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INVESTIGATION ON THE IN VITRO BIOLOGY OF *BACTROCERA CUCURBITAE*

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ABSTRACT

The study was conducted at the Fruit fly rearing laboratory in Agriculture Research Institute Tarnab, Peshawar during 2020. The study aimed was to investigate the life history parameters of *Bactrocera cucurbitae* on bitter gourd. The significant differences were observed in the life cycle of the *Bactrocera cucurbitae* when reared on bitter gourd. The mean incubation period, total larval period, pre pupal period, pupal period, adult male longevity, adult female longevity, fecundity, percent hatching, sex ratio and total life cycle for male and female were recorded as 1.20 $\pm$ 0.448, 1.50 $\pm$ 0.275, 1.54 $\pm$ 0.490, 3.50 $\pm$ 0.625, 6.02 $\pm$ 0.490, 1.05 $\pm$ 0.275, 5.75 $\pm$ 0.600, 11.02 $\pm$ 0.665, 2.90 $\pm$ 0.630, 1.80 $\pm$ 0.690, 10.23 $\pm$ 0.615, 15.00 $\pm$ 0.729, 33.50 $\pm$ 1.339, 32 to 35 eggs, 90%, respectively for bitter gourd.

INTRODUCTION

Fruit flies (Diptera: Tephritidae) are considered the main fruit pests worldwide (Ruiz *et al*. 2014). The dipteran family Tephritidae consists of over 4000 species, of which 700 belong to Dacine fruit flies (sub families of Tephritidae) (Fletcher, 1987). These pests are found in almost all fruit growing areas of the world and cause serious damage to fruits (Aluja and Mangan 2008). Fruit flies cause most of the damage to fruits and vegetables in the Indo-Pak subcontinent (Kapoor *et al.,* 1980).

It has been observed that there are three important species of fruit flies throughout the world including *Bactrocera zonata, B. dorsalis and B. cucurbitae* (White and Elson-Harris, 1992). *Bactrocera cucurbitae* is distributed widely in temperate, tropical and sub-tropical region of the world (Dhillon *et al.,* 2005; Sapkota *et al*., 2010). It is the only Tephritid species in India that is uniformly widespread, attacking a large array of cucurbit fruits (Dhillon *et al*., 2005). The first report on melon fruit fly was published by Bezzi (1913). Around 43 species described under the

genus *Bactrocera* from Asia, Africa, and Australia (Syed, 1969; Cavalloro, 1983; Munro, 1984; Fletcher, 1987).

In Pakistan, the important fruit flies that damage the veggies peach, melon and oriental fruits are *B. zonata*, *B. Dorsalis*, *B. cucurbitae* (John *et al*., 1997). Cucurbits are infested by several insect pests which are considered to be the significant block for economic production. Among them, cucurbit fruit fly is the serious pest responsible for considerable damage of cucurbits (Butani and Jotwani 1984). It has more than 81 host species, in which fruit losses can range from 30 to 100% (Dhillon *et al*., 2005). Cucumber is also the most preferred hosts of melon fruit fly.

Melon fruit fly, it infests wide range of fruits and vegetables. The first samples of *B. cucurbitae* in West Africa were taken in 1999 in Gambia by a team of researchers from the National Agricultural Research Institute (Sanyang, 2001). Senior-White (1924) listed 87 species of Tephritidae in India. Amongst these, the genus, *Bactrocera* (*Dacus*) causes heavy damage to fruits and vegetables in Asia (Nagappan *et al*., 1971).

The melon fruit fly is considered a federal quarantine pest in many countries, due to its highly invasive nature as majority of them cause extensive damage to many fruits and vegetables especially cucurbitaceous vegetables. They have been reported as the major limiting factor in obtaining high yields and good quality fruits of cucurbits. Their attack on cucumber not only reduces the yield but also affects the quality of cucumber and as a result, the marketability of the crop is reduced. In addition to direct losses, fruit fly infestation can result in serious losses in trade value and export opportunity due to strict quarantine regulations imposed by most importing countries (Chen and Ye, 2007).

The magnitude of losses varies from species to species which may range 30-100%, depending on the cucurbit species and season as well. Its abundance augments when the temperature falls below 32 °C and the relative humidity ranges between 60-70% (Dhillon *et al*. 2005). In Pakistan, cucurbit flies inflict around 7 million Rupees per annum (Khan *et al*., 1999).

Cucurbit fruit fly prefers young, green and tender fruits for egg laying. The females lay the eggs at 2 to 4 mm deep in the fruit pulp, and the maggots feed inside the developing fruits. The eggs are also laid in the corolla of the flower, and the maggots feed on the flowers. A few maggots have also been observed to feed on the stems. The full-grown larvae come out of the fruit by making one or two exit holes for pupation in the soil. The larvae pupate inside the soil at a depth of 0.5 to 15 cm. The depth up to which the larvae move in the soil for pupation, and survival

depend on soil texture and moisture (Jackson *et al*., 1998; Pandey and Misra, 1999). On different host the pupal stage varies from 7.7 to 9.4 days on bitter gourd, cucumber and spongy gourd (Gupta and Verma, 1995). In the course in hot weather early in the morning, the adult emerges in the largest number and throughout the cool climate emerges extra irregularly. Most of the adults emerge at morning time 8 to 10 (Jackson *et al.,* 1998). Keeping in view the above facts the current study was investigated the life history parameters of *Bactrocera cucurbitae* on bitter gourd.

MATERIALS AND METHODS

Study area

The present study was performed at the Fruit fly rearing laboratory at the Agricultural Research Institute, Tarnab, Peshawar.

Insect studied

Bactrocera cucurbitae was studied for mass rearing.

Methodology

The initial culture of *B. cucurbitae* was collected from infested melon fruits from the local fruit market Peshawar, Pakistan. The infested fruits were kept in 20 × 20 × 8 cm plastic trays on a 5 cm-thick layer of sieved moist sand to facilitate pupation. After every 3- 4 days, sand was sieved and newly formed pupae were collected. The pupae were kept in 10 cm-diam petri dishes (50 pupae/petri dish) lined with moist filter paper.

The newly emerged adult flies were collected and placed inside the rearing cages each 35 × 30 × 35 cm. Each rearing cage had wire mesh on 3 sides, glass on the top and a front at one side. A round trap door was provided in the plastic door to facilitate collection of adult flies for experimental purpose and also to provide food and water. The male and female flies were identified according to Drew and Raghu (2002).

On the bottom of each cage there was a 2 cm-thick layer of sieved sand with 5% moisture. A glucose solution (10% W/V) was provided inside the cage for adult feeding. This glucose solution was kept in a 50 ml beaker and a thumb sized water-soaked cotton swab was laid in such a way that half of it was immersed in glucose solution and remaining half stayed above rim of the beaker to keep the solution in reach of adult fruit flies. Slices of melon were kept inside each breeding cage for oviposition. These slices were replaced by fresh ones daily to avoid decay.

The entire fruit culture was maintained at mean temperature of 23.97 ± 0.66 °C and 16.17 ± 0.81 °C with mean relative humidity of 66.39 ± 1.66 and 74.07 ± 1.63%, respectively.

A binocular microscope was used to note the number of eggs present in each bitter gourd slice. This procedure was repeated until the death of the ovipositing females. The eggs collected were placed in 10 cm diam-petri dishes (50 eggs per petri dish) with moist filter paper at the bottom to prevent desiccation of eggs. After egg hatch, fresh bitter gourd slices were kept in each petri dish for feeding the young larvae. After 24 h melon slices were replaced.

Statistical analysis

The data was subjected to mean ± SE by using Statistix 8.1.

RESULTS AND DISCUSSIONS

Eggs

The incubation period varied from 1-2 days on different hosts. Results presented in table-1 showed that the incubation period of *B. cucurbitae* eggs ranged from 1 to 2 days for bitter gourd. The incubation period was 1.20 ± 0.448 days for bitter gourd respectively. The report on higher incubation period of *B. cucurbitae* on bitter gourd (1.43 days) reported by Patel (1989) and incubation period of *B. cucurbitae* 1.20 days on water melon (Shivakar and Dumbre, 1985) is not accordance with the present finding.

Hatching percentage

The hatching percentage differed on different hosts and ranged from 80.00 to 90.00% it is evident from the data in table-1 maximum egg hatching percentage of 90.00 was recorded when reared on bitter gourd. The observation on egg hatchability is in close agreement as reported by Patel (1989) is not in corroboration with present finding.

Maggots

The results on maggot revealed that, there were three larval instars of *B. cucurbitae* when reared on bitter gourd. The present finding are in conformity with the observation made by Manzar and Srivastava (2009) who reported the three larval instars of *B. cucurbitae* on bitter gourd.

First instar

The data in the table 1 indicated that the period of first instar development ranged from 1 to 2 days for all the three hosts. However, average minimum period of first instar larvae was 1.50 ± 0.275 days for bitter gourd.

Second instar

The data in table 1 indicated that the period of second instar development ranged from 1 to 2 days for all the three hosts. However, the average means development of second instar larvae were 1.54 ± 0.490 days, days for bitter gourd.

Third instar

Second instar larvae molted to third instar and become longer than the second instar. The third instar larval period ranged from 2 to 5 days on different hosts. However, the average means development of third instar larvae were 3.50 ± 0.625 (2 to 5 days) for bitter gourd respectively.

Total larval period

The larval period ranged from 6.02 to 8.09 days on different hosts. It is evident from the data presented in table 1 that the mean larval period was 6.02 ± 0.490 (5 to 7 days), 8.09 ± 0.330 (8 to 9 days) for bitter gourd. Bitter gourd recorded shorter larval period than rest of the hosts. Shorter larval period on bitter gourd (5.27 days) reported by Patel (1974), and Mir *et al* (2014) tally with the present finding.

Pre pupal period

The pre pupal periods *B. cucurbitae* on different hosts ranged from 1.08 to 1.12 days on different hosts. There was no impact of host on pre pupal period. The data in table 1 indicated that the pre pupal period varied from 1 to 2 days and the mean pre pupal period was 1.05 ± 0.275 days for bitter gourd Slightly longer pre pupal period on bitter gourd (0.56 day) reported by Patel (1989) and Mir *et al* (2014) is not in accordance with the present report.

Pupal period

The periods of pupa on different hosts differed significantly from each other and it varied from 5 to 11 days. Results on pupal period showed that the average means pupal period of 5.75 ± 0.600 (5 to 7 days), 7.10 ± 0.375 (7 to 8 days) for bitter gourd Thus, different hosts serving as food for larvae were found to have significant influence on the pupal period of *B. cucurbitae*, Patel (1974) and Mir *et al* (2014) has reported the shorter pupal period on bitter gourd (9.86 days) present finding. The reports on shorter pupal period of *B. cucurbitae* on bitter gourd (7.20 days) (Patel 1989) differ from the present report.

Pre oviposition

The data presented in the table 1 indicated that the females had a pre oviposition period of 9 to 13 days. However, the mean pre oviposition period was reported to be 11.02 ± 0.665 (10 to 12 days), 11.05 ± 0.500 (11 to 12 days) for bitter gourd respectively. The present investigation is

mostly is in agreement with the findings of Koul and Bhagat (1994) who also found it to between 10 to 15 days. The reports on longer pre oviposition period on bitter gourd (12.00 days) than on cucumber (10 to 15 days) (Patel, 1989) differed from the present results.

Oviposition

It is evident from the table 1 that significant difference in the oviposition periods of female *B. cucurbitae* when reared on all the three hosts. The oviposition period ranged from 2 to 5 days with the mean oviposition period of 2.90 ± 0.630 (2 to 4 days), 3.65 ± 0.705 (3 to 5 days) for bitter gourd. The longer oviposition period on bitter gourd (2.20 days) as reported by Patel (1989) and Mir *et al* (2014) is in agreement with the present results.

Post oviposition

It was observed that female fly lived for 1 to 3 days after completion of egg lying on all the three hosts. The mean post oviposition period of 1.80 ± 0.690, 1.84 ± 0.780 days was recorded for bitter gourd and cucumber respectively. The longer post oviposition periods on bitter gourd (0.50 days) than on cucumber reported by Patel (1989) are in corroboration with the present finding.

Longevity

Result in Table 1 showed that female lived for longer time than the male when reared on all the three hosts. The female longevity varied from 12 to 20 days with an average of 15.00 ± 0.729 (14 to 16 days), 17.21 ± 0.831 (16 to 19 days) for bitter gourd and cucumber respectively. Likewise, the males lived with range of 9 to 16 days and mean longevity was 10.23 ± 0.615 (9 to 11 days), 12.79 ± 0.653 (12 to 14 days) for bitter gourd and cucumber. Sisodiya (2007) reported shorter period of male adult on bitter gourd (10.60 days) than cucumber (12.00 days) is in accordance with the present report. However, Patel (1974) has reported shorter period of female adult bitter gourd (73.40 days) and male adult bitter gourd (67.80 days) which are not in tally with the present finding.

Fecundity

The female of *B. cucurbitae* deposited their eggs inside the epi or mesocarp region of ripening fruits. The females have an extremely slender and long aculeus that allowed them to gain access to a particular area for egg deposition. It becomes clear from the table 1 that the number of eggs laid by females when reared on bitter gourd, considerably. The fecundity of females ranged from 32 to 35 eggs (5 to 15 cluster) with a mean of 33.50 ± 1.339 eggs per five females on bitter

gourd, but at intervals of 1 to 5 days. Sisodiya (2007) has reported lower fecundity on bitter gourd (32.10 eggs). However, Patel (1974) has reported higher fecundity on bitter gourd (95.20 eggs) does not tally with present finding.

Total life cycle

The adult periods for female differed significantly on various hosts. The period from egg to the death of adult occupied by females was 28.05 ± 1.030 (27 to 30 days), 35.78 ± 1.812 (34 to 38 days) on bitter gourd. The report on shorter period of female on bitter gourd (14.70 days) (Patel 1989) which tally with the present finding. However, Patel (1974) than bitter gourd (73.40 days) does not tally with present finding.

The adult periods for male also differed significantly on different hosts. The period from egg to the death of adult occupied by male was 23.50 ± 1.318 (21 to 26 days), 30.17 ± 0.751 (29 to 32 days) on bitter gourd. Thus, a total life period of male was shorter than female recorded during present investigation. The report on shorter period of male adult on bitter gourd (10.10 days) reported by Patel (1989) tally with the present report (61.40 days) than bitter gourd (67.80) is not in accordance with the present finding (Table 1).

Table 1. Life cycle of Fruit Flies (*B. cucurbitae*)

S. No	Life stage	Bitter gourd Period (days)		
		Min	Max	Mean±S.D
1	Egg	1	2	1.20±0.448
Larvae				
2	I instar	1	2	1.50±0.275
	II instar	1	2	1.54±0.490
	III instar	2	5	3.50±0.625
	Total	5	7	6.02±0.490
3	Pre pupa	1	2	1.05±0.275
4	Pupa	5	7	5.75±0.600
Adult				
5	Pre oviposition	10	12	11.02±0.665
	Oviposition	2	4	2.90±0.630
	Post oviposition	1	3	1.80±0.690
Longevity				

6	**Male**	9	11	10.23±0.615
	Female	14	16	15.00±0.729
7	**Fecundity**	32	35	33.50±1.339
8	**Hatching%**	90.00		
Sex ratio and Total life cycle				
9	**Male**	21	26	23.50±1.318
	Female	27	30	28.05±1.030

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