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Parasitism preference of normal and irradiated host pupae of *Bactrocera zonata*, Saunders (Tephritidae: Diptera) and *Bactrocera cucurbitae*, Coquillett (Diptera: Tephritidae) by pupal parasitoid, *Dirhinus giffardii*, Silvestri (Chalcididae: Hymenoptera)

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Abstract

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Fruit flies of the genus, Bactrocera are destructive pests of fruits and vegetables. A number of insecticides are applied for their control which are a serious threat to the environment and human health. Awareness is now developing among the farmers towards more sustainable and environmentally safe management practices. In our present study parasitism potential of *Dirhinus giffardii* and effect of irradiation on host pupae emergence time was investigated. The results showed that maximum parasitism of 40.35 in free choice and 47.30 percent in the no choice test for pupae of *Bactrocera zonata* and 37.75 in free choice and 35.15 percent in no choice for the pupae of *B. cucurbitae*, respectively. Maximum parasitism of 58.4 percent was recorded on three day old pupae irradiated with 40 Gy dose. Moreover, *D. giffardii* emergence from parasitized irradiated pupae, sex ratio were slightly tilted in favor of female with maximum female ratio of 1.22: 1 and 1.19: 1 was observed on 60 Gy dose on *B. zonata* and *B. cucurbitae* respectively . On the basis of experimental results it is concluded that *D. giffardii* can effectively be used to control *B. zonata* and *B. cucurbitae* and irradiation could effectively be used to increase emergence time of the host pupae.

Keywords: pupae; host; parasitoid; fruit; flies

Introduction

Two dominant species of fruit flies, *Bactrocera zonata* and *Bactrocera cucurbitae* are the most devastating pests of horticultural crop mainly fruits and vegetables in almost all parts of Pakistan (Abdullah and Latif, 2001; Abdullah et al., 2002; Stonehouse et al., 2002). Different control approaches have been adopted for its management to minimize crop losses. Generally, insecticides are being applied for its control (Mian et al., 1986), but due to injudicious use of these chemical insecticides the pest acquires resistance against a number of groups and also deteriorates the ecological surroundings. Now days the focus is towards more safe and eco-friendly management practices, like Male Annihilation Technique (MAT), Bait Application Technique (BAT), Biocontrol and Sterile Insect Technique (SIT) among which biological control is an important tool in pest management program (Baranowski et al., 1993) because of its high adoptability under natural conditions. Among the fruit flies pupal parasitoid, *Dirhinus giffardii* has the potential to be exploited as bio-control agents against different fruit flies species in Pakistan.

Radiation is considered an important technique for the economical and mass culturing of natural enemies. Irradiation plays significant role in the production of natural enemies by increasing shelf life of their host that also results in increase parasitism rate (Mannion et al., 1994). The emergence of *Anastrepha suspense* was completely suppressed after irradiating larvae and no adverse effects were observed on the parasitism of larval as well as pupal parasitoid, *Diachasmimorpha longicaudata* (Ashmead), (Sivinski and Smittle, 1990). *Dichashmimorpha laungicaudata* emerged from irradiated pupae of *Anastrepha fraterculus* was biased towards female at every dose applied at 60, 80, and 100 Gy (Bachmann et al., 2015).

The present studies were conducted to evaluate rearing of *Dirhinus giffardi* on pupae of *Bactrocera zonata* and *Bactrocera cucurbitae* to find out its host preference, moreover, host pupae were irradiated at different radiation doses to increase shelf life of the host and the dose rate at which it is most preferred by the parasitoid for oviposition. In addition, the most suitable parasitoid age for parasitization and also to determine the sex ratio of the parasitoids reared on irradiated and normal pupae.

Materials and Methods

The experiments were conducted in the Nuclear Institute of Agriculture (NIA), Tandojam. *Bactrocera zonata* was reared on wheat bran based artificial diet, whereas *Bactrocera cucurbitae* was reared on Bottle gourd, *Lagenaria siceraria* (Molina) one of its natural host. The adults *Dirhinus giffardii* were provided 30% honey solution. The pupae of fruit flies and parasitoids were taken from their respective colonies. To determine the effect on shelf life, the pupae were also irradiated at different irradiation doses by using Gamma Radiation Source Co-60, Model GWXJ80 of Nuclear Power Institute of China (NPIC) at Nuclear Institute for Medicine and Radiopathy (NIMRA), Jamshoro. These pupae were exposed to a dose rate of 1.20 Gy per minute. The experiments were optimized at 27±2°C temperature and 65±5% R.H.

Parasitism preference

Pupal parasitism preference tests, free choice and no choice were carried out on two fruit fly species, *Bactrocera zonata* and *Bactrocera cucurbitae*. A set of 1200 healthy pupae from *Bactrocera zonata* and *Bactrocera cucurbitae* were collected from the culture already being maintained in the laboratory for each treatment. For free choice test 600 pupae, 300 each of *Bactrocera zonata* and *Bactrocera cucurbitae* were put in a Perspex cage along with 10 pairs of freshly emerged, *Drhinus giffardii*. For no choice test 300 pupae of each species were put in separate cages along with 5 pair of *Dirhinus giffardii* in both the cages. In both free choice and no choice tests, pupae were exposed to the parasites, for 24 h duration. After 24 h, the pupae were kept separately in Petri dishes for adult emergence. After emergence, parasitoids were counted and calculated on the basis of parasitism per female. This experiment was carried in three replications each of free choice and no choice test. The parasitism rate in both tests was analyzed statistically by using SPSS software.

Irridiation of the host pupae

The irradiation test was carried out to investigate its effects on the host pupae shelf life. A total of 14000 pupae of *Bactrocera zonata* were taken from the colony for each dose and exposed to radiation at different doses, 20, 30, 40, 50, 60, 70 and 80 Gy. Exactly 200 pupae each irradiated at single dose were exposed to 5 pairs of 2-days old parasitoids in Perspex cage for 24 h. At last the pupae were kept separately until adult emergence for 12 days. At emergence the rate of parasitism at different radiation doses with different ages was calculated. This experiment was carried out using CRD experimental design.

Effect of irradiated pupae on sex ratio of the parasitoid

Fresh pupae were irradiated at doses 20 to 80 Gy. Two days old parasitoids were exposed to irradiated pupae and also on normal pupae for 24 h. After irradiation these were kept separately in glass jars. At the time of emergence the sex ratio of the parasitoids, *Dirhinus giffardii* was calculated on both irradiated and normal pupae.

In each set of the experiment, four replications were kept for evaluation of the effects. All the experiments were analyzed in completely randomized design (CRD) by using the analysis of variance (ANOVA). Duncun's Multiple Range Test (DMRT) was used to distinguish among the means.

Results

Parasitism preference

The results indicated that the *D. giffardii* preferred the pupae of *B. zonata* compared to *B. cucurbitae* at all the host (pupae) ages tested. The parasitism rate was higher on *B. zonata* compared to the *B. cucurbitae* pupae in both the free choice and no choice tests Table 1. At the pupal age of 2-3 days, maximum parasitism on both the fruit flies pupae was recorded in free and no choice tests. The parasitism rate per female of *B. zonata* and *B. cucurbitae* was 40.35 and 37.75 in free choice and 47.30 and 35.15 percent in no choice respectively. The parasitism rate was the lowest on 5-6 days old pupae of both the fruit fly species which was 0.75 and

Table 1. Evaluation of mean (± SE) for parasitism preference (free choice and no choice) to pupae of the two fruit fly species, *Bactrocera zonata* and *B. cucurbitae*

Host age (days)	Mean parasitism per female			
	B. zonata		*B. cucurbitae*	
	Free choice	No choice	Free choice	No. choice
0-1	9.80 ^{d} ± 0.55	14.88 ^{d} ± 0.35	5.00 ^{c} ± 0.36	9.45 ^{d} ± 0.39
1-2	27.80 ^{b} ± 0.72	35.55 ^{b} ± 0.65	15.15 ^{b} ± 0.53	17.20 ^{b} ± 0.34
2-3	40.35 ^{a} ± 0.77	47.30 ^{a} ± 0.87	37.75 ^{a} ±2.53	35.15 ^{a} ± 0.38
3-4	25.70 ^{c} ± 0.50	22.35 ^{c} ± 0.43	17.70 ^{b} ± 0.81	14.70 ^{c} ± 0.31
4-5	7.60 ^{e} ± 0.43	7.30 ^{e} ± 0.44	6.00 ^{c} ± 0.26	2.50 ^{e} ± 0.21
5-6	0.75 ^{f} ± 0.10	0.30 ^{f} ± 0.13	0.70 ^{d} ± 0.13	0.45 ^{f} ± 0.10
6-7	0	0	0	0
7-8	0	0	0	0
LSD values	1.468	1.392	2.809	0.696

Means followed by different letters into the same column indicate a significant difference. Data was analyzed through analysis of variance followed by DMRT (P = 0.05)

0.30 on pupae of *B. zonata* and 0.70 and 0.45 on pupae of *B. cucurbitae* in the free and no choice tests, respectively. The results also confirmed that considerable rate of parasitism on pupae of both the species of fruit fly were recorded only up to the host age of 4-5 days. The present studies reflected that the parasitism rate of the parasitoid was comparatively higher in the no choice test on pupae of both the fruit flies and the most preferred age of the host pupae for parasitism was 2 to 3 days.

Irridiation of the host pupae:

The results revealed that irradiation doses had a significant effect and increased the host *B. zonata* pupal age for rearing of the parasitoid *D. giffardii*. The radiation doses of 20, 30 and 40 Gy resulted in maximum parasitism of 30.9, 48.6 and 58.4 per female. At the dose of 50 Gy, maximum parasitism per female (32.9) was recorded on the 2-days old pupae and continued up to the 10 days age of pupae (Table 2). The present studies established that the radiation doses of 40 and 50 Gy successfully increased the pupal duration and continuous parasitism was recorded on them up to 10-days, although the parasitism gradually decreased with the host age.

Effect of irradiated pupae on the sex ratio of the parasitoid:

Results established that irradiation of the fruit fly's pupae showed some effect on the sex ratio and it tilted slightly in favor of females. The effect was more prominent on higher irradiation doses. On the untreated control and a dose of 20 Gy, negligible effects on sex ratio of the parasitoids were recorded. Highest percentage of females was recorded when the parasitoids were offered irradiated pupae after irradiation at the doses of 40 and 60 Gy (Table 3). The parasitoids reared on *B. zonata*, showed maximum female percentage when subjected to the radiation dose of 60 Gy (1.22:1 fe-

Table 2. Effect of different doses of irradiation on the pupal duration of the host pupae

Dose (Gy)	Mean parasitism by *D. giffardii* on *B. zonata* one day old irradiated pupae of different ages									
	1	2	3	4	5	6	7	8	9	10
0	12.4^{a} ±0.26	37.9^{a} ±0.38	25.25^{e} ±0.38	5.2^{e} ±0.26	1.7^{f} ±0.13	0.2^{f} ±0.08	0.0	0.0	0.0	0.0
20	9.9^{b} ±0.37	30.5^{c} ±1.11	30.9^{c} ±0.61	8.75^{d} ±0.30	4.6^{d} ±0.18	1.8^{e} ±0.17	0.0	0.0	0.0	0.0
30	11.8^{a} ±0.18	36.2^{a} ±0.80	48.6^{b} ±0.90	19.55^{b} ±0.36	15.6^{b} ±0.26	10.4^{b} ±0.75	6.0^{c} ±0.26	2.4^{c} ±0.15	0.0	0.0
40	10.3^{b} ±0.37	31.7^{bc} ±0.95	58.4^{a} ±0.76	26.9^{a} ±0.49	21.5^{a} ±0.38	14.9^{a} ±0.30	9.9^{a} ±0.22	6.1^{a} ±0.13	2.3^{a} ±0.13	0.3^{a} ±0.05
50	10.7^{b} ±0.22	32.9^{b} ±1.42	27.8^{d} ±0.85	17.8^{c} ±0.17	12.0^{c} ±0.26	9.1^{c} ±0.17	8.0^{b} ±0.26	3.6^{b} ±0.10	1.2^{b} ±0.10	0.1^{a} ±0.10
60	3.6^{c} ±0.22	11.1^{d} ±0.66	14.7^{f} ±0.54	5.3^{e} ±0.26	4.6^{d} ±0.22	3.8^{d} ±0.16	1.5^{d} ±0.13	0.7^{d} ±0.10	0.0	0.0
70	2.7^{d} ±0.36	8.35^{e} ±0.36	9.80^{g} ±0.22	3.7^{f} ±0.17	2.9^{e} ±0.17	1.5^{e} ±0.13	0.7^{e} ±0.10	0.2^{e} ±0.05	0.0	0.0
80	1.2^{e} ±0.08	3.60^{f} ±0.18	4.70^{h} ±0.26	1.9^{g} ±0.13	0.6^{g} ±0.08	0.5^{f} ±0.13	0.1^{f} ±0.05	0.0	0.0	0.0
LSD value	0.781	2.368	1.845	0.728	0.705	0.836	0.481	0.250	0.168	0.114

Means followed by different letters into the same column indicate a significant difference. Data was analyzed through analysis of variance followed by DMRT (P = 0.05)

Table 3. Effect on sex ratio of *Derhinus giffardii* reared on 2-day old irradiated pupae of *B. zonata* and *B. cucurbitae*.

Radiation dose (Gy)	Reared on *B. zonata*			Reared on *B. cucurbitae*		
	Parasitoids emerged	Sex ratio Female : Male		Parasitoids emerged	Sex ratio Female : Male	
20	168	1.10	1	145	1.08	1
30	163	1.12	1	142	1.14	1
40	156	1.14	1	133	1.18	1
50	152	1.14	1	119	1.13	1
60	144	1.22	1	117	1.19	1
70	138	1.16	1	112	1.11	1
80	139	1.14	1	103	1.15	1
0 (Control)	172	1.05	1	151	1.01	1

males to males ratio) followed by treatment of 70 Gy where the ratio of females to males was 1.16:1. The radiation doses of 40, 50 and 80 Gy had also positive effect on the sex ratio of *D. giffardii* and higher percentage of females compared to males were recorded (1.14: 1). The numbers of females of the parasitoids reared on *B. cucurbitae* were also found maximum with the radiation dose of 60 Gy where the ratio of female to males was 1.19:1 followed by treatment of 40 Gy (1.18:1). Overall the sex ratio, female to male ratio were comparatively higher on the radiation dose of 40 and 60 Gy in comparison to the other radiation doses tested. At the higher doses (70 and 80 Gy) the sex ratio of females to males decreased. The studies revealed some positive correlation between the increased radiation doses and the production of more number of females up to the dose of 60 Gy. An identical trend in the sex ratio was observed in the parasitoids reared on the irradiated pupae of both the fruit fly species.

Discussion

These studies indicated a wide range of irradiation effects for rearing of the fruit fly pupal parasitoid, *D. giffardii*. The emergence of the adult fruit flies decreased with the increasing doses of radiation. The decreased emergence may be considered safe in case if some pupae are left un-parasitized in the augmentative biological control program. These studies provide significant support for irradiation of hosts before exposing to the parasitoids. The emergence of the parasitoids from the irradiated fruit fly pupae clearly demonstrated that the use of host irradiated pupae does not depict any negative effect on parasitoids. A number of studies conducted in Mexico have demonstrated a very high efficiency of the parasitoids cultured on irradiated hosts (Montoya et al., 2000).

Irradiation of host to prevent the emergence of adults of *B. zonata* and *B. cucurbitae* would be more useful as compared to the irradiation of already parasitized pupae. Irradiation of the host pupae before offering to the parasitoids has been successfully applied in Turkey in conjunction with mass trapping and mass releases of the parasitoid, Psyttalia concolor reared on irradiated Mediterranean fruit fly larvae (Hepdurgun et al., 2009). Greaney and Carpenter (1999) also reported irradiation of unparasitized host pupae by gamma radiation compared to the irradiation of parasitized pupae.

We observed that when irradiated 3 day old pupae at 40 Gy were used for parasitism, higher parasitism was achieved than the un-irradiated pupae. In a similar irradiation experiment, Sarwar et al. (2015) achieved higher parasitism with irradiated pupae of *B. zonata* by *D. giffardii*. He used a radiation dose of 80 Gy to host pupae, *B. zonata* and reported 95.35% parasitism of 24 h old pupae by *D. gaffardii*. Only 33.20% the un-irradiated pupae were parasitized by the parasitoid. The parasitism decreased by the parasitoid with the increasing dose of 120 Gy. Similarly, Morgan et al. (1986) applied gamma radiation to the pupae of *Musca domestica* and ceased their development. The irradiated pupae were then exposed to its parasitoid Spalangia endius Walker. In another similar study, Roth et al. (1991) applied irradiation to the pupae as host for the parasitoid.

The sex ratio in the parasitoids emerged from the irradiated pupae was also slightly tilted in favor of females which may be attributed to the fact that males are more sensitive to radiation than females. Hassan et al. (2009) also observed a significant discrepancy in the sex ratio of the parasitoids after rearing them on the gamma irradiated host pupae and recorded higher production of females than the male at all doses and all the host age groups of the host (pupae) they tested. Pupae irradiated at 40 or 50 Gy had higher percentage of parasitism as compared to the control and other doses. The observed result in regard to host dependent sex ratio may be due to differential sex specific mortality in males versus females as predicted by theoretical models (Charnov et al., 1981).

The pupae of both fruit fly species used in the present studies were almost of identical structure with great similar-

ity. But the rate of parasitism on *B. zonata* was significantly higher than on *B. cucurbitae*. Some physiological activities of the adult predators may affect the preference as reported by Tillman et al. (2004). Production of bio-control agents on large scale in an economical way is very important for augmentative release program. The present studies supported continuous production of *D. giffardii* on irradiated pupae of the fruit flies and will prove very useful in the bio-control program against fruit flies in different orchards. In general, the irradiation of fruit fly pupae for rearing of parasitoids does not cause any damaging effects on the emergence of the parasitoids in the rearing colony. Hence, the use of irradiation to enhance the adult emergence time of the host in the mass rearing facility of the parasitoids demonstrated a clear, positive and even indispensable for augmentative releases of the parasitoids to be used as a component of fruit fly integrated pest management program.

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