http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (13): 2478-2482, 2006
ISSN 1028-8880

Radiation Sterilization and Mating Competitiveness of Melon Fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) Male in Relation to Sterile Insect Release Method

[1]G. Nahar, [2]A.J. Howlader and [1]R. Rahman
[1]Institute of Food and Radiation Biology, Atomic Energy Research Establishment,
G.P.O. Box No. 3787, Dhaka, Bangladesh
[2]Department of Zoology, Jahangirnagar University, Savar, Dhaka, Bangladesh

Abstract: In the present research radiation sterilization dose of *Bactrocera cucurbitae* (Coquillett) was determined. Egg viability was reduced to 0.93% at a dose of 30 Gy. Cent percent sterility in both males and females was achieved at 40 Gy. Mating competitiveness and sexual or total competitiveness of males were measured quantitatively from direct observations and ratio test methods, respectively. In direct observation method the males treated up to 30 Gy were almost equally competitive as untreated males. In ratio test method the total competitiveness values (C) of treated males were estimated 0.73-0.91 at three different ratios from 1:1:1 to 3:1:1 under laboratory condition.

Key words: *Bactrocera cucurbitae*, sterilization, mating competitiveness

INTRODUCTION

Fruitflies of the genus *Bactrocera* are widely distributed and one of the most important pests of fruits and vegetables in Bangladesh. The popular cucurbit vegetables viz., sweet gourd, snake gourd, bitter gourd, ribbed gourd, sponge gourd, cucumber etc. are attacked by fruitfly (Alam, 1969). The practicability of the Sterile Insect Release Method (SIRM) for the control of pest species of insects has been demonstrated for a number of species in various parts of the world e.g., *Cochlionyia hominivorax* (Coquerel) in Florida (Knipling, 1960); *Dacus cucurbitae* (Coquillett) in the pacific (Steiner *et al*., 1965); *Ceratitis capitata* (Wiedemann) in Nicaragua (Rhode *et al*., 1971) and *Dacus tryoni* (Froggatt) in Australia (Andrewartha *et al*., 1967). Sterilization by using both chemicals and radiation for flies is getting popular. The induction of sterility, particularly by ionizing radiation, does not cause any problem.

Management of insect pests using ionizing radiation can be achieved by the application of lethal or sterilizing dose. Radiation can render the pest species sterile immediately or it can sub-sterilize the species, causing reduced reproduction in subsequent generations (Alam *et al*., 2001). It is essential to know the required sterilizing dose for the effective control of any pest population.

In control or eradication programme involving the sterile insect released method, the released sterile males must compete successfully with wild males for wild females. The success or failure of a sterile male technique programme depends on the ability of the released male to mate competitively in the field (Kaiser *et al*., 1979). Therefore, the competitiveness of the irradiated flies is of crucial importance that includes mating ability, number of mating, sperm transfer, activity, olfactory responses, vigor and longevity (Fried, 1971; Hooper and Katiyar, 1971).

The objective of this study is to determine the sterilization dose and to estimate the mating competitiveness of this harmful insect before planning any control measure. The irradiated and unirradiated males have been compared on various aspects of mating behavior for the effectiveness of using them in any proposed SIT programme.

MATERIALS AND METHODS

The insects used in this experiment were obtained from laboratory stock cultures reared at 28±1°C and 75±5% R.H. Adults were reared on diet made of sugar, torula yeast and casein in 2:1:1 ratio. Larvae were reared on a diet based on wheat bran, yeast and vegetable paste. Pupae were chosen as the object of irradiation for sterility test and treated at 10, 20, 30, 40 and 50 Gy of gamma radiation (dose rate 4166 Gy h^{-1} at 15 cm distance) from a Co^{60} source installed at IFRB, AERE, Savar, Dhaka. After irradiation the treated pupae were kept separately and observed for adult emergence. Soon after emergence, the adults were sexed to avoid the unwanted mating. From each dose of irradiated pupae the emerging adults (2-3 day old) were placed separately in 25×25×15 cm

Corresponding Author: G. Nahar, IFRB, Atomic Energy Research Establishment, P.O. Box No. 3787, Dhaka, Bangladesh

aluminum framed cages covered with netted cloths together with same aged untreated flies of the opposite sex. For each treatment there were 25 individuals of opposite sex. Each dose was replicated thrice. Same procedure was followed for all the doses applied. Eggs were collected twice a week during four consecutive weeks and carefully examined for hatching under binocular microscope (Nikon). The number of hatched and unhatched eggs were counted and recorded. Sterility was calculated in terms of percentage egg hatched.

Three day old pupae were treated by gamma-radiation with the same dose for mating competitiveness test. Preliminary tests were conducted to obtain base line data on mating performance. Soon after emergence adult flies from both treated and untreated groups were immobilized with cold and separated according to sex.

Direct observation method: In a test chamber (20×20×20 cm) a four-day-old untreated virgin female was placed with two unmated males of same age, one treated and the other untreated. The males emerged from the treated pupae were considered as treated male and were marked with a white color (vinyl matt emulsion, lily white color) on their thorax to distinguish it from the untreated one. Mating combinations were recorded for 5 h from 5 to 10 PM as melon fly preferred mating at dusk (Back and Pemberton, 1977; Kuba *et al*., 1984; Suzuki and Koyama, 1980; Kuba and Kuyama, 1982). For the assessment of mating competition, only the first mating combinations were considered. All the observations were taken at 28±1°C and 75±5% RH. Five replicates were used in each dose. Each replication consists of ten pairs of insects. Finally, the competitiveness was calculated using the formula:

$$C=\frac{i/n}{S/N}$$

Where, i = The number of mated irradiated males.
n = The number of mated normal males.
S = Number of sterile male used in the experiment
N = Number of normal male used in the experiment.

Ratio test method: The effect of radiation on the total competitiveness of male *B. cucurbitae* was determined by combining Treated Males (TM), Unmated Male (UM) and virgin Untreated Female (UF) in a desired ratio. Twenty-five treated males, twenty-five untreated males and twenty-five untreated females were caged in a 1:1:1 ratio. In this way the ratio used in this experiment were 1TM: 1UM: 1UF, 2TM: 1UM:1UF and 3TM: 1UM: 1UF. The treated insects used in this experiment were emerged from the pupae radiated two days before emergence at a dose of 30 Gy. Each set of insects was placed in a 25×25×15 cm^3-mating cage made of steel frame covered with mosquito net. The flies were provided with adult diet as described earlier and water was supplied regularly. At the age of 14 days, oviposition medium was supplied to each group of insect. After 24 h samples of 100 eggs were collected with fine camel hairbrush and kept in moist condition for hatching. Oviposition medium were made available to the females for 24 h twice a week for three weeks. The percentage egg hatch was recorded for each group of insects. Each assessment was made in triplicate. The ratio of 1UM: 1UF and 1TM: 1UF were used as checks. In calculating the expected egg hatch for each ratio, the following (Fried, 1971) formula was used.

$$\text{Expected \% egg hatch } H_e=\frac{U\times H_u+T\times H_t}{U+T}$$

Where, U = Number of untreated males.
T = Number of treated males.
H_u = % egg hatch from the cross U ♂♂ × U 0_+ 0_+
H_t = % egg hatch from the cross T ♂♂ × U 0_+ 0_+

For calculating the total competitiveness (C) of the sterilized males, the following equation (Fried, 1971) was used.

$$C=\frac{U}{T}\times\frac{H_u-H_o}{H_o-H_t}$$

Where, U = Number of untreated males,
T = Number of treated males
H_u = % egg hatch from the cross U ♂♂ × U 0_+ 0_+
H_o = Observed % egg hatch at different ratios
H_t = % egg hatch from the cross T ♂♂ × U 0_+ 0_+

RESULTS

Results of present study showed that mating of unirradiated females with those of irradiated males did not affect the production of eggs. But it seriously hampered the viability of eggs. Treatment of males at 10 Gy has reduced the viability to 45.33% (Table 1). Egg viability has reduced to 20.47% at 20 Gy and it reached only 0.93% at 30 Gy dose. The treatment of males at a dose of 40 Gy produced 100% sterilization. The exposure of females to gamma irradiation sharply reduced the production of eggs by the latter. Irradiation of females had an effect on both fecundity and fertility. Hatch of irradiated eggs declined with increasing dose to reach zero at 40 Gy. Only 50.64, 30.30 and 9.43% eggs hatched when females were exposed to 10, 20 and 30 Gy, respectively. Cent percent sterility of

Table 1: Sterility of *B. cucurbitae* irradiated at different dose of radiation

	Mating combination							
	TM × UF		TF × UM		UM × UF		Sterility (%)	
Dose (Gy)	Egg laid	Hatch (%)	Egg laid	Hatch (%)	Egg laid	Hatch (%)	Male	Female
00	00	00.00	00	00.00	2732	79	00.00	00.00
10	1132	45.33	1582	53.33	00	00	54.67	46.67
20	1219	20.47	859	33.88	00	00	79.53	66.12
30	1167	0.93	280	9.43	00	00	99.07	90.57
35	1085	0.61	103	1.25	00	00	99.39	98.75
40	1107	00.00	00	00.00	00	00	100.00	100.00
50	1195	00.00	00	00.00	00	00	100.00	100.00

TM = Treated male, UF = Untreated female, TF = Treated female, UM = Untreated male

Table 2: Assessment of mating competitiveness of radiation-treated males of *Bactrocera cucurbitae* by direct observation

Dose	Mating combination			Competitiveness
(Gy)	T♂ × U0+	U♂ × U0+	Unmated	(C) value
10	18.2	18.8	13.0	0.96
20	16.8	20.2	13.0	0.83
30	15.2	17.4	17.4	0.087
40	8.2	18.2	23.6	0.45
50	7.8	20.0	22.0	0.39

T ♂ = Treated male, U 0+ = Untreated female, U♂ = Untreated male

Table 3: Estimation of total competitiveness (C) values of irradiated (30 Gy) *Bactrocera cucurbitae* males

Ratio T ♂:U♂:U0+	% egg hatched	Expected % egg hatched	Competitiveness (C) value
0:1:1	72.372±2.412	-	-
1:0:1	8.037±5.40	-	-
1:1:1	51.585±4.22	47.2	0.9140
2:1:1	35.88±4.426	33.47	0.8353
3:1:1	21.462±2.07	28.61	0.7387

T ♂ = Treated male, U 0+ = Untreated female, U♂ = Untreated male

female *B. cucurbitae* was achieved at a dose of 40 Gy. With the increasing dose up to 30 Gy, sterility increased rapidly. Total sterility was achieved at 40 Gy for both males and females.

Determination of mating competitiveness

Direct observation: The relationship between radiation dose and mating competitiveness evaluated by the method of direct observation indicated that 10, 20 and 30 Gy radiation treated males were almost as successful as untreated males (Table 2). On the other hand 40 Gy and 50 Gy treated males were 2 or 3 times less competitive than the untreated insects. The C-values of 40 and 50 Gy treated males were 0.45 and 0.39, respectively. The C-value reduced significantly at 40 and 50 Gy, indicating less competitive as compared to 10, 20 and 30 Gy treated males. It was found that 10, 20 and 30 Gy treated males were equally compatible with untreated females in the presence of untreated males.

Ratio test method: The observed and expected mean±SE percentage hatch for different fly ratio and the estimated total competitiveness values of radiation treated males of *B. cucurbitae* are presented in Table 3. As the ratio of radio-sterilized males increased, percentage hatching of untreated females declined. It was found that the decline in percentage hatching was not as great as that from T ♂♂ × U 0+ 0+ mating (8.03±5.40). The C-values (0.73-0.91) by ratio test method indicated that the population of 30 Gy radiation treated males must be increased from 1.09 (i.e., 1/0.91) to 1.35 (i.e., 1/0.74) times to achieve the effect on the reduction in percentage hatching that T ♂♂ × U 0+ 0+ mating would have given.

DISCUSSION

Unirradiated females mated to irradiated males did not affect the production of eggs, but it seriously hampered the rate of egg hatch. In the present experiment, males irradiated at 20 and 30 Gy produced sterility at 79.60 and 99%, respectively. Complete sterility in male was achieved at 40 Gy. Similar data has also been reported for other tephritids. Hooper (1975) reported 95 and 99% sterility in *C. Capitata* at 7 and 11 krad respectively. About 95 and 99% sterility when 3 krad and 7.5 krad doses were applied to *Dacus tryoni.* The doses applied for those insects were higher than that applied in the present study.

The mating competitiveness calculated from the direct observation in *B. cucurbitae* revealed that males emerged from pupae irradiated at 10 to 30 Gy are equally capable of mating as control. The competitiveness values calculated in the present study at 10 Gy was 0.96 and that at 30 Gy was 0.87. Steiner *et al.* (1962), Feron (1966) and Katiyar and Ramirez (1969) also made similar observations and reported that gamma irradiation at 6-10 krad applied to matured pupae had little or no effect on the mating ability of irradiated male *Ceratitis capitata* which agrees with the present observation. On the other hand, Ohinata *et al.* (1977) reported that when male *C. capitata* was irradiated 2 days before eclosion the competitiveness of male was significantly reduced, but when same dose was applied 2 days after eclosion of the flies had no adverse effect on mating competitiveness. Teruya and

Zukeyama (1979) estimated the competitiveness of *Dacus cucurbitae* by direct counting method. They stated that doses from 10 to 100 Gy did not reduce competitiveness significantly while Fried's method was applied. According to them the mating competitiveness values of males irradiated with 7 and 12 Kr were less than unity, but not significant. The C-values estimated were reported as 0.92 at 1 krad and that at 30 krad was 0.06. The C-values calculated at 1krad by these authors were very similar to the present study but that estimated at 30 krad was 0.06, which suggested less competitive attitude. Islam and Gordon (1992) stated that the greater mating competitiveness of the treated males did not always correspond to the greater total competitiveness value of the same males as revealed from their study with *Musca domestica* sterilized by hempa. Technological methodology for the attainment of induced sterility may be the cause of their variation.

The C values in the present experiment, estimated from different ratios (1:1:1 to 3:1:1) used were 0.73-0.91 that is closer to unity. Hooper and Katiyar (1971) estimated C-values of irradiated males of the Mediterranean fruitfly *Ceratitis capitata* (Weid) based on the mean values of egg-hatch percentages of the untreated, treated and observed fly ratios and obtained a negative correlation between the dose and the competitiveness.

Fried (1971) and Hooper and Horton (1981) proposed procedures to quantify sterile-insect total competitiveness based on egg-hatch resulting from competitive mating tests. Based on those calculations, C-values of X-ray sterilized European corn borer males, *Ostrinia nubilalis* (Hiibner) (Lepidoptera: Pyralidae) were found to be 0.3-0.4 (Fried, 1971), those of irradiated (5 krad) males of *C. capitata* 0.52 and irradiated (7 krad) cucumber fly, *Dacus cucumis* French 0.68 (Hooper and Horton, 1981). Brower (1982) calculated C-value (0.91-0.93) of irradiated males of the tobacco moth, *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae) to be as good that of untreated males. C-value of chemo sterilized *Culex quinquefasciatus* male was found 0.96 (El-Gazzar and Dame, 1983) and that of males of the greater wax moth, *Galleria mellonella* (Linnaeus) (Lepidoprera: Pyralidae), sterilized by radiation was 0.91 (Eischen *et al*., 1984). These findings are in good agreement with the present results at the different ratios from 1:1:1 to 3:1:1.

The sterilization dose for both male and female was determined as 40 Gy. The competitive mating tests (direct observation) gave an estimate of mating competitiveness of radio-sterilized males and the percentage hatching data obtained from three ratios of treated and untreated males, gave a measure of the total competitiveness of the treated males. A large number of sterilized males are usually released in order to compensate for their reduced sexual competitiveness (caused by laboratory rearing and irradiation). The consequences of the strategies of sterile insect release method need much more attention and warrant through study.

REFERENCES

Alam, M.S., A.T.M.F. Islam and S. Islam, 2001. Sterilization and subsequent effects of Gamma radiation on fecundity, egg viability and longevity of the hide beetle, *Dermestes maculatus* Degeer. Bangladesh J. Zool., 29: 59-64.

Alam, M.Z, 1969. Pests of Cucurbit Vegetables. In: Insect Pests of Vegetables and Their Control in East Pakistan. Agril. Infor. Seru, Dept. Agric. Dhaka, pp: 87-110.

Andrewartha, H.G., J. Munro and N.L. Richardson, 1967. The use of sterile males to control populations of Queensland fruit fly, *Dacus tryoni* (Frogg.) (Diptera: Tephritidae). II Field experiment in New South Wales. Aust. J. Zool., 15: 475- 499.

Back, E.A. and C.E. Pemberton, 1977. The melonfly in Hawaii. U.S. Dept. Agric. Bull., 491: 1-64.

Brower, J.H., 1982. Mating competitiveness of irradiation- sub sterilized males of the tobacco moth. J. Econ. Entomol., 75: 454-457.

Eischen, F.A., A. Deitz and J.H. Brower, 1984. Effect of aging on the mating competitiveness of irradiated male greater wax moth (Lepidoptera: Pyralidae). J. Econ. Entomol., 77: 1534-1536.

El-Gazzar, L.M. and D.A. Dame, 1983. Effects of combinations of irradiation and chemo sterilization on mating competitiveness of *Culex quinquefasciatus* Say. J. Econ. Entomol., 76: 1331-1334.

Feron, M., 1966. Sterilisation de la mouche mediterraneenne des fruits, *Ceratitis capitata* Wied., par irradiation des pupes aux rayons gamma. Ann. Epiphyt., 17: 229-239.

Fried, M., 1971. Determination of sterile-insect competitiveness. J. Econ. Entomol., 64: 869-872.

Hooper, G.H.S. and K.P. Katiyar, 1971. Competitiveness of gamma-sterilized males of the Mediterranean fruit fly. J. Econ. Entomol., 64:1068-1071.

Hooper, G.H.S., 1975. Sterilization of *Dacus cucumis* French (Diptera : Tephritidae) by Gamma radiation. I. Effect of dose on fertility, survival and competitiveness. J. Aust. Ent. Soc., 14: 81-87.

Hooper, G.H.S. and I.F. Horton, 1981. Competitiveness of sterilized male insects: A method of calculating the variance of the value derived from competitive mating tests. J. Econ. Entomol., 74: 119-121.

Islam, M.S. and R.P. Gordon, 1992. Two methods of estimating competitiveness of chemosterilized males of *Musca domestica* (Diptera: Muscidae). Bull. Entomol. Res., 82: 521-524.

Kaiser, P.E., L. Doald, E.L. Ronald, J.A. Seawright and D.A. Dame, 1979. Mating competitiveness of chemosterilized males of a genetic sexing strain of *Anopheles albimanus* in laboratory and field tests. Mosquito News, 39: 768-775.

Katiyar, K.P. and F. Ramirez, 1969. Sterilizationof the mediterranean fruit fly and itsapplication to fly eradication. In the Application of Nuclear Energy toAgriculture, Triennial Report, July 66-30 June 69, for Contract AT (30-1) 2043 of Inter-American Institute of Agricultural Science of the OAS., Turrialba, Costarica, pp: 90-105.

Knipling, E.F., 1960. The eradication of the screw worm, Sci. Am., 203: 54-61.

Kuba, H. and J. Koyama, 1982. Mating behavior of wild melon flies, *Dacus cucurbitae* Coquillett (Diptera : Tephritidae) : Comparative studies of one wild and two laboratory strains. Applied Ent. Zool., 17: 559-568.

Kuba, H., J. Koyama and R.J. Prokopy, 1984. Mating behavior of wild melonflies, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae) in a field cage: Distribution and behavior of flies. Applied Ent. Zool., 19: 367-373.

Ohinata, K., M. Ashraf and E.J. Harris, 1977. Mediterranean fruit flies: Sterility and sexual competitiveness in the laboratory after treatment with gamma irradiation in air, carbon dioxide, helium, nitrogen or partial vacuum. J. Econ. Entomol., 70: 165-168.

Rhode, R.H., J. Simon, A. Perdomo, J. Gutierrez, C.F. Dowling Jr. and D.A. Lindquist, 1971. Application of the sterile insect release technique in Mediterranean fruit fly suppression. J. Econ. Entomol., 64: 708-713.

Steiner, L.F., W.C. Mitchell and A.H. Baumhover, 1962. Progress of fruit-fly control by irradiation sterilization in Hawaii and the Mariana Islands. Intl. J. Applied Radiat. Isotop., 13: 427-434.

Steiner, L.F., H.J. Harris, W.C. Mitchell, M.F. Fujimoto and L.D. Christenson, 1965. Melon fly eradication by over flooding with sterile flies. J. Econ. Ent., 58: 519-522.

Suzuki, Y. and J. Koyama, 1980. Temporal aspects of mating behavior of the melon fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae): A comparison between laboratory and wild strains. Applied Ent. Zool., 15: 215-224.

Teruya, T. and H. Zukeyama, 1979. Sterilization of the melon fly, *Dacus cucurbitae* Coquillett, with gamma radiation: Effect of dose on competitiveness of irradiated males. Applied Ent. Zool., 14: 241-244.