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Studies on the field efficacy of gamma irradiated male *M. vitrata* (Fabricius) (Lepidoptera: crambidae) in comparison with chemical spray

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Abstract
In recent years *Maruca vitrata* is becoming predominant insect pest in all pigeon pea growing areas of India. It was controlled primarily through use of chemical insecticides. But their effectiveness is hindered by the tight larval webbing that reduces pesticide exposure. So an eco friendly or autocidal method is one of the best options. In an effort to continue the development of a sterile insect release program against the spotted pod borer, the present study was conducted in field cages, during flowering stage of the pegion pea crop, ten pairs of normal *M. vitrata* were released in all the four blocks. Later irradiated males release and multinemor 0.15 per cent spray was imposed each of them alone and in combination. Results suggest that efficacy of gamma irradiated male *Maruca* moths in comparison with multinemor spray indicated combined effect irradiated moths and multinemor at 0.15 per cent spray which recorded significantly lower incidence, pod damage and high grain yield as compared gamma irradiation or multinemor 0.15 per cent spray each of them alone.

Keywords: *M. vitrata*, sterile insect technique, gamma irradiation, multinemor spray

Introduction
Pigeon pea *Cajanus cajan* (L.) Millsp. is one of the protein-rich legumes of the semi-arid tropics grown throughout the tropical and subtropical regions of the world. pigeon pea can be grown between 14^0N to 28^0N lattitude, with a temperature ranging from 26^0 to 30^0 in the rainy season . Globally, it is grown on 4.7 m ha area with an annual production of 3.69 mt in about 50 countries [3].

Despite the importance of pigeon pea in semi-arid regions of the world, little concerted research effort has been directed towards pigeon pea crop improvement. A number of factors are responsible for the poor productivity including pod borer damage, wilt incidence, lack of knowledge about IPM, non- availability of good quality seed, non-availability of biocontrol agents, poor crop husbandry, majorly insect pest and disease/damage [19].

Pigeon pea crop is attacked by a wide variety of pests. Insect pest complex cause adequate economic damage leading to very low yield levels of 500 - 800 kg/ha as against the potential yield of 1800 - 2000 kg/ha [12, 29].

The most important pests are pod borer, *Helicoverpa armigera* (Hubner), spotted pod borer, *Maruca vitrata* **(**Fabricius). The gram pod borer and the pod fly, *Malanagromyza obtusa* (Malloch) are the major pest species inflicting damage to the economic parts of the plant. The spotted pod borer, *Maruca vitrata* (Lepidoptera: Crambidae) is an important pantropical pest of grain legumes and has a wide distribution throughout Africa, Asia, South America, and the southern states of Australia [26].

In recent years *Maruca vitrata* (Fabricius) (Lepidoptera:crambidae) is becoming predominant insect pest in all pigeon pea growing areas of India. This pest is a major factor responsible for heavy yield loss in early and medium late maturing pigeon pea genotypes [23, 25]. Larvae feed by remaining inside the webbed mass of leaves, flowers and pods. This typical feeding habit protects larvae from natural enemies and other adverse factors including insecticides. In pigeon pea yield loss varied between 70 –80 per cent yield loss [27]. The cryptic behaviour of the larvae, the cost, development of resistant genotypes, and the environmental risks associated with excess use of chemical insecticides impose serious limitations to control this insect pest [4]. A multifaceted approach is required for the control of this pest because other control measures are inadequate when applied alone.

The sterile insect technique (SIT) has been used for Lepidoptera but insects in this order are radio-resistant, presumably due to their holokinetic chromosomal configuration [6]. Therefore, lepidopterans require large doses of radiation for sterilization, leading to somatic damage and reduced competitiveness in the irradiated insect.

A favoured alternative to using fully sterile moths in SIT is the use of F_1 sterility. F_1 survivor progeny of sub-sterile parental (P) male moths result when sub-sterilizing doses of radiation are applied to the P males. The resulting F_1 progeny are more sterile than the irradiated parent, and the irradiated moths are more competitive as a result of receiving a lower dose of radiation. Inherited sterility in the progeny of treated males has been shown to have potential in suppressing populations of lepidopteron pests [20, 10, 21, 11].

The degree of competitiveness of irradiated males is of crucial importance in eradication or suppression experiments evaluating the sterile-male release method. If the sterilizing treatment reduced competitiveness, then the number of insects to be released must be increased to effectively attain the over flooding ratio considered necessary to produce a downward trend in the target population. Thus, an evaluation of the competitiveness of males having the desired level of sterility is an integral part of research into the sterile male release technique. The lack of data on efficacy of irradiated male *Maruca vitrata* in comparison with chemical spray has prevented the attempt of any large-scale field trials.

The purpose of the present study was to provide much needed information on efficacy of irradiated adult males of *M.vitrta* in comparision with chemical spray. Gamma dose of 150Gy was selected for this studies based on the previous studies conducted by my senior Pratap gowda on radio-biological studies of *Maruca vitrata* (Fabricius) (LEPIDOPTERA: CRAMBIDAE). He reported that this 150 Gy dose induced 49.9 ±4.1 per cent corrected sterility in parental and 66.7±2.74 per cent in F_1 population.

2. Materials and Methods

Present investigations on “Studies on the field efficacy of gamma irradiated male *M. vitrata* (Fabricius) (Lepidoptera: crambidae) in comparison with chemical spray” were carried outduring 2016-17 at University of Agricultural Sciences, Raichur, Karnataka, India.

Rearing of *M. vitrata* on semi synthetic diet

Maruca vitrata larvae were reared on semi synthetic diet as suggested by Baramappa (2015). The ingredients used for preparing the diet are listed in Table 1. The detailed procedure of rearing is explained below.

Field collected larvae were reared on pigeon pea flowers and pods under the laboratory conditions of 26 ± 1 °C temperature with a photoperiod of 14:10 L:D and 60 ± 10 per cent relative humidity to establish initial culture. The pupae obtained along with webs were kept in a plastic container with a cotton padding (bedding material) for adult emergence. Newly emerged adults were released in wooden cages of 60 x 30 x 30 cm size and were fed with honey solution (10 %) soaked in cotton and kept inside the cage. Fresh tender twigs and inflorescence of pigeon pea were placed in water filled container with hole on the lid to hold tender twigs upright. The container with such inflorescence were kept in cages for egg laying, inflorescence were changed daily. The flowers, flower buds and tender leaves were examined for the presence of egg masses and such egg masses were placed on moist filter paper (Whatmann no. 41) kept in Petri plates. This larval culture was then reared on artificial diet in a box of 10 cm diameter for one generation to obtain pure culture. This pure culture was further utilized for investigations on radio-biological studies in the laboratory conditions mentioned above.

The field experiment was conducted to study the efficacy of gamma irradiated male *M. vitrata* in comparison with chemical spray. Pigeon pea crop was raised in 360 square meter area as per package of practices. Prior to flowering stage the crop was covered with nylon nets of size 60 x 60 m^2. There were four treatments; for each treatment five replications were maintained. In all the four treatments ten pairs of normal *M. vitrata* were released and treatments were imposed as mentioned below. Observations were made on different parameters *viz*., number of webs, number of pods damaged and grain yield per plot.

Table 1: Release of ten normal pair of *M. vitrata* in all 4 treatments at flowering stage.

Treatments	Treatments details*
T_1	Untreated control (No spray/ no release)
T_2	Spray of neem 3ml / l (Multinemor @ 0.15%) (three days after release of normal population)
T_3	Release of irradiated male five times of natural male(50 irradiated males) on same day of natural release
T_4	Combination of both neem spray and irradiation (T_2+ T_3)

2.1 Number of live webbings /plant

This study was conducted in field cages, during flowering stage of the crop for all the four blocks ten pairs of normal *M. vitrata* moths were released. Then irradiated males were released at the ratio of 1:5 (five times more than that of natural males and neem was sprayed according to the treatments as mentioned in the above. These irradiated males were released during flowering stage of the crop (139 days after sowing). Number of live webbings per plant was recorded at weekly interval 10 days after the release of irradiated males. In each treatment, five replications were made for recording observations. Observation on number of live webbings per plant was made on weekly interval, while taking observation from each replication five plants were randomly selected to check number of live webbings per plant. Observation on live webbings per plant was continued until crop was matured.

2.2 Number of damaged pods /plant

After pod maturation, number of damaged pods per plant was recorded in each replication and treatments. Five plants were randomly selected from each replications, observation on number of damaged pods per plant was taken at weekly interval until harvesting.

2.3 Grain yield (kg/360 m^2)

Grain yield was recorded in terms of kg per plot. Grain yield in each treatment was calculated by multiplying the grain yield from all five replication in each treatment (Each treatment consists of five replications).

3. Results

3.1 Number of live webbings /plant

This study was conducted in field cages, during flowering stage of the crop and ten pairs of normal *M. vitrata* were released in all the four blocks. Later irradiated males were released at the ratio of 1:5 *i.e* (five times more than that of natural males and multinemor at 1500ppm was sprayed according to the treatments. Observation on number of live webbings per plant was made on weekly interval and the incidence was recorded at 7, 14, 21 and 28 days after release (Table 2).

Seven days after release, the number of webbings per plant varied significantly in all the treatments. The highest number of webbings per plant was recorded in control (1.69 ±0.02) and lowest of 0.96±0.02 webbings per plant was recorded in treatment where 1:5 irradiated moths was released followed by spray. On 14 day after release the mean number of webbings per plant declined from 1.39±0.06 in control to 0.84 ± 0.06 in treatment 4. Similarly, on 21 days after release the webbings per plant showed gradual decrease from 1.25±0.06 in control to 0.65± 0.03 in T_4. Twenty eight days after release, significantly lower webbings per plant(0.44± 0.06) was recorded in irradiated release and spray block compared to control where it was 0.74±0.02 webbings /plant (Fig 1). Overall combined effect of irradiated moths and bio rational spray recorded significantly lower incidence as compared to control. (The lower incidence indicated that the most effective method of *M.vitrata* population suppression would integrate the use of multinemor 1500 ppm followed by the release of sterile males). Undoubtedly the use of SIT itself could also be applied, but integrated effect of sterile insect technique and bio rational insecticides would give higher pest population suppression.

3.2 Number of damaged pods /plant

Five plants were randomly selected from each replication in all the four treatments and observation on number of damaged pods per plant was taken at harvesting stage. Significantly highest pod damage was recorded in control (23.20± 0.21), followed by treatment T_2 (19.47±0.07) and T_3 (17.80 ±0.15). The pod damage was found to be lowest in treatment T_4 (14.80±0.10). Overall combined effect of irradiated moths and multinemor 1500ppm recorded significantly lower pod damage as compared to rest of the treatments (Table 3).

3.3 Grain yield (kg/ 360 m^2)

Grain yield in terms of kg per plot in each treatment was calculated by multiplying the grain yield from all five replication in each treatment (Each treatment consists of five replications).

Pigeon pea grain yield per plot recorded in all four treatments varied from 2.86 kg in control to 5.67 kg in T_4 where the sterile release followed by (multinemor 1500 ppm) spray was taken. The grain yield obtained from treatment T_2 (3.10.kg) and T_3 (3.48 k) were on par with each other. Significantly highest grain yield of 5.67kg per plot was obtained from T_4 (Fig 2). Overall combined effect irradiated moths and multinemor 1500 ppm spray recorded significantly higher grain yield per plot as compared to control (Table 2).

4. Discussion

The success of any insect mass release program in inducing subsequent generations depends, on the comparative competitiveness of the released sterile insects and the native populations, Keeping the above context in view, the present investigations were carried out on studies on the field efficacy of gamma irradiated male *M. vitrata* (Fabricius) (Lepidoptera) in comparison with chemical spray. The results of the findings are discussed here under with relevant literature.

The present study indicated the combined effect of gamma irradiation and multinemor at 0.15 per cent spray. Results indicated that combined effect induced more remarkable effects as compared to gamma irradiation or multinemor 0.15 per cent spray each of them alone.

The number of webbings per plant in control significantly differed in all the treatments. The highest number of webbings per plant 1.26 was recorded in control with lowest of 0.72 number of webbings per plant was recorded in T_4 (irradiated moth release followed by multinemore at 0.15 per cent spray). Significantly highest pod damage was recorded in control (23.20± 0.21 per cent). The pod damage was found to be lowest in T_4 (14.80±0.10 per cent). The grain yield per plot was found to be highest in T_4 (5.67kg) when compared with the control (2.86kg). Overall, combined effect irradiated moths and multinemor spray recorded significantly lower incidence, lower pod damage and higher grain yield as compared to control. This is just because irradiated moths at the ratio of 1:5 produced 27.74 per cent egg hatch, 81.64 percent control of reproduction and 84.07 sterility in the progeny, remaining population might have suppressed by mutinemor spray at 0.15 per cent (Fig. 1and 2).

These results suggested that the most effective method of *M.vitrata* population suppression would integrate the use of multinemor at 0.15 per cent spray followed by the classic SIT. Undoubtedly the use of SIT itself could also be used alone to suppress the pest but larval stage can-not controlled. So combined effect of SIT and multinemor spray might have controlled the pest effectively.

Compatible control methods, such as plant extracts, that possess a source for naturally occurring substances which act as feeding and insect growth deterrents, may be synergistic when integrated with the SIT. The present data indicate that the treatment combination of irradiation and plant essential oils resulted in less fecundity, fertility and more larval mortality compared with the use of irradiation alone as suggested by(Mohamed, 2004 and Mohamed, 2013).

Present results are in confirmation with the reports of Ouye and Graham (1967) [22] they made studies on SIT of pink bollworm and achieved 81.1 per cent control in field cages. It was accomplished by a single release of 9:1 ratio of sterile to normal males. Similar tests provided 98 per cent reduction in the normal population of pink boll worm with a seasonal average ratio of seven sterile to one normal male, released on a daily basis.

Present study is also in line with Stephen *et al*. (2005) [28] who made a study on sterile insect release program against the invasive cactus moth, *Cactoblastis cactorum* (Berg). Results indicated that an over flooding ratio as low as 5:1 effectively suppressed *C. cactorum* in field cages.

Similarly, the combined effects of gamma irradiation and bio insecticides on Greater Wax Moth, *Galleria Mellonella* have been studied by several authors (Sallam *et al*., 1991; Mohamed, 2004; Mohamed *et al*., 2004; El-Nagar *et al*., 2004; El-Shall and Mohamed, 2005; Mohamed, 2006 and Mohamed, 2013) [24, 14, 17, 7, 8, 15, 13] Result indicated that combined effect of gamma irradiation and bio insecticides provide better efficacy compared to each of them alone.

Present findings are also in accordance with the reports of (Mohamed *et al*., 2014) [18] who studied the impact of essential oils of marjoram and lemon grass in conjunction with gamma irradiation against the Greater Wax Moth, *Galleria Mellonella.* Results indicated that percentage of larval mortality increases in the case of combined treatment of radiation and aromatic oils of the two plants than in the case of both of them solo. The percentage of pupation and emergence was less in the case of combined treatment.

These findings are also in confirmation with those obtained by Ali (1992) [2] who studied the effect of gamma irradiation (100Gy) in combination with the (Lc50) of botanical oils (canola, sunflower and sesame) against the 3rd larval instar of *A. ipsilon* resulted from (irradiated males crossed with irradiated females), or from non-irradiated males crossed with irradiated females or from irradiated males crossed with non-irradiated females. Results obtained indicated that the combined effect of gamma irradiation (100Gy) and the (Lc50) of botanical oils tested markedly affected the different biological aspects of *A. ipsilon*. Increased the larval and pupal mortality; prolonged the larval and pupal duration; reduced the percentage of adult emergence; decreased the weight of the resulted pupae and caused malformation percentages among the resulted pupae and adults.

Similarly, Mohamed (2012) [16] evaluated the larvicidal activity of essential oils derived from two essential plants and / or gamma irradiation against early fourth instar larvae of the greater wax moth (GWM), Galleria mellonella L. The oil of two plants Marjoram, Origanum majorana and Lemon Grass, Cymbopogon proximus were tested. The two essential oils were evaluated further for the determination of their LC10, LC50 and LC90 values based on probit analysis, five of gamma irradiation doses (100,150,200,250 and 300 Gy) and the combined effect of them and essential oil were tested. Results indicated that percentage of larval mortality increases in the case of combined treatment of radiation and aromatic oils of the two plants than in the case of both of them solo.

Present findings are also in accordance with the reports of Ahmad *et al*. (2002) [1] who studied by releasing of partially sterilized pink boll worm adults at three week intervals at a ratio of 50:1 treated to normal. It reduced the larval infestation to sub-economic levels inside field-cages. These studies showed that the number of progeny produced by the partially sterile moths was limited and caused no significant damage to the crop.

Table 2: Field efficacy of gamma irradiated male *M. vitrata* in comparison with chemical spray

Number of webbings per plant						
S. No	**Treatment**	**7 DAR****	**14 DAR****	**21 DAR****	**28 DAR****	**Mean**
1	T1- UTC (No spray and no irradiated male release)	1.69^{a} ±0.02	1.39^{a} ±0.06	1.25^{a} ±0.06	0.74^{a} ±0.02	1.26
2	T2- Biorational spray (3 day after release) Neem 1500 ppm (3ml/l)	1.32^{ab} ±0.01	1.27^{ab} ±0.01	0.90^{ab} ± 0.05	0.68^{ab} ± 0.02	1.04
3	T3- Release of irradiated male (5 times of natural male) same day	1.29^{b} ±0.05	1.01^{ab} ±0.02	0.79^{b} ± 0.02	0.52^{ab} ± 0.03	0.90
4	T4- (T2+T3) spray after 3 days	0.96^{b} ±0.03	0.84^{b} ± 0.06	0.65^{b} ± 0.03	0.44^{b} ± 0.06	0.72
	F Value	F = 12.26* df= 3,16	F= 6.09* df= 3, 16	F = 7.27* df=3, 16	F = 5.71* df = 3, 16	

Means ± SE followed by same letter within a column are not significantly different at P≤ 0.01 level ; For statistical analysis by ANOVA, the count data were transforme using squre root *i.e.* (** : √× +0.5); In all the four blocks ten pairs of normal moths were releases before imposing treatment. Irradiated males were released 139 days after sowing of pigeon pea crop; DAR : days after releas *Significantat P ≤0.01 level

Table 3: Field efficacy of gamma irradiated male *M. vitrata* in comparison with chemical spray

S. No.	**Treatments**	**Mean number of damaged pods per plant**	**Grain yield (kg/ 60x60 m^2)****
1	T1- UTC (No spray No release)	23.20^{a} ± 0.21	2.86^{b} ± 0.06
2	T2- Biorational spray (3 day after release) Neem 1500 ppm (3ml/l)	19.47^{ab} ±0.07	3.48^{b} ±0.16
3	T3- Release of irradiated male (5 times of natural male) same day	17.80^{ab} ±0.15	3.10^{b} ±0.09
4	T4- (T2+T3) spray after 3 days	14.80^{bc} ±0.10	5.67^{a} ±0.01
	F - Value	F=7.25* Df=3,16	F = 10.19* df= 3, 16

Means ± SE followed by same letter within a column are not significantly different at P≤ 0.01 level ; For statistical analysis by ANOVA, the count data were transformed using squre root *i.e.* (** : √× +0.5); Irradiated males were released 175 days after sowing of pigeon pea crop; DAR : days after release. * Significant at P ≤0.01 level

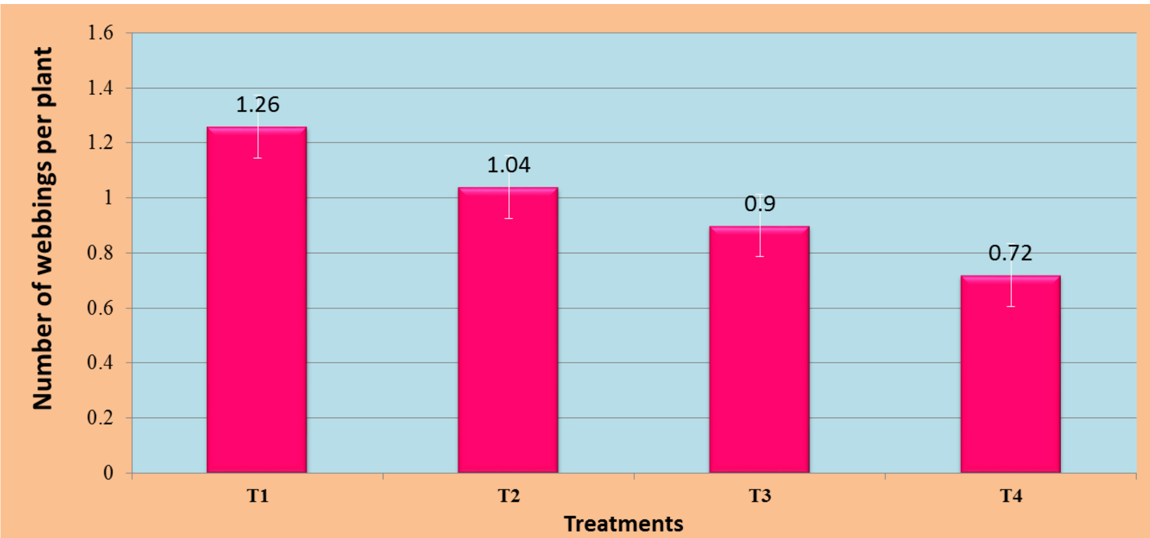

Fig 1: Effect of gamma radiation (150Gy) on incidence of *M. vitrata* in pigeon pea under net house condition

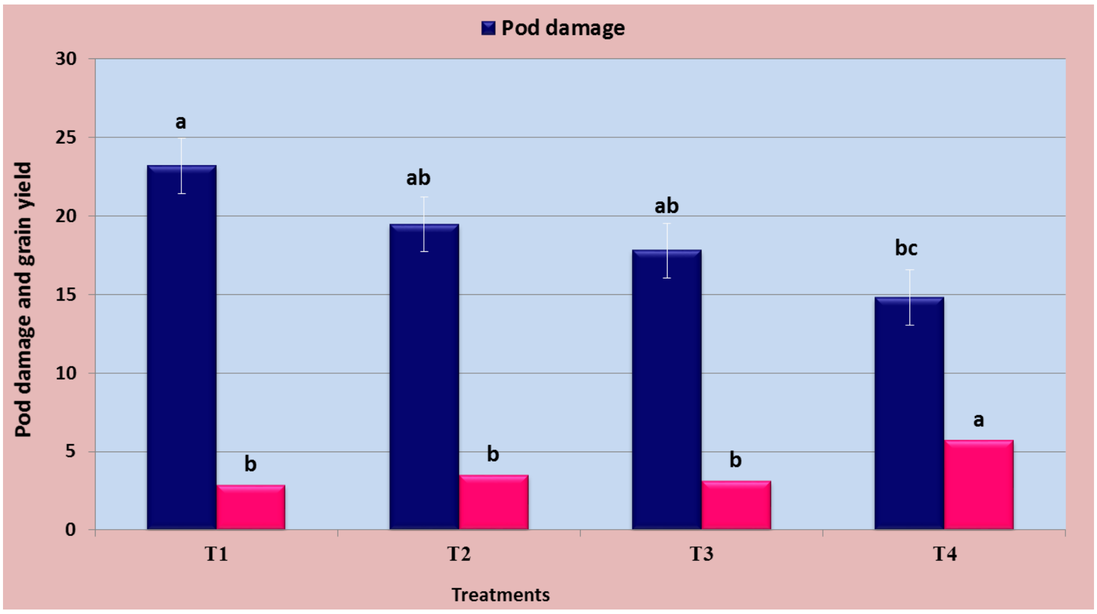

Fig 2: Effect of gamma radiation on pod damage and grain yield of pigeon pea under net house Condition

5. Conclusion

Efficacy of gamma irradiated male *Maruca* moths in comparison with multinemor spray indicated combined effect irradiated moths and multinemor at 0.15 per cent spray which recorded significantly lower incidence, pod damage and high grain yield as compared to control. The lower incidence indicated that the most effective method of *M. vitrata* population suppression would integrate the use of multinemor spray followed by the release of sterile males. Undoubtedly the use of SIT itself could also be applied, but integrated effect of sterile insect technique and multinemor insecticides would give higher pest population suppression.

The demonstrated potential of sterility to reduce the reproductive ability of *M. vitrta* and to perform compatibly with multinemor strategy suggested further studies to evaluate the economic and biological potential of this approach at higher ratios to achieve more suppression in commercial agro ecosystems.

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