradually declined and insignificant difference was observed from P+CL- males. Nearly 43.0 and 32.0% of P+CL+ males initiated and participated in leks, where as in P+CL-, 38.7 and 28.0% of sterile males were involved in initiation and participation of leks, respectively. In P+CL+ and P+CL-, 33.3 and 29.3% of males were engaged in emission of pheromones (male calling), respectively. The mating success in all the treatments was increased at 11 days of age, when compared to 10 days of age. The sterile males from P+CL+ achieved high of mating success (30.3). At 3-days after exposure, the mating parameters recorded for all the four treatments continued to decline. Further, the P+CL+ males involved in lek initiation, lek participation, male calling, and mating success reduced from 34.7, 25.3, 26.7 and 21.3% (at 3 DAT) to 17.3, 12.0, 13.3 and 13.3% (at 5 DAT). These results indicated that, P+CL+ sterile males showed better performance and were effectively involved in all the mating parameters at only one day of exposure to cue lure. From the second day onwards, sexual performance of P+CL+ males were similar with the remaining treatments (Table 3).

Sterile males fed with yeast hydrolysate and had accessed to cue lure (P+CL+) at 1 DAT were actively involved in forming leks (60.0%) and nearly 50.0% of males participated in leks and 45.3% called for mates. Significantly lower percentage i.e. 38.7, 29.3 and 32.0 of P+CL- males were involved in lek initiation, participation and male calling, respectively. P+CL+ males had successful matings (43.0%) with FAI of 0.84. While in the remaining treatments, the mating success was in the range of 8.0-17.3 % with FAI of 0.28-0.45 only. The mating potency of P+CL+ sterile males started declining 2-day after exposure to cue lure, as the percentage of male involvement in lek initiation, participation and calling behaviour was reduced to nearly ¾ times and does not remain significantly different from those sterile males which were fed with amino nitrogen molecules but never exposed to synthetic cue lure in the laboratory (P+CL-), however, their mating success was 36.0% with the FAI of 0.65. Less percentage of P-CL+ males were involved in mating, compare to P-CL- males. The sexual performance of sterile males keeps on decreasing with increase in the period of the treatment of flies. The percentage of lek initiation, lek participation, male calling and mating success decreased from 35.7 to 18.7, 24.0-11.7, 24.0-9.3 and 32.0 -21.3% from 3 DAT to 5 DAT, respectively, in P+CL+ sterile males. The similar trend was observed in all the treatments also. These results indicate that, an exposure of sterile males fed with yeast hydrolysate and cue lure had enhanced the mating behaviour for almost one day by increasing their ability in forming leks and emission of pheromones to attract their female mates for successful matings (Table 2).

At one day after exposure, nearly 47.0% of P+CL+ males initiated leks, to which about 36.0% of males joined and 30.7% were involved in calling. In P+CL- treatment, 25.3% of sterile males were involved in lek

Table 2. Influence of adult dietary protein and cue lure on mating behavior of 10-14 days old sterile males of *B. cucurbitae*

Treatment	Lek initiation (%)	Lek participation (%)	Male calling (%)	Mating success (%)	FAI	Lek initiation (%)	Lek participation (%)	Male calling (%)	Mating success (%)	FAI	Lek initiation (%)	Lek participation (%)	Male calling (%)	Mating success (%)	FAI
	10 Days Old					12-days old					14-days old				
						1 DAT									
P+CL+	54.7a (33.19± 2.40)	43.3a (25.68± 1.54)	42.7a (25.27± 1.70)	38.7a (22.75± 4.62)	0.80a (0.46± 0.01)	60.0a (37.0± 3.32)	49.7a (29.64± 3.14)	45.3a (26.99± 2.28)	43.0a (25.62± 2.67)	0.84a (0.48± 0.03)	46.7a (27.85± 2.27)	36.0a (21.10± 1.42)	30.7a (17.87± 2.12)	32.0a (16.26± 1.38)	0.77a (0.44± 0.01)
P-CL+	14.7c (8.43± 1.55)	10.7c (6.12± 2.03)	8.0c (4.58± 1.33)	5.3b (3.05± 0.77)	0.31b (0.17± 0.03)	17.3c (9.98± 0.78)	9.3c (5.35± 0.77)	10.7c (6.12± 0.77)	8.0b (4.60± 1.33)	0.28c (0.15± 0.01)	16.0b (9.20± 1.34)	9.7b (5.35± 0.77)	9.3b (5.35± 0.77)	5.3b (3.05± 0.77)	0.17c (0.09± 0.01)
P+CL-	32.0b (18.66± 1.40)	26.7b (15.47± 1.59)	24.0b (13.88± 1.36)	10.7b (6.12± 0.77)	0.38b (0.21± 0.02)	38.7b (22.75± 1.67)	29.3b (17.06± 1.59)	32.0b (18.66± 1.40)	17.3b (9.98± 0.78)	0.45b (0.25± 0.02)	25.3b (14.68± 1.57)	16.0b (9.20± 1.34)	16.0b (9.20± 1.34)	9.3b (5.35± 0.47)	0.35b (0.20± 0.02)
P-CL-	21.3bc (12.31± 0.78)	13.3c (7.66± 0.77)	12.0c (6.89± 1.33)	8.0b (4.58± 1.33)	0.33b (0.18± 0.02)	28.0bc (16.26± 1.38)	17.3bc (9.98± 1.55)	20.0c (11.53± 1.35)	12.0b (6.89± 1.33)	0.31c (0.17± 0.01)	20.0b (11.53± 1.35)	14.7b (8.43± 1.55)	12.0b (6.89± 1.33)	6.7b (3.82± 0.38)	0.22c (0.12± 0.02)
						2 DAT									
P+CL+	42.7a (25.28± 2.23)	32.0a (18.66± 1.40)	33.3a (19.46± 0.81)	30.3a (17.91± 3.49)	0.70a (0.40± 0.01)	44.0a (26.23± 3.97)	34.7a (20.33± 2.96)	32.0a (18.70± 2.80)	36.0a (21.14± 2.84)	0.65a (0.40± 0.03)	32.0a (18.66± 1.40)	22.7a (13.10± 1.57)	22.7a (13.11± 2.07)	29.3a (17.05± 0.80)	0.61a (0.34± 0.01)
P-CL+	12.0c (6.89± 1.33)	9.3c (5.35± 0.77)	6.7c (3.82± 0.77)	8.7b (4.58± 1.33)	0.32c (0.18± 0.02)	13.3c (7.66± 1.33)	10.7b (6.12± 0.77)	8.0c (4.58± 0.00)	10.7b (6.12± 0.77)	0.32b (0.18± 0.03)	12.7b (7.66± 0.77)	8.0b (4.58± 1.33)	8.0b (4.60± 0.01)	8.0c (4.58± 1.33)	0.24c (0.13± 0.03)
P+CL-	38.7a (22.75± 1.67)	28.0ab (16.26± 1.38)	29.3a (17.05± 0.80)	18.7ab (9.20± 1.34)	0.54ab (0.30± 0.03)	30.7ab (17.86± 1.61)	21.3b (12.31± 0.78)	21.3ab (12.31± 0.78)	22.7ab (13.11± 2.07)	0.51ab (0.29± 0.03)	21.3ab (12.33± 2.07)	13.3b (7.66± 0.77)	13.3ab (7.66± 0.77)	15.7b (10.75± 0.78)	0.49ab (0.28± 0.04)
P-CL-	25.3b (14.67± 0.79)	17.3bc (9.99± 2.06)	17.0b (9.98± 0.78)	12.0b (6.89± 1.33)	0.40bc (0.22± 0.01)	21.3bc (12.31± 0.78)	17.3b (9.98± 0.78)	14.7bc (8.43± 0.77)	14.7b (8.43± 0.77)	0.39b (0.22± 0.01)	16.0b (9.20± 1.34)	9.3b (5.35± 0.77)	12.0b (6.90± 1.33)	13.3bc (7.66± 2.04)	0.33bc (0.18± 0.04)
						3 DAT									
P+CL+	34.7a (20.28± 0.81)	25.3a (14.67± 0.79)	26.7a (15.48± 2.09)	21.3a (12.33± 2.07)	0.52a (0.29± 0.02)	35.7a (20.33± 2.96)	24.0a (13.91± 2.73)	24.0a (13.91± 2.73)	32.0a (18.66± 1.40)	0.49a (0.28± 0.03)	28.0a (16.30± 2.37)	20.0a (11.53± 1.35)	14.7a (8.43± 1.55)	20.0a (11.53± 2.35)	0.40a (0.23± 0.03)
P-CL+	9.3c (5.35± 1.53)	5.3c (3.05± 0.77)	5.3b (3.05± 0.77)	8.0a (4.58± 1.33)	0.27c (0.15± 0.02)	10.7b (6.12± 0.77)	8.0b (4.58± 1.33)	5.3b (3.05± 0.77)	9.3c (5.35± 0.77)	0.22c (0.12± 0.01)	6.7b (3.82± 0.77)	5.3b (3.05± 0.77)	4.0b (2.29± 1.32)	6.7b (3.82± 0.77)	0.16c (0.09± 0.01)

(*contd...*)

(*contd...*)

P+CL-	28.0ab (16.26± 1.38)	17.3ab (9.98± 1.55)	21.3a (12.33± 2.07)	16.7a (10.76± 2.05)	0.46ab (0.26± 0.01)	25.3ab (14.69± 2.10)	18.7ab (10.77± 2.05)	14.7ab (8.43± 1.53)	21.3b (12.31± 0.78)	0.39ab (0.22± 0.01)	14.7ab (8.45± 2.79)	10.7b (6.12± 0.77)	12.0ab (6.90± 1.33)	13.3ab (7.66± 0.77)	0.32ab (0.18± 0.02)
P-CL-	22.7b (13.11± 2.07)	12.0bc (6.89± 2.30)	14.7ab (8.43± 0.77)	12.0a (6.89± 1.33)	0.37bc (0.21± 0.02)	14.7b (8.43± 0.77)	12.0ab (6.89± 1.33)	8.0b (4.58± 1.33)	12.0c (6.90± 1.33)	0.32bc (0.18± 0.02)	12.0b (6.89± 1.33)	6.7b (3.82± 0.77)	9.3ab (5.35± 0.77)	10.7ab (6.12± 0.77)	0.19bc (0.10± 0.01)
							4 DAT								
P+CL+	28.0a (16.26± 1.38)	22.7a (13.09± 0.78)	20.0a (11.53± 1.35)	18.7a (11.53± 1.35)	0.52a (0.29± 0.03)	26.7a (15.47± 1.59)	18.7a (10.75± 0.78)	18.7a (10.75± 0.78)	26.7a (15.47± 1.59)	0.40a (0.23± 0.02)	10.7a (6.12± 0.77)	12.0a (6.89± 1.33)	9.3a (5.35± 0.77)	17.3a (9.99± 2.06)	0.35a (0.20± 0.02)
P-CL+	8.0b (4.58± 1.33)	2.7c (1.52± 0.76)	4.0b (2.29± 0.00)	6.7c (3.82± 0.77)	0.25c (0.14± 0.02)	5.3c (3.05± 0.77)	2.3b (1.52± 0.76)	2.7c (1.52± 0.76)	5.3b (3.05± 0.77)	0.28bc (0.14± 0.02)	4.0a (2.29± 1.32)	2.7b (1.53± 0.76)	1.3b (0.76± 0.01)	4.0b (2.30± 1.32)	0.07c (0.03± 0.02)
P+CL-	20.0ab (11.53± 1.35)	14.7b (8.43± 0.77)	13.3ab (7.66± 1.54)	16.0ab (9.21± 1.34)	0.42ab (0.24± 0.01)	22.7ab (13.11± 2.07)	13.3a (7.66± 0.77)	12.0ab (6.89± 1.33)	20.0a (11.53± 1.35)	0.32ab (0.18± 0.01)	8.0a (4.58± 1.33)	9.3ab (5.35± 0.75)	5.3ab (3.05± 0.77)	12.0ab (6.90± 1.33)	0.25ab (0.14± 0.01)
P-CL-	13.3b (7.66± 2.04)	8.0bc (4.58± 1.33)	9.3b (5.35± 1.53)	9.3bc (5.35± 0.77)	0.30bc (0.17± 0.02)	12.0bc (6.89± 1.33)	6.7b (3.82± 0.77)	6.7bc (3.82± 0.77)	9.7b (5.35± 0.77)	0.21c (0.12± 0.01)	6.7a (3.82± 0.77)	4.0b (2.30± 0.00)	4.0b (2.29± 0.00)	5.3b (3.05± 0.77)	0.16bc (0.09± 0.02)
							5 DAT								
P+CL+	17.3a (10.00± 2.79)	12.0a (6.89± 2.20)	13.3a (7.66± 2.04)	13.3a (10.75± 0.78)	0.38a (0.21± 0.02)	18.7a (10.75± 0.78)	11.7a (6.89± 1.33)	9.3a (5.35± 0.77)	21.3a (12.31± 0.78)	0.30a (0.17± 0.02)	8.0a (4.58± 1.33)	6.7a (3.82± 0.77)	6.4a (3.82± 0.77)	14.7a (8.43± 1.55)	0.26a (0.14± 0.01)
P-CL+	2.7b (1.52± 0.76)	1.3a (0.76± 0.01)	2.7b (1.52± 0.76)	4.0b (2.29± 1.32)	0.11b (0.06± 0.03)	3.7c (1.53± 0.76)	2.7b (1.53± 0.76)	1.3b (0.76± 0.01)	2.7c (1.52± 0.76)	0.05c (0.03± 0.01)	2.7a (1.52± 1.35)	1.3b (0.76± 0.01)	1.3b (0.76± 0.04)	1.3b (0.76± 0.09)	0.02b (0.01± 0.01)
P+CL-	13.3ab (7.66± 1.54)	8.0a (4.58± 1.33)	9.3ab (5.35± 0.77)	10.7ab (6.12± 0.77)	0.33a (0.19± 0.01)	15.3ab (7.70± 0.77)	10.7a (6.12± 0.77)	6.7ab (3.82± 0.77)	13.3b (7.66± 0.77)	0.24ab (0.13± 0.01)	5.3a (3.05± 0.77)	4.0ab (2.29± 0.00)	4.0ab (2.30± 0.00)	6.7ab (3.82± 0.77)	0.14ab (0.08± 0.02)
P-CL-	8.0ab (4.58± 1.33)	2.7a (1.52± 0.76)	2.7b (1.52± 0.76)	6.7b (3.82± 0.77)	0.24ab (0.13± 0.01)	9.3b (5.35± 0.77)	5.3ab (3.05± 0.77)	5.3ab (3.05± 0.77)	5.3c (3.05± 0.77)	0.17bc (0.09± 0.02)	5.3a (3.05± 0.77)	2.7ab (1.52± 0.76)	2.7ab (1.52± 0.76)	4.0b (2.30± 1.32)	0.06b (0.03± 0.01)

Within a column, means followed by the same letter are not significantly different (α=0.05; Tukey test, PROC ANOVA). Figures in parentheses are Arc Sine transformed values ± standard error.

initiation and 16.0% each in lek participation and male calling. The mating behaviour of P-CL- and P-CL+ sterile males was not significantly different from P+CL- males. Significantly high percentage of mating success (32.0%) was recorded from P+CL+ compare to P+CL-(9.3%), P-CL-(6.7%) and P-CL+(5.3%). The FAI (0.77) was high for P+CL+ males followed by P+CL- (0.35). At 2-days after exposure, lekking and calling abilities of sterile males from all the four treatments decreased and continued to decline. In P+CL+, the performance of sterile males in initiation and participation of leks decreased from 32.0 to 8.0% and 22.7-6.7%, respectively during the observation period ranged from 2 DAT to 5 DAT. Similarly the percentage of male calling and mating success were also decreased to 6.4 and 14.7. The percentage of lek initiation, lek participation, male calling and mating success by P+CL- males decreased at 2-DAT i.e. from 21.3, 13.3, 13.3 and 18.7% to 5.3, 4.0, 4.0, and 6.7% at 5-DAT, respectively. The mating parameters recorded for P-CL- and P-CL+ males were statistically on par with P+CL- throughout the experiment. However, the least percentage of P-CL+ males participated in lekking and calling behaviours (Table 2).

The sterile males applied with methoprene on the day of emergence and accessed to adult diet consists of yeast hydrolysate (M+P+) attained sexual maturity at the age of 5-days. Sterile males from M+P+ has initiated and participated in leks by calling their mates at 2-days earlier than the males from M+P-, M-P+ and M-P- treatments. These results are in agreement with the findings of Collins et al. (2015) who reported that exposure of adult flies of *B. tryoni* to methoprene and having access to the diet containing yeast hydrolysate advanced the sexual maturation for 2-days. Sterile males of *A. fraterculus* treated with methoprene attained sexual maturity earlier than untreated males and exhibited lekking behaviour and attractiveness to females as mature wild males (Segura et al., 2009; Liendo et al., 2013). The sterile males from M+P- and M-P- started participation in mating behaviour at 8-DAT and slightly increased their performance up to the age of 10-days. While on 11 and 12-days of age, least number of males was involved in mating behaviour due to lack of protein in their diet. Sterile males of *B. tryoni* fed on protein diet had higher levels of mating propensity than sugar fed males (Perez-Staples et al., 2008).

In the present study, high percentage of M+P+ sterile males had initiated (57.0) and participated (48.0) in leks. Similar results were reported by Yuval et al. (1998) that application of methoprene and exposure to protein increased male lek initiation and participation in *C. capitata*. The males that were accessed to protein without methoprene application (M-P+) performed better than M-P- and M+P- (protein deprived) males. Nearly 36.0 and 26.0% of protein fed sterile males (M-P+) involved in lek initiation and lek participation (Fig. 1). These results are comparable with the earlier reports that sterile males of *C. capitata* fed on protein diet are more likely to start and participate in leks compare to the males fed on protein deprived diet (Taylor and Yuval, 1999; Joachim-Bravo, 2009). The reason could be that protein-rich diet increased the sexual signaling of methoprene treated sterile males, in which 43.0% of M+P+ males were engaged in emission of pheromones to attract their mates. Methoprene treated and protein fed sterile males were more attractive to their mates with female acceptance index (FAI) of 0.82 at which M+P+ males achieved higher mating success(35.0%). Bachmann et al. (2017) also opined that young and aged females tended to mate more frequently with methoprene treated-males as they released larger amounts of pheromonal compounds *A. fraterculus*. Similar results were reported in *C. capitata* that methoprene and protein showed additive effect which enhanced the pheromone calling and mating success (Yuval et al., 1998).

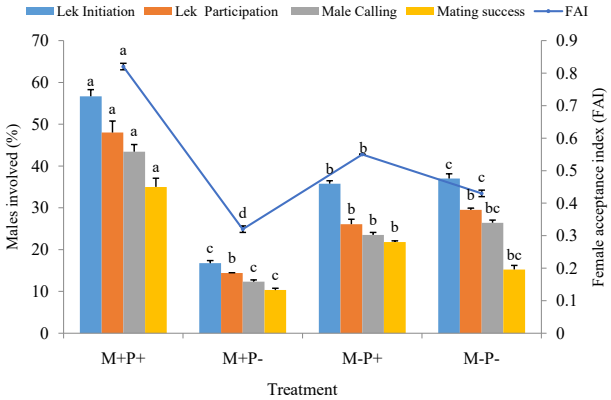

Fig. 1. Sexual performance of sterile males of *B. cucurbitae* exposed to methoprene (M+/M-) and adult dietary protein (P+/P-)

Only protein fed sterile males (M-P+) called earlier and had frequent matings (22.0%) as compared to males fed on sugar alone (i.e. M-P-) or treated with methoprene only (i.e. M+P-). It clearly shows that the diet containing yeast hydrolysate significantly increased the male signaling to attract females for mating. Aluja et al. (2001) found that males of *A. ludens* fed on combination of protein and dry sucrose were sexually active and had higher mating success. Sterile males of

C. capitata fed on diet containing yeast hydrolysate expended more energy during male calling than males fed on sucrose only (Warburg and Yuval, 1997; Kaspi et al., 2000). Role of protein on mating success of sterile males of *C. capitata* and found that protein fed sterile males secured highest copulation rates than the males fed on protein deprived diet (Joachim-Bravo et al., 2009). Sterile males of *B. tryoni* fed on protein diet achieved higher levels of mating probability and longer copulations than males fed on protein-deprived diet (Perez-Staples et al., 2007).

In the present study, application of methoprene and access to the protein (yeast hydrolysate) diet had showed a synergistic effect on mating behaviour of sterile males of melon fly. Hence, M+P+ males had initiated and participated more in aggregations. Synergistic interaction of methoprene and protein could accelerate the sexual maturity in sterile males of *Z .cucurbitae* and enhanced their mating success. Males of *A. suspensa* exposed to methoprene and hydrolyzed yeast (M+P+) emitted pheromones and attracted more females and had more successful mating than males of other treatments (Pereira et al., 2009) 31. Males of *Z. cucurbitae* from M+P+ treatment had higher mating propensity up to the age of 18-days. These results were on par with the report of Shelly et al. (2005) found that males of *B. dorsalis* fed on yeast hydrolysate up to 12-days had mating propensity up to 21 days of age. Recently, Bachmann et al. (2017) found that exposure of *A. fraterculus* males to methoprene and protein during pre-maturation period enhanced the male mating success by increased pheromone production. Further they found that methoprene treatment enhanced male sexual competitiveness even after the sexual maturation phase, and the effect did not decrease until males were older than 20 days.

The existence of male lures was reported approximately 100 years ago and most widely used in detection and management of tephritids. Typically, males emit pheromone to attract females and recent evidence indicates that naturally occurring male lures may function as precursors in pheromone synthesis. The idea that synthetic parapheromones mimic the male sex pheromone appears reasonable and shown to be valid (Wee et al., 2007).

Cue lure (4-[p-Acetoxyphenyl]-2-butanone) is a parapheromone and highly attractive to *B. curcubitae* males. Sterile males of *B. cucurbitae* fed with yeast hydrolysate during pre-copulatory period of 10-days and then exposed to cue lure (P+CL+) had mating advantage over the males fed on either sugar only (P-CL-) or cue lure alone (P-CL+). It was followed by the males (P+CL-) that were allowed to feed on protein diet without cue lure exposure (Fig. 2). The sterile males exposed to yeast hydrolysate for 12-days period following cue lure feeding were more profoundly involved in lek initiation, lek participation and male calling. Cue lure feeding increased the attractiveness of protein fed males to females and increased the mating success by 2- and 3-times higher compare to P-CL- and P-CL+ males, respectively (Fig. 3). Exposure of 14-day old protein fed males to cue lure enhanced mating performance compare to the males from other treatments (Fig. 4). These results were supported by previous reports that ingestion of methyl eugenol and trimedlure increased the mating success through increased level of male signaling in *B. dorsalis* and *C. capitata* males 35. Sterile males from P+CL+ had highest FAI due to their increased attractiveness to their mates at all the ages tested. Females readily accepted 10 and 12-days old P+CL+ sterile males with FAI of nearly 0.6, followed by P+CL- males. Where as in P-CL+ males were least preferred by their mates with FAI ranged between 0.13-0.25 (Fig. 5).

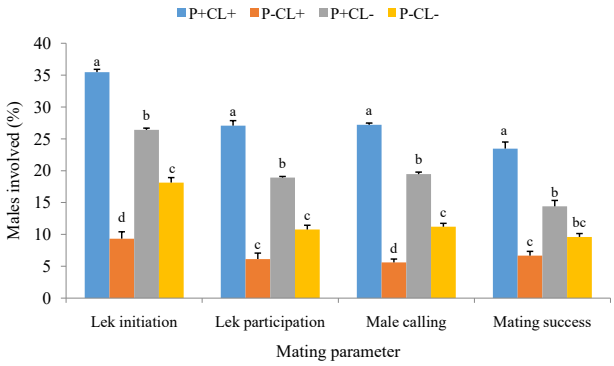

Fig. 2. Sexual performance of 10-days old sterile males exposed to dietary protein (P+/P-) and cue lure (CL+/CL-)

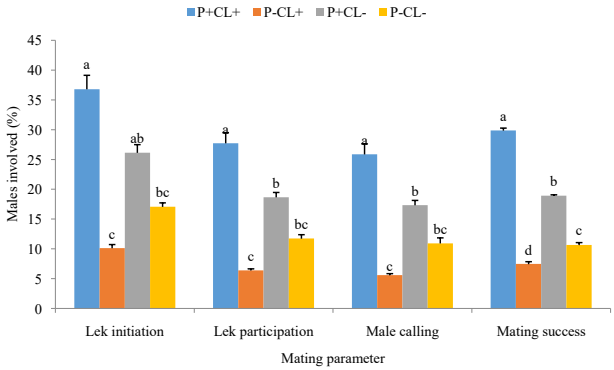

Fig. 3. Sexual performance of 12-days old sterile males exposed to dietary protein (P+/P-) and cue lure (CL+/CL-)

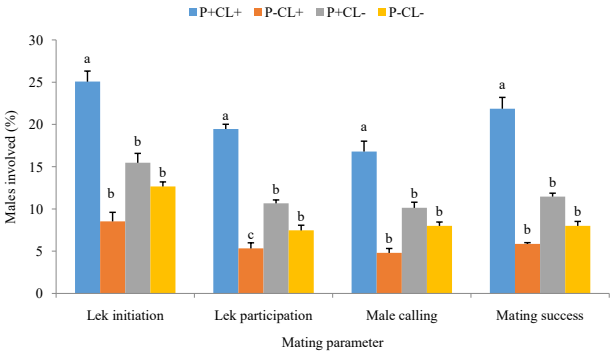

Fig. 4. Sexual performance of 14-days old sterile males exposed to dietary protein (P+/P-) and cue lure (CL+/CL-)

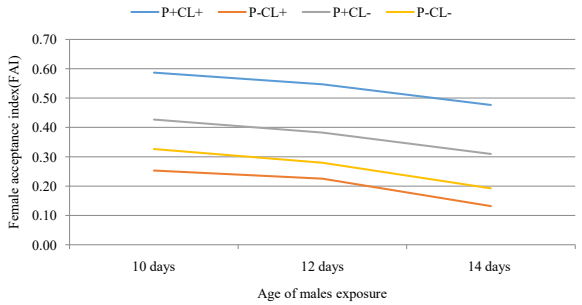

Fig. 5. Influence of adult dietary protein (P+/P-) and cue lure (CL+/CL-) on Female Acceptance Index of *B. cucurbitae* sterile males of different ages

Among the different age of males exposed, 10 and 12-days old P+CL+ males performed better in all the parameters tested as compared to 14-days old P+CL+ males. Exposure of cue lure to 12-days old protein fed males increased the lek initiation, lek participation, male calling and mating success by 28.0, 38.0, 47.0, and 34.0% over 14-days old P+CL+ sterile males. These results tally with the findings of Shelly and Villalobos (1995) that sterile males of 10-12 days old exposed to cue lure highly involved in male signalling and had high mating success on the day following exposure to cue lure. These results showed an additive effect of interaction of protein-rich diet and cue lure exposure on mating success of *B. cucurbitae.* Khoo and Tan (2000) that cue lure-fed males of *B. cucurbitae* attracted more females compared to males deprived of cue lure. The similar results were reported by Shelly et al. (1996) that the sterile *B. philippinensis* males exposed to methyl eugenol (ME) before release achieved more number of matings with females over wild males. In laboratory studies, it is also confirmed that exposure of P+ sterile males to ME+ increased the percentage of mating with wild females in *B. philippinensis* (Obra and Resilva, 2011). In all the age group exposed, sterile males fed on protein diet (P+CL-) had more matings than males fed only on sugar (P-CL-) or cue lure alone (P-CL+). Less percentage of P-CL+ males were involved in mating, compare to P-CL- males. Sterile males exposed to cue lure alone were least preferred by females for mating as cue lure treated males were not matured sexually due to lack of protein in their diet. It indicates that cue lure alone has adverse effect on FAI for sterile males in absence of protein in their pre-release diet. These results were on par with the findings of Orankanok et al. (2011) that sterile males fed on sugar–yeast hydrolysate combinations achieved significantly more matings than males fed only water in *B. dorsalis* and more matings than males fed only sugar, only yeast hydrolysate or only water in *B. correcta*. Recently, Haq et al. (2016) examined the effect of methyl eugenol treatment on the incidence of pairing in *B. dorsalis* and *B. carambolae* and found that Methyl eugenol feeding enhanced the competitiveness and increased the number of matings in *B. dorsalis*.

The positive influence of cue lure on sexual performance of *Z. cucurbitae* was short-lived compared to methoprene. Feeding of sterile males of *Z. cucurbitae* with protein-enriched diet up to 10-12 days of age and then exposure to cue lure enhanced their mating performance by increasing male signaling (male calling) and male attractiveness to females. The combinations of methoprene application and yeast hydrolysate-sugar diet showed an additive effect on increased mating success in *Z. cucurbitae* sterile males and a clear effect of methoprene exposure confirming the potential of this approach to improve operational SIT application against melon fly.

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AUTHOR CONTRIBUTION STATEMENT

KS and PGS conceived and designed research. PGS conducted experiments. SRK provided timely guidance in planning of the experiments. BP provided gamma radiation facilities.

CONFLICT OF INTEREST

No conflict of interest.

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