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Population fluctuation of adult males and pupal radiosensitivity of the melon fly, *Zeugodacus cucurbitae* (Coq.) (Diptera: Tephritidae)

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Abstract

The experiment was conducted to study the population fluctuation of adult males of melon fly, *Zeugodacus cucurbitae* (Coq.) in different backyard vegetable gardens of Rajshahi University Campus from July 2014 to January 2015 and radiosensitivity of late age pupae to gamma radiation for determining the appropriate sterility dose. On average, the highest (77.19±19) fruit fly population per trap/month was recorded in August and the lowest was (12.29±0.70) in January. From the experimental results, it was observed that temperature, relative humidity and host availability influenced the melon fly population. It was noted that gamma radiation significantly prolonged the pupal duration ($p<0.01$). The longevity of irradiated flies was significantly decreased gradually at higher irradiation doses ($p<0.01$). The sterility percentage of the melon fly increased as the dose increased. Cent percent male sterility was achieved at 60 Gy. Therefore, sterile males should be released at optimum ratios at right time for getting the maximum output.

Keywords: population fluctuation, melon fly, sterility, radiosensitivity, cuelure

Introduction

Fruit flies (Diptera: Tephritidae) are arguably the most destructive insect pests of fruits, vegetables and other ornamental plants throughout the world [1-4]. About 70 species of fruit flies are considered important agricultural pests, and many others are minor or potential pests [5]. The melon fly, *Zeugodacus cucurbitae* (Coq.) is a major pest widespread in Asia [6], infesting primarily Cucurbitaceae at the flowering and fruiting stages [7, 8]. *Z. cucurbitae* (23.6%) is the 2nd dominant species among the cucurbit pests available in Bangladesh [9]. In Bangladesh, available host plants for *Z. cucurbitae* are watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai), winter melon (*Benincasa hispida* (Thunb.) Cogn,), melon (*Cucumis melo* L.), ivy gourd (*Coccinia grandis* (L.) Voigt), tomato (*Lycopersicon esculentum* Mill.), cucumber (*C. sativus* L.), gac fruit (*M. cochinchinensis* Spreng.), pumpkin, calabash, angled luffa (*Luffa acutangua* (L.) Roxb.), smooth luffa, balsam-apple (*Momordica balsamina* L.), spiny gourd (*M. dioica* Roxb. Ex Willd.), bitter gourd (*M. charantia* L.), eggplant (*Solanum melongena* L.), and snake gourd (*Trichosanthes cucumerina* L.) [7, 10, 11], with losses estimated at 10-30% of annual agricultural produce. Considering the economic and quarantine importance control of this pest is frequently uttering most of the countries of the world [12]. As the flies bear unusual biological characteristics, so controlling these disastrous pests is quite difficult only through the application of chemical pesticides. Again, it is also established that before developing insect pest management program for a specific agro-ecosystem, it is necessary to have basic information on the incidence of the pest concerning weather parameters which help in determining the appropriate time of action and suitable method of control. Pest population monitoring throughout the year is one of the most vital elementary information in designing IPM concept for sustainable agriculture.

The Sterile Insect Technique (SIT) has practically applied for several key pests of agricultural importance [13]. It is a species-specific, environmentally safe process with the potentials for suppression of the target pest population from a defined area. SIT is usually applied as a component of area-wide IPM [14, 15]. The SIT involves rearing of a large number of the target species, exposing them to gamma rays to induce sexual sterility and then releasing them into the target area.

The released sterile males will mate with wild females to prevent them from reproducing. So far, there are limited scientific data available regarding the population fluctuation of melon fly in Rajshahi area and management of melon fly, *B. cucurbitae* population using SIT in Bangladesh. The present study was therefore undertaken to determine the male sterility dose for possible field applications of SIT and to study the population fluctuation of adult males of the melon fly.

Materials and Methods

Population fluctuation of adult males

The experiment was conducted in backyard vegetable gardens of Rajshahi University campus (GPS Coordinates- 24° 22' 26.40" N, 88° 36' 4.10" E), Rajshahi, Bangladesh. Seven traps baited with Cuelure were hung on the trees or bamboo poles about 1.8 meters above the ground and maintained from July 2014 to January 2015. Traps were made of two-litre empty plastic drinking water bottles, with two triangular lateral holes (approximately 25×30×30 mm) at mid-height of the bottles. A lure plug containing 2 g of lure (Scentry Biologicals, Billings, Montana, USA) and 50% of a 25 × 90 mm strip containing 10% dichlorvos (2,2–dichlorovynil dimethyl phosphate) (Vaportape® II, Hercon Environmental, Emingsville, Pennsylvania, USA) were suspended from the trap's ceiling with a hook made of tie wire. The dead flies were sorted out and counted at every seven days interval. Monthly average temperature and relative humidity were collected from Regional Meteorological Station, Shyampur, Rajshahi. Collected data were analyzed by using Minitab and Microsoft Excel software. The monthly mean population of male melon fruit flies was separated by Tukey's pairwise comparison test.

Pupal radiosensitivity and male sterility dose determination

Stock culture:

Laboratory cultures of *Z. cucurbitae* were maintained in the fruit fly laboratory, Insect Biotechnology Division, Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka. Larvae were maintained on artificial diet. Adult melon flies were stocked in a steeled frame cage (60×50×45 cm) covered with a nylon net. The culture was usually supplied with an artificial diet (yeast extract: sugar: casein/ 1:2:1) and water-soaked cotton. In general, 2000-3000 adult fruit flies were maintained in a stock cage. The temperature and relative humidity of the rearing room was maintained at 28±2ºC and 70-80% respectively.

Pupae collection and irradiation:

The larvae of *Z. cucurbitae* were kept in plastic bowls with saw dusts at the bottom for pupation. After pupation, the saw dusts were sieved in order to collect the dust-free pupae. Two hundred (200) pupae of the same ages (six days old) were transferred into Petri dishes and irradiated in a Co^{60} gamma irradiator of IFRB, AERE, Savar, Dhaka. The applied radiation doses were fluctuating from 10 to 70 Gy at a rate of 10 Gy per minute. Each dose group had four replications and this was repeated five times. One control batch was also maintained with an equal number of replications.

Sterility dose determination

Fifty (50) male flies were collected from each of 10, 20, 30, 40, 50, 60 and 70 Gy treated 6-day old pupae and fifty females were also isolated from the control batch and allowed to mate with individual dose-treated males in small rearing cages (12×10×24 cm). After two days of mating, eggs were collected from a piece of sweet gourd placed inside the rearing cage as an egg-laying medium and allowed to hatch. The experiment was repeated five times.

Results and Discussion

The population fluctuation of male melon fly at different months of the year and trapped by using cuelure baited traps has been presented in Table 1. Monthly population fluctuation of male melon fly varies significantly (p<0.001). The highest fruit fly population was recorded in August (77.19±19) and the lowest was in January (12.29±0.70). The results revealed that melon fly populations around the year fluctuate much from time to time. The higher population density was recorded during the hot months while low or very low during the cooler months. Fluctuation in the fly population may be due to the prevalence of congenial environmental conditions and/or fruiting and flowering time of the hosts concerned. From the Co-efficient of correlation (r) value, it is observed that the fruit fly population mostly varies with temperature (r=0.52), relative humidity (r=0.39) and host availability. In the month of December-January, a few fruit fly was captured in the trap. This is because of the low temperature and relative humidity and there was no host available in the surroundings.

Table 1: Monthly average trapped male melon fly, average temperature and relative humidity at Rajshahi University campus, Rajshahi

Month	No. of trapped Fruit fly/trap (Mean±SE)	Temperature °C (Mean±SE)	Relative humidity (%) (Mean±SE)
July'14	27.14d±3.90	28.20±3.90	84.00±7.64
August'14	77.19a±19	29.7±3.20	80.00±4.65
September'14	20.43e±3.87	28.70±3.41	80.00±3.56
October'14	32.29c±3.94	27.10±3.70	78.00±5.75
November'14	46.50b±0.50	23.90±4.40	77.00±7.54
December'14	25.23d±0.50	19.80±4.50	69.00±1.36
January'15	12.29f±0.70	17.4±2.21	68.00±1.54

Means followed by different letters differ significantly by Tukey's pairwise comparison test

It has been observed that during warm and rainy months (July, August) the melon flies were more active as compared to that of dry and winter (December, January) months [16]. A significant positive correlation was also observed between fruit flies trapped and temperature. It also studied that the population fluctuation of *B. dorsalis* in Chapainawabganj area and reported that the highest oriental fly population was recorded in July and the lowest from January [17]. They also reported that temperature, relative humidity, rainfall and host availability influenced the fruit fly population. Another investigation denoted that the population peak of the *B. cucurbitae* appeared in July-August and declining was observed in December depending on the host maturity, temperature and rainfall [18]. Several workers conducted some parallel studies on the effect of meteorological parameters on the frequency of the melon flies [19, 20].

Determination of sterility dose of male melon fly

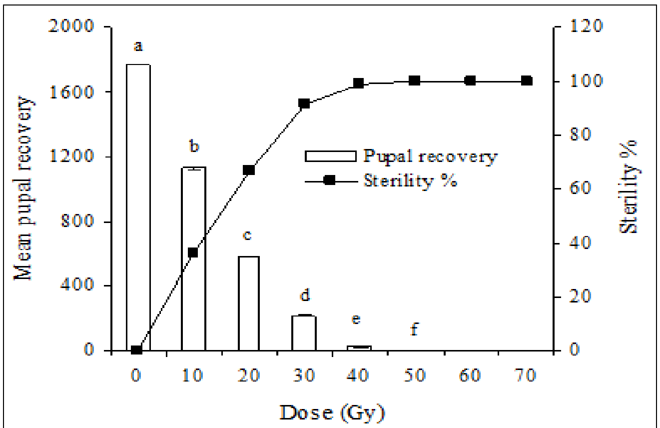
Six days old pupae were irradiated and subsequently emerged male adults were allowed to mate with unirradiated virgin females. The eggs laid onto the host fruit were maintained for larval hatching, development and pupation. A sharp decrease in pupal recovery was recorded as radiation doses increased (Fig. 1). But, no pupa was found in rearing comprising 60 and 70 Gy treated male flies. Significant prolongation ($P<0.001$) of pupal duration was noted with an increment of radiation doses (Table 2).

Table 2: Pupal duration and adult longevity of melon fly at different doses of gamma radiation

Dose (Gy)	Pupal duration in days (Mean ± SE)	Longevity in days (Mean ± SE)
0	8.25±0.023a	110.27±0.74 a
10	9.25±0.011b	106.39±0.503 b
20	9.42±0.026c	101.17±0.096c
30	9.57±0.007d	96.34±0.69d
40	9.68±0.002e	92.58±0.30e
50	9.68±0.006e	92.16±0.18e
60	9.64±0.019e	91.21±0.54e

Means followed by different letters differ significantly by Tukey's pairwise comparison test

Pupal duration of control flies was 8.25±0.023 days, while those of 60 Gy treated male groups required 9.64±0.019 days at laboratory condition. The percent of sterility attained in the F_1 generation was 33.84, 46.55, 64.21, 86.54, 90.63, 100 and 100 at 10, 20, 30, 40, 50, 60 and 70 Gy dose treatment of adult male respectively (Fig. 1). Sterility rate increased gradually with the increase of radiation doses. Complete sterility of male melon fly was observed at 60 Gy. The longevity of irradiated males was significantly decreased gradually at higher irradiation doses. The adult longevity of irradiated males at 60 Gy was 91.21±0.54 days, while that of the unirradiated control group was 110.27±0.74 days (Table 2). Considering the sterility rate and adult longevity, 60 Gy appeared as an appropriate sterilizing dose for male melon fly with possible use in the field-level application of melon fly SIT.



A column with different letters differs significantly by Tukey's pairwise comparison test.

Fig 1: Average pupal recovery and sterility percentage of melon fly at different doses of gamma irradiation.

Many researchers determined the sterility dose by irradiating pupae 2-3 days before adult emergence [21-24]. Determination of the sterility dose is important for the suitability of releasing them in the fields for SIT. In case of gamma radiation for sterilization to many fruit fly species, the optimum doses of were successfully determined [25, 26]. Though, picking an ideal sterilization dose for SIT application, an equilibrium condition is required to reach between the range of mating competitiveness and sterility of males [27, 28]. In the present study the sterility attained in F_1 generation was 36.18, 66.67, 91.49, 98.56, 99.75, 100 and 100 at 10, 20, 30, 40, 50, 60 and 70 Gy respectively. It has reported that in case of 5-days old pupae of melon fly the sterility percentage was 53 and 82 at 20 and 40 Gy respectively [22]. Another study reported that males of melon flies irradiated at 20 and 30 Gy produced 79.60 and 99% sterility respectively [29]. Larvae are usually more radiosensitive than pupae, which might be the reason behind attaining a higher percentage of sterility at a relatively lower dose of radiation at the pupal stage. The sterilizing dose for male *B. cucurbitae* was testified by several researchers [30, 22, 23] as 50-60 Gy which is closely matched with the findings of this current study. The reasons to have a reduced number of pupal recovery at higher doses is related to the damage of genetic materials [30, 12]. Significant prolongation of pupal duration following radiation may be due to hyperstimulation of the mechanism of repair to slightly damaged tissues [30]. The outcome of this study is also supported by many researchers who reported that gamma radiation extends the pupal duration of the melon fly [30, 25, 23]. Parallel outcomes were described for irradiated Caribbean fruit fly *Anastrepha suspense* [31]. This study revealed that that female flies treated with lower radiation doses (15, 20 and 25 Gy) resulted in higher or equivalent survival rates to the control flies while the higher radiation doses (30, 50 and 70 Gy) triggered pointedly more mortality. In another study [32], it is found that the male *Bactrocera zonata* flies, evolving from irradiated pupae with 10, 30, 50, 70 or 90 Gy were paired with untreated females, the mean number of deposited eggs was found as 211.3, 197.3, 184, 173 and 161.3 eggs /female as likened to 220 eggs/ female in the control experiment. This current investigation also followed the result of Nahar and Ahmed [33]. In their studies, they irradiated *B. cucurbitae* at different doses. the male and female mortality rate was 98% and 92% respectively in 3.00 Kr dose of radiation; while the result was 96% and 94% respectively in 4.00 Kr dose.

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