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EFFECT OF MALE IRRADIATION AND ITS MATING STATUS ON THE REMATING PROPENSITY, INSEMINATION QUALITY AND REPRODUCTIVE BEHAVIOUR OF MALE *SPODOPTERA LITURA* (F.)

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ABSTRACT

In this study various factors, viz., male irradiation, its mating status and post mating interval were studied on the remating propensity, insemination quality and reproductive behaviour of a noctuid pest *Spodoptera litura* (F.) that may govern the efficacy of radiation mediated inherited sterility (IS) technique proposed to control this pest. The male irradiation (substerilizing 130 Gy and sterilizing 250 Gy) affected the copulation duration, mating success and sperm transfer during mating. Multiple matings of male significantly depleted the sperm in fully sterile male followed by substerile male than control. During remating with virgin female, the reproductive performance of mated irradiated male, was more affected at 24 hr than at 48 hr inter mating interval, which might be due to associated physiological impact on mating behaviour and insemination quality. This study might be crucial in simulation modelling and optimization of successful implementation of IS programme.

Key words: *Spodoptera litura*, male irradiation, 130 Gy, 250Gy, insemination quality, apyrene and eupyrene sperm depletion, F1 sterility technique, multiple mating

For many insects, the optimal reproductive strategies differ between males and females, where males benefit from increased mating frequency (Parker and Birkhead, 2013) whereas the females maximize their egg production by selecting fewer but high quality mates. By acquiring multiple matings, males generally maximize mating success by providing an opportunity for each mating to result in an offspring. The multiple mating in males often has a significant impact on their lifelong reproductive success. According to recent studies, the males can experience significant costs such as sperm depletion and reduced survival due to multiple mating (Wedell et al., 2002). In remating males, successful fertilization may depend on their ability to replenish ejaculatory substances during each mating (Dewsbury 1982). The time interval between first and subsequent matings had a strong effect on the size and protein content of ejaculates (Bissoondath and Wiklund, 1996). The males transfer nutrients to females via ejaculates/ spermatophore, or other nuptial gifts during mating or copulation (Thornhill 1976; Thornhill and Alcock 1983) but the male reproductive potential has been limited by spermatogenesis (Wedell et al., 2002). During mating, the male moths transfer spermatophore containing sperm and accessory gland products to females. The male could affect a recipient's reproductive strategy (Dewsbury, 1982) by affecting female fertility, offspring size or quality, female remating behavior and female longevity (Parker and Simmons, 1989; Boggs, 1990; Wedell, 1996). The nutrients enclosed in spermatophores have been found in the eggs and soma of mated females (Boggs and Gilbert, 1979; Greenfield 1982; Wiklund et al. 1993). According to a meta-analysis of Lepidopterans, increasing mating frequency results in a steady decline in male ejaculate (sperm counts and/or spermatophores mass), which has a negative influence on female fecundity and fertility (Torres-Vila and Jennions, 2005).

The tobacco cutworm *Spodoptera litura* (F.) (Lepidoptera: Noctuidae), is a polyphagous noctuid agricultural and forest pest species. The chemical pesticides are the common method to control the pest population of *S. litura* but it has resulted in an increased resistance towards the insecticides and also affected the human health and environment (Shad et al., 2012). In this context, an eco-friendly bio-rational radio-genetic technique of pest control, commonly known as sterile insect technique (SIT) could be an appropriate option for pest management, which involves mass-rearing, sterilization and release of male moths in the infected area where sterile males must mate with wild females

and prevent them from reproducing by transferring inviable sperm in a sufficient ejaculate including accessory gland fluid (Knipling, 1955; Robinson, 2005). In intrinsically radioresistant Lepidoptera, the use of a high dose of gamma radiation to induce full sterility in male moths may diminish competitiveness and ejaculate quality in irradiated moths. Therefore, an alternate strategy known as F1 or Inherited sterility technique (IS), as a modified SIT was proposed to overcome the limitations of SIT by releasing sub-sterile males with the ability to cause greater sterility in their F1 generation to be used in pest suppression. Gamma radiation often resulted in reduced sperm transfer, which might be drastic if irradiated males mated repeatedly. Male multiple matings (polygamy) could result in aspermia (Hooper, 1989) and possible ejaculate depletion. For example, the sterile males of *Bactrocera cucurbitae* and *B. tryoni* rapidly reached sperm depletion after three consecutive matings (Haynes and Mitchell, 1977; Kuba and Itô, 1993; Radhakrisnan et al., 2009). These findings implied that the combined effect of irradiation and multiple male matings might result in a smaller quantity and/or lower quality of the ejaculate, which could affect the female post-mating behaviour and induction of sterility in F1 generation which might eventually influence the efficiency of this radio-genetic technique. In the present study, an attempt was made to ascertain the effects of male mating history and irradiation on the male reproductive investment in the form of ejaculates transferred during mating, and the insemination quality in the spermatophore (in terms of apyrene and eupyrene sperm transferred) during consecutive matings was examined with an inter-mating interval of 24 and 28 hr (post first mating) in relation to reproductive performance.

MATERIALS AND METHODS

A continuous culture of *S. litura* was maintained in the insectary at the Department of Zoology, University of Delhi, India. The larvae were reared on a semisynthetic diet under ambient environmental conditions with temperature of 27.0± 1°C, 75± 5% RH, and a photoperiod of 12:12 hr (light: dark), also precautionary measures were taken to avoid microbial infection (Seth and Sharma, 2001). Freshly emerged male moths (0-1 day old) were exposed to substerilizing dose of 130 Gy, proposed for the F1 sterility technique against *S. litura* and fully sterilizing dose of 250 Gy (Seth and Sehgal, 1993; Seth and Sharma, 2001) at a dose rate of 0.625-0.359 KGy/ hr in a Co^{60} Gamma Chamber-5000 (GC-5000) located in the radiobiological unit of the Institute of Nuclear Medicine and Allied Sciences (INMAS), Ministry of Defense, New Delhi. For control, the unirradiated male moths were exposed to the same environmental conditions as the irradiated males except the irradiation exposure. To examine the effect of male mating history and irradiation on ejaculate quality, the newly eclosed adults were placed in cohorts of 12-15 pairs in a Perspex-nylon cage (45 x 30 x 30 cm) in the following combinations: [i] unirradiated females (N♀) × unirradiated males (N♂) as control, [ii] N♀ × irradiated males ($T♂_{130}$), irradiated at substerilizing dose,130 Gy and [iii]] N♀ × irradiated males ($S♂_{250}$), irradiated at fully sterilizing dose, 250 Gy. During scotophase, the cages were inspected every 15 min for mating pairs under red light. Individuals observed to mate during that time were collected and held for use in remating trials. Once copulation was terminated, the females were immediately put into a freezer, whereas males were transferred to holding cages where they were fed *ad libitum* until the day they were to be remated.

For remating trials, since no males mated twice in a day; all males mated for the first time (control or irradiated mated male moths) were given the opportunity to remate either after 24 or 48 hr post initial mating with normal females in a cohort of 10-12 pairs/ cage (45x 30x 30 cm). For the purpose of remating, the mated males and virgin females were kept in cages and were inspected every 15 min for mating pairs. Once a copulating pair was detected, the time of initiation and cessation of copulation was recorded to determine the copulation duration. Immediately after mating termination, the females were dissected for the presence of spermatophore in the bursa copulatrix. To examine the number of sperm transferred during mating of moths in a specific regimen, the female moths were dissected in Belar's saline. To count the number of eupyrene sperm bundles, the contents of the spermatophores were spread on a microscope slide for examination by phase contrast microscope. Whereas to determine the number of apyrene sperm (loose sperm) the content of spermatophore was diluted in known volume of Belar's saline and ten 10 ul aliquots were removed from each spermatophore to count the apyrene sperm number by Haemocytometer, and an average reading of these ten aliquots constituted one replicate. The biological replication was performed seven times for examining the ejaculate quality related to sperm number and an average reading in a cohort of 4-5 females in each regimen (control and irradiated) after inter-mating intervals of 24 and 48 hr post initial mating with virgin male constituted one replicate. For ascertaining the

mating and remating success, each replicate comprised of 12-15 pairs of moths in a specific regimen and nine replicates were conducted to compute the results.

Mated females from each regimen were placed in a cage and were provided with an oviposition substrate of castor leaf and 10% honey as a source of food. Each cage was monitored for ovipositional response and the eggs laid were recorded daily. For each experimental regimen, an average reading of oviposition per female from each cage of 4-5 pairs constituted one replicate, and an average reading from 6-7 egg samples from each such cage for testing egg hatchability constituted one replicate. This experiment was repeated seven times for each condition. All statistical analyses were performed using Graph Pad Prism software (version 9.0). One-way ANOVA, followed by Tukey's multiple comparisons was performed on different regimens to analyze the differential effect of multiple matings of irradiated males with different inter-mating interval (24 and 48 hr after first mating) on the male ejaculate quality and other reproductive parameters, in relation to control. The percentage data were arcsine transformed before ANOVA and the data in the table were back transformations.

RESULTS AND DISCUSSION

In this study the effect of multiple matings of males with different inter-mating interval (24 and 48 hr after first mating) was observed on the copulation duration and mating success in irradiated male moths with respect to control (unirradiated male moths). In control (unirradiated moths), the copulation duration with virgin females was found to be affected by the male mating history, the previously mated males took longer time than the virgin males (Table 1). The duration spent in copula during remating (with virgin female) increased to ca. 83 min at post mating interval of 24 hr and ca. 72 min at post mating interval of 48 hr in comparison to first mating in control (untreated male moths) The number of days between consecutive matings significantly affected the copulation duration of second matings. The copulation duration was also influenced by the male irradiation status. The duration of mating of treated males (with virgin untreated females) was increased by ca. 21% in sub-sterile male (130 Gy) and ca. 36% in fully sterile male (250 Gy) in comparison to control (first mating with unirradiated male. The copulatory period of mated irradiated male moth was also increased during remating in both the radiation regimen. The average duration spent in copula during remating was increased by 30-35% at intermating interval of 24 hr and by 15-25% at intermating interval of 48 hr in comparison to first mating of irradiated male moths (130 and 250 Gy) (Table 1). The mating duration of the irradiated male moths (with virgin normal females) was shortened as the post first mating interval was increased. However, the copulation duration was notably increased during the remating of normal as well as irradiated male moths.

The prolonged copulation during remating might be a form of mate guarding (Drummond, 1984) whereby the male ensures that the physiological transition of the female from sexually receptive to a non-receptive state is initiated. An extended copulation might also indicate inadequate ejaculate quantity or difficultly in transferring spermatophore during mating (Sims, 1979). Also, involving the female in a lengthy copulation might effectively limit the females to copulate only once in a day (Vera et al., 2003). The male mating history also influenced the mating success of males, as the remating

Table 1. Influence of irradiation and male mating status on the copulation duration of *S. litura* during its sequential matings

Radiation regimen	Copulation duration (min)			
	First mating (V♂xV♀)	Remating		
		24 hr later (M♂xV♀)	48 hr later (M♂xV♀)	
N♀xN♂	55.33± 2.93 Aa	83.43± 2.92 Ac	71.91± 1.47 Ab	F(2,18)=35.34*
N♀xT♂$_{130}$	67.02± 2.02 Aa	90.71± 4.07 ABc	78.57± 4.04 Ab	F(2,18)=11.38*
N♀xS♂$_{250}$	75.36± 2.14 Ba	98.64± 4.55 Bb	92.86± 3.43 Bb	F(2,18)=12.73*
	F(2,18)=17.61*	F(2,18)=3.79*	F(2,18)=11.36*	

Means ± SE followed by same small letters within a row not significantly different at $p<0.05$; Means± SE followed by same capital letters within a column not significantly different at $p<0.05$ (One way ANOVA followed by Tukey's post hoc test); n=7, N-normal (unirradiated), V-virgin, M- mated, T♂$_{130}$- Male moth irradiated at 130Gy; S♂$_{250}$- Male moth irradiated at 250Gy; *-denotes a significant difference at $p \leq 0.05$

success of mated males was found to be decreased as compared to the first mating of the virgin males. Similar observation was also noticed in *D. melanogaster* (Markow, et al., 1978), *Cnephasia jactatana* (Jimenez and Wang, 2004), *Heliothis virescens* (Klepetka and Gould, 1996) and *Spalangia endius* (King and Fischer, 2009). Gamma radiation also influenced the mating success of male. The fully-sterile males were less successful in mating as compared to the sub-sterile males and unirradiated males (Table 2). The reduced mating success as a function of increasing gamma dose was also reported in *Epiphyas postvittana* (Stringer et al., 2013). The remating success of mated irradiated males was also reduced as compared to first mating of the virgin irradiated males. Further, the remating success of irradiated (130Gy, 250Gy) as well as normal (unirradiated) male moths at 24 hr post first mating interval was markedly reduced as compared to 48h post mating interval (Table 2).

These results suggested that even though the males of *S. litura* were capable of multiple matings, they did not remate with same propensity at all intermating intervals, that might be attributed to the limited amount of the ejaculate resources to be invested over consecutive matings. In this study, the effect of consecutive matings with different inter-mating interval (24 and 48 hr after initial mating) on the sperm number in successive ejaculates of the *S. litura* was also examined in irradiated (sub-sterile and fully sterile) male moths in comparison with control (unirradiated male moths). The amount of eupyrene sperm bundles and apyrene sperm transferred during mating via spermatophores was significantly reduced in the mated male moths in compared to virgin male moths (Table 3, 4) as also reported by Seth et al. (2002b) in *S. litura*. The ability of a mated male to transfer the sperm presumably might get reduced after each mating, indicating that male would fail to replenish their sperm supply fully after mating

Table 2. Influence of irradiation and male mating status on the mating success of *S. litura* during its sequential matings

Radiation regimen	Mating success (%)			
		Remating		
	First mating (V♂xV♀)	24 hr later (M♂xV♀)	48 hr later (M♂xV♀)	
N♀xN♂	72.22± 3.6 Aa	52.78± 3.5 Ab	58.33± 2.9 Ab	F(2,24)=8.95*
N♀xT♂$_{130}$	61.11± 3.7 ABa	41.67± 2.4 Bb	55.56± 3.5 Aab	F(2,24)=9.49*
N♀xS♂$_{250}$	47.22± 2.9 Ba	33.33± 2.6 Bb	41.67± 2.5 Aab	F(2,24)=6.85*
	F(2,24)=13.43*	F(2,24)=11.53*	F(2,24)=8.88*	

Means± SE followed by same small letters within a row not significantly different at p<0.05; Means± SE followed by same capital letters within a column not significantly different at p<0.05 (One way ANOVA followed by Tukey's post hoc test); n=9; An average reading for mating success from a cohort of 12-15 pairs constituted one replicate; N-normal (unirradiated), V-virgin, M- mated, T♂$_{130}$- Male moth irradiated at 130 Gy; S♂$_{250}$- Male moth irradiated at 250 Gy; *-denotes a significant difference at p ≤ 0.05

Table 3. Influence of irradiation and male mating status on the eupyrene sperm transferred by males of *S. litura* during its sequential matings

Radiation regimen	Eupyrene sperm bundles transferred to female via spermatophore			
		Remating		
	First mating (V♂xV♀)	24 hr later (M♂xV♀)	24 hr later (M♂xV♀)	
N♀xN♂	351.7± 8.72 Aa	86.14± 7.636 Ac	199.9± 11.37 Ab	F(2,18)=202*
N♀xT♂$_{130}$	308.4± 10.03 Ba	69.29± 6.69 Ac	158.6± 10.36 Bb	F(2,18)=60.44*
N♀xS♂$_{250}$	159.3± 12.18 Ca	29.86± 4.95 Bc	64.43± 5.47 Cb	F(2,18)=66.43*
	F (2,18)=106.2*	F (2,18)=19.63*	F (2,18)=54.26*	

Means ± SE followed by same small letters within a row ot significantly different at p<0.05; Means± SE followed by same capital letters within a column not significantly different at p<0.05 (One way ANOVA followed by Tukey's post hoc test); n=7; An average reading for eupyrene sperm bundles transferred from a cohort of 4-5 pairs constituted one replicate; N-normal (unirradiated), V-virgin, M- mated, T♂$_{130}$- Male moth irradiated at 130Gy; S♂$_{250}$- Male moth irradiated at 250Gy; *-denotes a significant difference at p ≤ 0.05

Table 4. Influence of irradiation and male mating status on the apyrene sperm transferred by males during its sequential matings.

Radiation regimen	Apyrene sperm x 10^3 transferred to female via spermatophore			
		Remating		
	First mating (V♂xV♀)	24h later (M♂xV♀)	48h later (M♂xV♀)	
N♀xN♂	281.8±7.12 Aa	127.4± 6.40 Ac	231.3± 11.13 Ab	F (2,18)=104.6*
N♀xT♂$_{130}$	227.1± 7.59 Ba	98.21± 9.08 Bc	171.9± 11.79 Bb	F (2,18)=30.82*
N♀xS♂$_{250}$	126.3± 8.37 Ca	49.37± 4.25 Cc	86.29± 4.27 Cb	F (2,18)= 57.52*
	F (2,18)=78.72*	F (2,18)=32.43*	F (2,18)=56.68*	

Means ± SE followed by same small letters within a row not significantly different at $p<0.05$; Means ± SE followed by same capital letters within a column not significantly different at $p<0.05$ (One way ANOVA followed by Tukey's post hoc test); n=7; An average reading for apyrene sperm transferred from a cohort of 4-5 pairs constituted one replicate; N-normal (unirradiated), V-virgin, M- mated, T♂$_{130}$- Male moth irradiated at 130Gy; S♂$_{250}$- Male moth irradiated at 250Gy; *-denotes a significant difference at $p \leq 0.05$

and males need time to mobilize and/or replenish it. The extent of decline in sperm supply during remating was influenced by the interval between consecutive matings. With increase in inter-mating interval there was a significant increase in sperm number observed in irradiated and normal males as noticed in the present study.

Seth et al. (2002b) showed in *S. litura* that the decrease in the number of eupyrene sperm bundles in the duplex on the day after mating might be correlated with the estimates of the number of bundles transferred to the female in the spermatophore during mating. The increased sperm transferred during remating of male after an interval of 48 hr could be due to accumulation of sperm into duplex as Seth et al. (2002b) showed that the rate or extent of sperm descent from the testis to the upper vas deferens (UVD) in adult male *S. litura* did not get affected by mating.

Male moths irradiated with high dose of fully sterilizing gamma radiation (250 Gy) transferred significantly less sperm than male irradiated with substerilizing, 130 Gy. Irradiated males mated to normal females showed similar trend in sperm (eupyrene sperm bundles and apyrene sperm) transfer during remating as observed in unirradiated male (Table 3, 4). Also, the irradiated male moths transferred significantly less amount of sperm during their first as well as subsequent matings when compared with consecutive matings of unirradiated males. Likewise, the increased sperm depletion with subsequent matings of the irradiated male moths was also noticed in *Bactrocera tryoni* (Radhakrishnan et al., 2009). The reduced number of sperms transferred could be due to effect of irradiation on sperm production and/ or sperm descent from the testes (Seth et al., 2002a). The extent of decline in sperm transfer was found to be influenced by irradiation dose (130, 250 Gy) as well as the interval between the consecutive mating (24, 48 hr post initial mating) in *S. litura.*

In Lepidoptera, the newly emerged adult males have sperm and late spermatid stages (Holt and North 1970; Ashrafi and Roppel, 1973). The radiosensitivity of germ cells has been classified, in decreasing order, as follows: spermatogonia cells, spermatocytes, spermatids and the most resistance stage is the mature sperm (Hodges, 1980). Therefore, if newly emerged adult males are irradiated, their spermatids would be more affected and would suffer greater damage than the mature sperm which might be one of the reasons in the decline of sperm transfer observed during subsequent mating of irradiated moths and the effect was more pronounced at high dose of gamma radiation (250 Gy). Further, the effect of male mating history on the oviposition behavior and egg viability was investigated in *S. litura* by pairing normal females with virgin and mated males at different intermating interval of 24 and 48 hr vis-à-vis first mating. The oviposition (eggs laid/ mated female) and egg fertility were reduced in case of females crossed with mated than virgin male. These reproductive responses (ovipositional response and egg fertility) in *S. litura* were more adversely affected due to remating of the mated male at 24 than 48 hr intermating interval (Table 5, 6). Similar influence of the male mating history on the oviposition of females was observed in *Colias eurytheme* (Rutowski et al., 1987), *Ostrinia nubilalis* (Royer and McNeil, 1993) and *Helicoverpa armigera* (Hou and Sheng, 1999), as well as the egg viability of *H. virescens* (Henneberry and Clayton, 1985), *O. nubilalis* (Royer and McNeil, 1993)

Table 5. Influence of irradiation and male mating status on the oviposition of females of *S. litura* crossed with these males during its sequential matings

Radiation regimen	Oviposition per female			
	Remating			
	First mating (V♂xV♀)	24 hr later (M♂xV♀)	24 hr later (M♂xV♀)	
N♀xN♂	1685± 31.13 Aa	933.1± 25.96 Ac	1489± 24.42 Ab	F(2,18)=203.9*
N♀xT♂$_{130}$	1118± 27.64 Ba	378.2± 34.24 Bc	871.3± 30.29 Bb	F(2,18)=148.8*
N♀xS♂$_{250}$	741± 28.04 Ca	181± 26.46 Cc	389± 18.28 Cb	F(2,18)=132.2*
	F(2,18)=268.9*	F(2,18)=179.2*	F(2,18)=493.6*	

Means ± SE followed by same small letters within a row are not significantly different at p<0.05; Means ± SE followed by same capital letters within a column not significantly different at p<0.05 (One way ANOVA followed by Tukey's post hoc test); n=7; An average reading for oviposition (eggs/mated female) from a cohort of 4-5 pairs constituted one replicate; N-normal(unirradiated), V-virgin, M- mated, T♂$_{130-}$ Male moth irradiated at 130Gy; S♂$_{250-}$ Male moth irradiated at 250Gy; *-denotes a significant difference at p ≤ 0.05

Table 6. Influence of irradiation and male mating status on the egg fertility in females crossed with these males during its sequential matings

Radiation regimen	Egg fertility (%)			
	Remating			
	First mating (V♂xV♀)	24 hr later (M♂xV♀)	24 hr later (M♂xV♀)	
N♀xN♂	85.48± 3.55 Aa	67.03± 2.89 Ab	76.41± 3.94 Aab	F(2,18)=6.99*
N♀xT♂$_{130}$	45.49± 2.03 Ba	11.32± 2.5 Ac	29.47± 1.03 Bb	F(2,18)=85.71*
N♀xS♂$_{250}$	0Ca	0Ca	0Ca	F(2,18)=1
	F(2,18)=328.1*	F(2,18)=264.5*	F(2,18)=268.6*	

Means ± SE followed by same small letters within a row not significantly different at p<0.05 level; Means ± SE followed by same capital letters within a column not significantly different at p<0.05 level (One way ANOVA followed by Tukey's post hoc test); n=7; An average reading for egg fertility from a cohort of 4-5 pairs constituted one replicate; N-normal(unirradiated), V-virgin, M- mated, T♂$_{130-}$ Male moth irradiated at 130 Gy; S♂$_{250-}$ Male moth irradiated at 250 Gy; *-denotes a significant difference at p ≤ 0.05.

and *Epiphyas postvittana* (Foster and Ayers, 1996). In these examples, both oviposition and egg viability of female mated to previously mated males got decreased.

The irradiation of male moths had a gradational effect on the ovipositional response and egg fertility with drastic impact at 250 Gy (fully sterilizing dose) than 130 Gy (partially sterilizing dose) (Table 5,6). The oviposition and egg fertility of female copulated with mated irradiated male moths was significantly reduced than those mated with virgin irradiated male moths. The most apparent explanation for the effect of male mating history on female ovipositional response might be the reduced ejaculate quality in form of sperms transferred to female during insemination. The present findings showed that females might alter their investment on egg production based on male insemination quality. The females who receive low sperm count are more likely to have lower reproductive output, demonstrating cryptic female choice through differential allocation based on the quality of her mate and/ or the time and energy they devote to attract another mate (Wedell, 1996; Wedell and Karlsson, 2003). Even though the male reproductive success could be related to the number of females they would mate, the multiple matings assessed in *S. litura* in this study indicated a lower sperm count with reduced fertilization ability. Similar observation was also reported in *S. littoralis* (Sadek, 2001). Further, as per the present study, the irradiated males seemed to exert its sterilizing effect in its first mating through dominant lethal mutations (DLM), although further added impact on its reproductive performance was apparent in its sequential remating, presumably due to associated physiological adversity in sperm dynamics.

In this study, the findings reflected that male mating history (virgin or mated) and irradiation affected the ejaculate quality and sperm transfer that would be vital in the induction of sterility and mating competitiveness of the irradiated moths to be employed in IS programme.

The results also indicated that the mating could be costly to male as copulation depleted the male access to ejaculate constituents as evidenced by the fact that copulation durations were longer and ejaculates smaller in matings involving the mated males. Hence, this study might help in better understanding of SIT/ IS technique for *S. litura* and optimize its operational logistics.

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AUTHOR CONTRIBUTION STATEMENT

RKS conceived and designed research. NA conducted experiments. NV and MS assisted in the conducting experiments and validation of data. RKS and NA analyzed the data and involved in writing, editing and reviewing the manuscript. All authors read and approved the manuscript.

CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

REFERENCES

Ashrafi S H, Roppel R M. 1973. Radiation-induced partial sterility related to structurally abnormal sperms of *Plodia interpunctella* (Lepidoptera: Phycitidae). Annals of the Entomological Society of America 66(6): 1309-1314.

Bissoondath C J, Wiklund C. 1996. Effect of male mating history and body size on ejaculate size and quality in two polyandrous butterflies, *Pieris napi* and *Pieris rapae* (Lepidoptera: Pieridae). Functional Ecology 457-464.

Boggs C L, Gilbert L E. 1979. Male contribution to egg production in butterflies: evidence for transfer of nutrients at mating. Science 206(4414): 83-84.

Boggs C L. 1981. Selection pressures affecting male nutrient investment at mating in heliconiine butterflies. Evolution 931-940.

Boggs C L. 1990. A general model of the role of male-donated nutrients in female insects reproduction. The American Naturalist 136(5): 598-617.

Dewsbury D A. 1982. Ejaculate cost and male choice. The American Naturalist 119(5): 601-610.

Drummond B A. 1984. Multiple mating and sperm competition in the Lepidoptera. Sperm competition and the evolution of animal mating systems. pp. 291-370.

Foster S P, Ayers R H. 1996. Multiple mating and its effects in the lightbrown apple moth, *Epiphyas postvittana* (Walker). Journal of Insect Physiology, 42(7): 657-667.

Greenfield M D. 1982. The question of paternal investment in Lepidoptera: male-contributed proteins in *Plodia interpunctella*. International Journal of Invertebrate Reproduction 5(6): 323-330.

Haynes J W, Mitchell E B. 1977. Fractionated irradiation of boll weevils during pupal development: effect of sperm depletion and transfer as measured by female responsiveness. Journal of Economic Entomology 70(4): 411-412.

Henneberry T J, Clayton T E. 1985. Tobacco budworm moths (Lepidoptera: Noctuidae): effect of time of emergence, male age, and frequency of mating on sperm transfer and egg viability. Journal of Economic Entomology 78(2): 379-382.

Hodges R J. 1980. Spermatogenesis in normal and X-irradiated *Dermestes frischii* Kugelann (Coleoptera) and the effects on breeding of X-irradiation of the males. United Kingdom

Holt G G, North D T. 1970. Effects of gamma irradiation on the mechanisms of sperm transfer in *Trichoplusia ni*. Journal of Insect Physiology 16(12): 2211-2222.

Hooper G H S. 1989. The effect of ionizing radiation on reproduction. World crop pests 3. pp.153-164.

Hou M, Sheng C.1999. Fecundity and longevity of *Helicoverpa armigera* (Lepidoptera: Noctuidae): effects of multiple mating. Journal of Economic Entomology 92(3): 569-573.

Jiménez-Pérez A. Wang Q. 2004. Male remating behavior and its effect on female reproductive fitness in *Cnephasia jactatana* Walker (Lepidoptera: Tortricidae). Journal of Insect Behavior 17(5): 685-694.

King B H, Fischer C R. 2010. Male mating history: effects on female sexual responsiveness and reproductive success in the parasitoid wasp *Spalangia endius*. Behavioral Ecology and Sociobiology 64(4): 607-615.

Klepetka, Gould F. 1996. Effects of age and size on mating in *Heliothis virescens* (Lepidoptera: Noctuidae): implications for resistance management. Environmental Entomology 25(5): 993-1001.

Knipling E F. 1955. Possibilities of insect control or eradication through the use of sexually sterile males. Journal of Economic Entomology 48(4): 459-462.

Kuba H, Itô Y. 1993. Remating inhibition in the melon fly, *Bactrocera* (= Dacus) *cucurbitae* (Diptera: Tephritidae): copulation with spermless males inhibits female remating. Journal of Ethology 11(1): 23-28.

Markow T A, Quaid M, Kerr S. 1978. Male mating experience and competitive courtship success in *Drosophila melanogaster*. Nature 276(5690): 821-822.

Parker G A, Simmons L W. 1989. Nuptial feeding in insects: theoretical models of male and female interests. Ethology 82(1): 3-26.

Radhakrishnan P, Pérez-Staples D, Weldon C W, Taylor P W. 2009.

Multiple mating and sperm depletion in male Queensland fruit flies: effects on female remating behaviour. Animal Behaviour 78(4): 839-846.

Parker G A, Birkhead T R. 2013. Polyandry: the history of a revolution. Philosophical Transactions of the Royal Society B: Biological Sciences 368(1613): 20120335.

Robinson A S. 2005. Genetic basis of the sterile insect technique. Sterile insect technique (pp. 95-114). Springer, Dordrecht.

Royer L, McNeil J N. 1993. Male investment in the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae): impact on female longevity and reproductive performance. Functional Ecology. pp. 209-215.

Rutowski R L, Gilchrist G W, Terkanian B. 1987. Female butterflies mated with recently mated males show reduced reproductive output. Behavioral Ecology and Sociobiology 20(5): 319-322.

Sadek M M. 2001. Polyandry in field-collected *Spodoptera littoralis* moths and laboratory assessment of the effects of male mating history. Entomologia Experimentalis et Applicata 98(2): 165-172.

Seth R K, Sehgal S S. 1993. Partial sterilizing radiation dose effect on the F 1 progeny of *Spodoptera litura* (Fabr.): Growth, bio-energetics and reproductive competence. Management of insect pests: Nuclear and related molecular and genetic techniques.

Seth R K, Sharma V P. 2001. Inherited sterility by substerilizing radiation in *Spodoptera litura* (Lepidoptera: Noctuidae): Bioefficacy and potential for pest suppression. Florida. Entomologist 84(2): 183-193.

Seth R K, Kaur J J, Rao D K, Reynolds S E. 2002a. Sperm transfer during mating, movement of sperm in the female reproductive tract, and sperm precedence in the common cutworm *Spodoptera litura*. Physiological Entomology 27(1): 1-14.

Seth R K, Rao D K, Reynolds S E. 2002b. Movement of spermatozoa in the reproductive tract of adult male *Spodoptera litura*: daily rhythm of sperm descent and the effect of light regime on male reproduction. Journal of Insect Physiology 48(1): 119-131.

Shad S A, Sayyed A H, Fazal S, Saleem M. A, Zaka S M, Ali M. 2012. Field evolved resistance to carbamates, organo-phosphates, pyrethroids, and new chemistry insecticides in *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). Journal of Pest Science 85: 153-162.

Sims S R. 1979. Aspects of mating frequency and reproductive maturity in *Papilio zelicaon.* American Midland Naturalist. pp.36-50.

Stringer L D, Sullivan N J, Sullivan T E, Mitchell V J, Manning L A M, Mas F, Hood-Nowotny R C, Suckling D M. 2013. Attractiveness and competitiveness of irradiated light brown apple moths. Entomologia Experimentalis et Applicata 148(3): 203-212.

Thornhill R, Alcock J. 1983. The Evolution of Insect Mating Systems. Harvard University Press, Cambridge, MA

Thornhill R. 1976. Sexual selection and paternal investment in insects. The American Naturalist 110 (971): 153-163.

Torres-Vila L M, Jennions M D. 2005. Male mating history and female fecundity in the Lepidoptera: do male virgins make better partners? Behavioral Ecology and Sociobiology 57(4): 318-326.

Vera M T, Cladera J L, Calcagno G, Vilardi J C, Mcinnis D W. 2003. Remating of wild *Ceratitis capitata* (Diptera: Tephritidae) females in field cages. Annals of the Entomological Society of America 96(4): 563-570.

Wiklund C, Kaitala A, Lindfors V, Abenius J. 1993. Polyandry and its effect on female reproduction in the green-veined white butterfly (*Pieris napi* L.). Behavioral Ecology and Sociobiology 33(1): 25-33.

Wedell N. 1996. Mate quality affects reproductive effort in a paternally investing species. The American Naturalist 148(6): 1075-1088.

Wedell N, Gage M J, Parker G A. 2002. Sperm competition, male prudence and sperm-limited females. Trends in ecology and evolution 17(7): 313-320.

Wedell N, Karlsson B. 2003. Paternal investment directly affects female reproductive effort in an insect. Proceedings of the Royal Society of London. Series B: Biological Sciences 270 (1528): 2065-2071.

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