Efficacy of Gamma Radiation on the Productivity of *Bactrocera oleae* Gmelin (Diptera: Tephritidae)

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***Abstract*—**The olive fruit fly, *Bactrocera oleae* Gmelin (Diptera: Tephritidae), is one of the most serious pests in olive orchards in growing province in Iran. The female lay eggs in green olive fruit and larvae hatch inside the fruit, where they feed upon the fruit matters. One of the main ecologically friendly and species-specific systems of pest control is the sterile insect technique (SIT) which is based on the release of large numbers of sterilized insects. The objective of our work was to develop a SIT against *B. oleae* by using of gamma radiation for the laboratory and field trial in Iran. Oviposition of female mated by irradiated males is one of the main parameters to determine achievement of SIT. To conclude the sterile dose, pupae were placed under 0 to 160 Gy of gamma radiation. The main factor in SIT is the productivity of females which are mated by irradiated males. The emerged adults from irradiated pupae were mated with untreated adults of the same age by confining them inside the transparent cages. The fecundity of the irradiated males mated with non-irradiated females was decreased with the increasing radiation dose level. It was observed that the number of eggs and also the percentage of the egg hatching was significantly ($P < 0.05$) affected in either IM x NF crosses compared with NM x NF crosses in F_1 generation at all doses. Also, the statistical analysis showed a significant difference ($P < 0.05$) in the mean number of eggs laid between irradiated and non-irradiated females crossed with irradiated males, which suggests that the males were susceptible to gamma radiation. The egg hatching percentage declined markedly with the increase of the radiation dose of the treated males in mating trials which demonstrated that egg hatch rate was dose dependent. Our results specified that gamma radiation affects the longevity of irradiated *B. oleae* larvae (established from irradiated pupae) and significantly increased their larval duration. Results show the gamma radiation, and SIT can be used successfully against olive fruit flies.

***Keywords*—**Fertility, olive fruit fly, radiation, SIT.

I. Introduction

THE punctual date of olive coming to Iran is not clear but what is for sure is that olive has been in Iran for many years now and can be finding in most of Iran areas. According to the collected data, this plant has been planted for over 900 years in three cities of Iran: Zanjan, Gilan, and Ghazvin. Average production of olive in Iran was 42098 tons per year in 2014 [1].

Bactrocera oleae Gmelin is one of the most harmful and widespread pests in olive plantations in a number of provinces in Iran [2]. It is monophagous on olives which develop several generations per year [3], [4]. The females lay eggs in the summer when the olive is at least 8 mm in diameter by creating a hole with the ovipositor in the mesocarp of the olive, laying only one egg per green olive fruit, and about 200 to 250 eggs are laid in a lifetime and many generations grow throughout summer and fall [5], as larval development is completed within the fruits. The larvae feed upon fruit pericarp, causing in a major quantitative and qualitative damage in the production of olive and oil. Portion of the production is also missing due to premature falling of the injured fruit. Also, the oil attained from infected olives has a high level of acidity and a minor shelf life; therefore the value of the olive oil decreased by up to 80% and caused the refusal of total products of table olives [6]. SIT is one of the main environment-friendly and species-specific systems for the control of *B. oleae* which is a biologically-based technique for controlling of key insect pests of agricultural and medical/ veterinary importance and is based on the release of large numbers of sterilized insects [7]. It is also a kind of "birth control" in which wild female insects of the pest population do not reproduce when they are fertilized by released, sterilized males to the extent that FAO defined this technique as "a method of pest control using area-wide releases of sterile insects to reduce reproduction in a field population of the same species" [8].

Applying the sterile males technique shows the successes on the eradication of the New World Screwworm (*Cochliomyia hominivorax* Coquerel) from north and central America [9], the tsetse fly (*Glossina austeni* Newst.) from Unguja Island in Zanzibar, Tanzania [10]; the melon fly (*Bactrocera cucurbitae* Coquillett) from Japan [11]; the pink bollworm (*Pectinophora gossypiella* Saunders) from California, San Joaquin Valley [12] and the Queensland fruit fly (*Bactrocera tryoni* Froggatt) from western Australia [13].

In this technique, sterilization is made due to the effects of irradiation on the reproductive organs of the insects, then the number of fertile mating is decreased by a competition among the wild and sterile released males, which finally induced a decline in the whole population density. Therefore, to promote the potential use of SIT, broad indication is essential about productivity of females matted by irradiated males which is the main objective of our study.

II. Material and Methods

A. Sampling and Adult Culture

The rearing culture of *B. oleae* was initiated from infested fruits composed from the Roodbar (Fig. 1) and Qazvin which was kept at 27±1 °C with 16:8 (L:D) photoperiod and 60%

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RH. in insectarium. Artificial diet for larval stages was developed by Tsitsipis and Kontos [14] and Tzanakakis [15] with a little change consisting of 1100 mL distilled water, 60 g soy hydrolysate, 150 g brewer's yeast, 40 g sugar, 40 mL olive oil, 15 mL Tween, 4 g Nipagin, 1 g potassium sorbate, 9 mL HCl, and 550 g cellulose powder. Also, the adults were fed on a diet of yeast hydrolysate: sucrose (1:3).

B. Rearing

The eggs were collected daily at 24-h intervals from paraffin net of cages. Development time and percentage of egg hatching were studied daily using stereomicroscope. Hatched larvae were carefully collected on a wet black filter paper and transferred to larval diet and when developed to the third instar, the dishes were moved to larger boxes containing a layer of sterile sand and sawdust (Fig. 2).

C. Irradiation Treatment

The pupae were irradiated by Co-60 gamma source (by 0, 50, 70, 90, 100, 120, 140, and 160 Gy) at Nuclear Science and Technology Research Institute at a dose rate of 0.3 Gy/sec. The required dose was obtained by treating the pupae with different exposure times. Five replicates of 100 pupae for each dose were used. The control group was treated in similar manner but not exposed to irradiation treatment.

D. Effect of Gamma Radiation on the Flies Productivity

The mean pupal duration and newly appeared adults were noted daily. 100 emerged males from irradiated pupae were mated with the same number of the untreated females. The cages were placed in the rearing room under the same conditions of light and temperature (27±1 °C with 16:8 (L: D) photoperiod and 60% RH). The adults were allowed to mate and lay eggs on the waxed net of the wall of each cage until all flies died. Egg papers were incubated at 27±1 °C for three days and then observed for egg hatch under a stereomicroscope. The total number of eggs laid, the number of hatched eggs and also the larval mortality were recorded. Each treatment was replicated five times.

E. Statistical Analysis

All developmental and survivorship tests were replicated five times. Analyses of variance (ANOVA) were used to test the effects of treatment on the developmental time or survival rate. Then, the means were separated by Duncan's Multiple Range Test (P<0.05) (SPSS 16).

III. RESULTS

Fecundity and hatching of eggs derived irradiated pupae are concise in Figs. 3 and 4. Fecundity of the irradiated males copulated with non-irradiated females was declined with the increasing radiation doses markedly. Fewer eggs in all crossing of IM x NF at all doses of radiation suggest that the males were susceptible to gamma radiation. It was observed that gamma radiation reduced number of eggs significantly (P<0.05) from 185 eggs per female in control to 45 eggs per female by using 160 Gy, and this reduction displayed at all doses of irradiation in IM x NF crosses compared with NM x NF crosses.

However, the results showed that there was no statistically significant difference in the number of laying eggs among those females mated with irradiated males at the dose of 50 with 70 and 90 Gy and also between those females mated with irradiated males at the dose of 120 and 140 Gy (P<0.05). Egg hatching rates from the females mated with the irradiated males, and controls are shown in Fig. 4. The egg hatching percentage declined obviously with the increase of the radiation dose and no eggs could be hatched in 100-160 Gy which demonstrated that egg hatching depends on dose of radiation. The females mated with the fertile wild-type males displayed the maximum effective hatch rate at 75.8%. The lowest hatch rate of 5.6 % was noted in the eggs from females mated with males irradiated at 90–160 Gy. There was a significant difference in hatch rates between treatments and control (P<0.05).

Fig. 1 The olive plantations – Roodbar

Fig. 2 Rearing of *Bactrocera oleae* in insectarium

The larvae of the F_1 generation were powerfully affected by radiation at all doses (50-100 Gy). The results in Fig. 5 specified that the mean duration of the larvae was significantly different (P<0.05) at any irradiation dose compared to that of the non-irradiated (control) one. The larval developmental time was significantly (P<0.05) extended in all crosses with the increasing dose of the radiation when compared with the control.

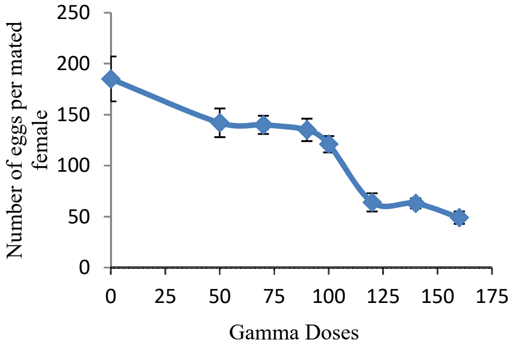

Fig. 3 Effects of gamma radiation on fertility of *Bactrocera oleae*

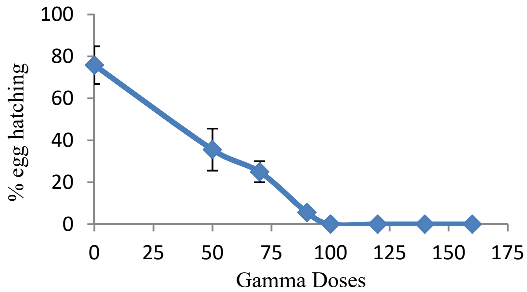

Fig. 4 Effects of gamma radiation on egg hatching of *Bactrocera oleae*

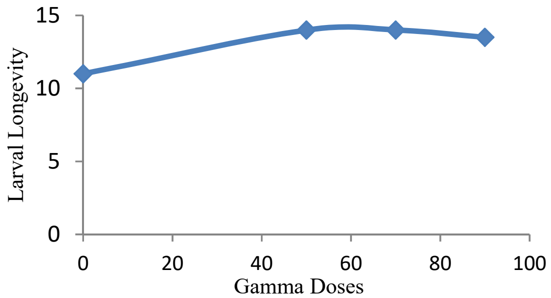

Fig. 5 Effects of gamma radiation on larvae longevity of *Bactrocera oleae*

IV. Discussion

The results specified that the gamma radiation produced significant reduction in percentage egg hatching of the female crossed with irradiated males compared with control. Consequently, the amount of the doses is inversely proportional to the hatchability of the eggs. Also, the irradiation of pupae to 90 and 100 Gy even produced total sterility of the eggs. These results are in agreement with the results of Shehata et al. [16], which showed that a dose of 60–90 Gy has the most deleterious effect on the male gonads of *Bactrocera zonata*. Tephritids flies have comparatively similar sensitivity to the gamma irradiation, with the requiring dose of < 100 Gy to have proper levels of sterility [17]. Similar results were presented for *Anastrepha fraterculus* (Wiedemann) with doses of 90–100 Gy [18], or *B. cucurbitae* (Coquillett), and *B. zonata* with the dose rate of 70–90 Gy [19] (Cited by [20]). It was concluded that these differences may be due to genus of flies, age of pupae, methods of investigation and radiation source. In the case of receiving low radiation doses, sterility did not produce the proper effect; however, those that received too high doses might be affected by the competitive and effectiveness of the irradiated insects for SIT.

The results illustrated that the length of larval development in the progeny of irradiated pupa was significantly longer and increased with increasing the radiation doses. Furthermore, fewer progeny of the treated pupa survived when compared to the larval survival in the control. Consequently, our findings are consistent with those obtained for many other insect species [21], [22].

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