Research Article



The Mortality Rate of Pupae and Adult of Fruit Fly *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae) Affected by Different Submerging Time and Soil Types under the Laboratory Treatment

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Abstract | Three separate experiments were carried out to investigate the effect of submerging time under water, pupal age and soil types on mortality and rate of emergence of fruit flies pupae to adult stage. Pupae of *Bactrocera cucurbitae* Coquillett at different ages by hour i.e., 12, 24, 48, 72 and 96 (hrs) were submerged underwater for (1 and 24 hrs). The rate of pupal emergence to adult stage and mortality was investigated. Results showed that emergence and mortality of *B. cucurbitae* was significantly affected by both submerging time and pupal age. The 12 hrs pupae were more sensitive than 72 hrs pupae, with minimum adult emergence rate and maximum mortality. Results from various submerging times (1, 2, 3 and 4 hrs) on same age pupae also showed similar trends in pupal's mortality and emergence to adult stage. In further investigation on the effect of four soil texture classes such asclay loam, silt clay loam, sandy loam and pure sand as control, low emergence rate and high mortality was recorded from silt clay loam texture soil as compared to only sand. Hence it is concluded that pupal age, soil texture and under water pupal submergence time has direct effect on the rate of emergence and mortality rate of fruit fly pupae in the soil. Intermittent irrigation of fruit fly host plants standing in clay loam or silt clay loam soil texture may help in controlling the overwintering pupae in the orchards.

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Keywords | Bactrocera cucurbitae, Cultural control, Flood irrigation, Mortality, IPM

Introduction

The cucurbits such as cucumber, bitter gourd, sponge gourd, bottle gourd, snake gourd, pointed gourd and pumpkins are the major vegetables grown across Pakistan. Among several biotic factors, responsible for the low productivity of cucurbits, the fruit fly *B. cucurbitae* is most destructive insect pest of fruits and vegetables over the last several decades in many countries of the world including Pakistan. This species of fruit fly is cosmopolitan and distributed in

the tropical, subtropical and temperate region of the world (Dhillon *et al.*, 2005).

The fruit fly *B. cucurbitae* (Coquillet) (Tephritidae: Diptera) lay eggs inside the fruits tissue at 2 to 4 mm and they prefer to infest soft-skinned young and green fruits. After hatching, the young larvae start feeding inside the fruit tissues. The last instar larvae leave the fruit and fall to the soil for pupation and pupation occurs inside the soil surface at the depth of 0.5 to 15 cm (Dhillon *et al.*, 2005). The soil texture





and moisture content play an important role in the movement of larvae and also affect the pupal survival in the soil (Jackson *et al.*, 1998).

Fruit fly has been reported to damage over more than 80 plant species, but crops such as cucumber, the bitter gourd (Momordica charantia), muskmelon (Cucumis melo), snap melon (C. melo var. momordica) and snake gourd (Trichosanthes anguina) are the most preferredhost plants (Ellwood et al. 1999; Dhillon et al., 2005). In Pakistan, the fruit flies cause annual loss to fruit and vegetable over US\$ 200 million (Stonehouse et al., 1998). The extent of damage is variable ranging from 30 to 100% depending on season and different cucurbits species (Sapkota et al., 2010). It has also been reported that *B. cucurbitae* infestation vary from 41 to 89% in Momordica charantia (Rabindranath and Pillai, 1986), in Trichosanthes anguina 90%, 60 to 87% in pumpkin fruits (Hollingsworth et al., 1997) and 28.55% infestation in watermelon (Singh et al., 2000b).

Because of their particular life history, most of the management strategy have focused on adults stage of fruit fly that includes bait traps (McQuate et al., 2005; Vargas et al., 2007), pheromone traps (using cue lures as male attractant) (Leblanc et al., 2011; Vargas et al., 2012; ; Mafra-Neto, 2013; Tan et al., 2014), sterile insect technique (Hendrichs et al., 2002; Dyek et al., 2006) and chemical control (Singh et al., 2000a). However, some attention has been paid to the possibility of fly control by targeting late-instar larvae and pupae in soil type and moisture (El-Gendy and AbdAllah, 2019; Li et al., 2019) which could be the major lethal factors during the pupal period of fruit fly species. Application of entomopathogenic nematodes (Steinernema feltiae) was found effective to suppress late larval instars of fruit fly in soil. During field tests in Hawaii, drench applications of entomopathogenic nematode (S. feltiae) to the soil increased the mortality of Mediterranean fruit fly larvae by 99.5% (Lindegren et al., 1990).

The exposure of fruit fly pupae to unfavorable environmental conditions negatively affect pupal survival. (Hennessey and Michael, 1994; Jackson *et al.*, 1998). Soil texture and moisture content plays an important role in provision of nutrients to the plants but it may negatively affect the hibernating fruit flies pupae in such soils. Research on the influence of soil texture and moisture content on the survival of Sarhad Journal of Agriculture

fruit fly pupae hibernating underneath the host plant may provide useful clues in their control. Knowledge on the effect of host plant irrigation, timing, soil moisture content and field saturation is necessary for devising new strategies useful in the cultural control of fruit flies. In addition, the effect of flood irrigation under natural conditions may also play important role on the survival fruit fly species. Keeping in view the above hypothesis, the present study was conducted to investigate the effect of different types of soils and water submerging timeson the different and uniform age *B. cucurbitae* pupae as a cultural control measure.

Materials and Methods

Establishment of fruit flies culture

The current research work was conducted under laboratory condition at the Nuclear Institute for Food and Agriculture (NIFA), Tarnab Peshawar, Pakistan. Fruit fly culture was established under laboratory condition 26±2°C and 70% R.H. The fruit fly infested fruits and vegetables were collected from the field and placedon pupal substrates having saw dust in the bottom for fruit fly pupation. After four to five days, the saw dust of the pupal substrate was sieved and pupae were collected. The collected pupae were placed in fruit fly rearing cage for adult emergence. After emergence, adults inside the cage were provided with artificial adult diet. The adult started mating after 10-12 days. Vegetable (as host) was placed inside the rearing cage for female to lay eggs. After two or three days, the host in which the eggs were laid inside the cage was transferred to the pupal substrate for pupation. The same methodology was repeated to maintain the fruit fly stock culture for further experiments.

Effect of different submerging time on B.cucurbitae pupae of different ages

Pupal substrate was prepared from clay loam soil. The pupal substrate was filled in plastic PEPSI bottles which served as pupal cage. Both end of the bottles was cut off at the tapering position. Lower end of the cage was covered with muslin cloth. One third of the pupal cages was filled with pupal substrate. Fifteen pupae of the required age (12, 24, 48, 72, 96 hrs.) were obtained from the stock culture and placed in each cage at the depth of 15 cm. The pupal age and submerging time served as treatment (five treatments) and three replicates per treatment. The upper opening of the cage was covered with muslin



cloth and tightened with rubber band. Plastic tubs were filled with tap water and pupal cages were submerged in water up to the marked pupal position in the cage for various durations (0, 1, 24 hrs.). After submerging for the required period, the pupae cages were taken out of the water and waited for pupae to emerge in the cage. Data on the adult emergence was recorded by counting the number of adults emerged in each cage. All emerging adults of *B. cucurbitae* were further transferred to adult cages and provided with 10% sugar and honey as artificial diet. Their mortality and longevity was recorded up to 3 months.

Effect of submerging time on uniform (24 hrs) old pupae of B. cucurbitae

Fifteen pupae of uniform (24 hr) age were obtained from the stock culture and placed in pupal cage at the depth of 15 cm. as in experiment above. The upper opening of the cage was covered with muslin cloth and tightened with rubber band. Each cage with known number of same age pupae was submerged in water tubs for 0, 1, 2, 3 and 4 hr duration. Pupae in the control were not submerged. After submerging for the required duration, the pupae cages were taken out from their respective tubs and placed in laboratory. Data on the adult emergence was recorded from each cage as in the first experiment. All emerging adults of B. cucurbitae were transferred to adult cages and provided with 10% sugar and honey as artificial diet. The adult's mortality and longevity was recorded up to 3 months on regular basis.

Effect of various soil types on uniform (24 hrs) old pupae of B. cucurbitae

Separate experiment was carried out to ascertain the effect of various soil types on emergence of uniform 24 hrs old pupae of *B. cucurbitae* under laboratory conditions at 26 ±2 °C and 70% RH. Four different types of pupal cages as in experiment 1 and 2 were prepared with various texture pupal substrates from soils i.e clay, silt clay loam, sandy loam and pure sand as control. Fifteen uniform age (24 hrs) old pupae of B. cucurbitae were obtained from the stock culture maintained in the laboratory and placed at a depth of 15 cm. in each cage with different soil textures. The upper end of the cage was covered with muslin cloth and tightened with rubber band. The pupae remain in the soil till adult emergence. The data on adult emergence and mortality was recorded for up to 2 months. The experiment comprised of 4 soil textures as treatments 3 replications of each treatment.

Results and Discussion

Effect of different submerging time on B.cucurbitae pupae of different ages

Results revealed that the percent emergence of B.cucurbitaepupae to adult wassignificantly affected by both submerging time and pupal age (Table 1). It is clear from the data that all the tested pupal age showed similar adult emergence of 93% with 0 submerging time. However, significant differences were found in mean percent adult emergence with respect to submerging time. The adult emergence was maximum (43.88%) at 1hour submerging and minimum (29.39%) when the pupae were submerged for 24 hrs. The adult emergence was lowest (31.10%) for 12 hrs old pupae at 1 hr submerging time followed by 32.90, 37.66, 55.50 and 62.20% adult emergence for 24, 48, 72, and 96 hrs old pupae respectively. Significant differences were found in mean percent pupal emergence to adult stage with respect to pupal age. The adult emergence increased with respect to increase in pupal age. Minimum number (6.66%) of adults emerged from 12 hrs old pupae when submerged for 14 hrs followed by 24 hrs old pupae (7.0%), 48 hrs old pupae (24.46%), 72 hrs old pupae (48.90%) and 96 hrs old pupae (59.96%). The adult emergence with respect to submerging time was lowest (43.68%) for 12 hrs old pupae while highest (71.82%) for 96 hrs old pupae. The present findings are in agreement with Pablo et al. (2008) who reported that young pupae were more susceptible than old pupae when immerse in rain water for different submerging time. EsKafi and Fernandez (1990) reported that when moisture content reached to the saturated point, the adult emergence of C. capitata dropped drastically. According to Hulthen and Clark (2006), the most significant factor affecting pupae was extremes in soil moisture. 85% pupal mortality occurred at 0% soil moisture and 30% pupal mortality occurred at 100% soil moisture. The good rate of pupal survival in soils with low moisture is most likely an adaptation to semi-desert climate in the Mexican northeast where this species originated. Similar results have been reported by Bressannto (2001) for pupal mortality due to desiccation in A. obliqua, (Hou et al., 2006) in C. capitata and (Jackson et al. 1998) in B. dorsalis. In addition, Fitt (1981) reported that survival of Dacus opiliae were drastically reduced in soils with moisture content below 10%.

In the present work, adult's emergence from soil with



more submerging times threatened pupal survival. When moisture content retained for long, adult emergence dropped drastically, perhaps due to a lack of oxygen, as pointed out by Eskafi and Fernandez (1990) for *C. capitata*. The survival of the pupal stage of *B. dorsalis* was inhibited when soil moisture reached the field capacity (Hou *et al.*, 2006). In our research, under normal condition with 0 submerging level, no effect on the emergence rate was recorded.

Table 1: Percent of the meantime of adult emergence of *B. cucurbitae affected by submerging time and pupal age.*

Pupal age (hrs)	Submerging time (hrs)			Percent of the meantime of adult emergence
	0	1	24	
12	93.30 a	31.10 de	6.66 f	43.68 d
24	93.30 a	32.90 e	7.00 f	44.60 d
48	93.30 a	37.66 d	24.46 e	51.81 c
72	93.30 a	55.56 b	48.90 bc	65.82 b
96	93.30 a	62.20 b	59.96 b	71.82 a
Mean per- cent adult emergence	93.30 a	43.88 b	29.39 с	

Means followed by different letters in respective column are significantly different at 5% level of significance followed by LSD Test; LSD (0.05) for submerging time: 3.917; LSD (0.05) for pupal age: 5.05; LSD (0.05) for submerging time x pupal age: 8.75

Post emergence adult's mortality of B. cucurbitae affected by submerging time and pupal age

Results given in Table 2 showed that post emergence percent adult mortality of B. cucurbitae was significantly affected by both submerging time and pupal age. With the progression in pupal age, post emergence adult's mortality decreased. Similarly, with the increase in submerging time, post emerged adult mortality increased. Data indicates that non-significant difference in post emerged adult mortality was found with 0 submerging time. The post emergence adult mortality was minimum (1.9%) at 0 submerging time, followed by 1 hr submerging and maximum at (3.2%) for 24 hrs. submergence time. It indicates that post emergence adult mortality is also affected with pupal age and submerging time. The post emergence adult mortality was found to be maximum (3.0%) for 12 hrs old pupae when submerged for 1 hr followed by 24 hrs old pupae (2.%), 48 hrs old pupae (2.3%), 72 hrs old pupae (2.0%) and 96 hrs old pupae (2.0%). The post emergence adult mortality was highest (3.7%) for 12 hrs old pupae3.33 % for24 hrs submerging time

followed by 2.66 % for 48 hrs old pupae respectively.

Table 2: Post emergence adult's mortality (%) of B. cucurbitae affected by pupal age and water submerging time.

Pupal age (hrs)	Submerg	ing time (Means percent	
	0	1	24	adult mortality
12	2.33 b-d	3.00 abc	3.66 a	3.00 a
24	2.00 cd	3.33 ab	3.33 ab	2.88 ab
48	2.00 cd	2.33 b-d	2.66 a-d	2.33 bc
72	1.66 d	2.00 cd	3.33 ab	2.33 bc
96	1.66 d	2.00 cd	3.00 a-c	2.22 с
Mean percent adult mortality	1.93 c	2.53 b	3.20 a	

Means followed by different letters in respective column are significantly different at 5% level of significance followed by LSD test; LSD (0.05) for submerging time: 0.48; LSD (0.05) for pupal age: 0.62; LSD (0.05) for submerging time x pupal age: 1.08

Significant differences were also found in mean percent adult emergence with respect to pupal age and submerging time. The younger pupae were found to be more susceptible with maximum mortality rate as compared to the old pupae. Similarly, the adult mortality with respect to submerging time was lowest 2.2% for older (96 hrs) pupae while highest 3.0% for youngest (12 hrs) pupae.

Effect of different submerging time on uniform age (24 hrs) old pupae of B. cucurbitae

Results presented in Table 3 revealed that *B. cucurbitae* adult emergence was significantly affected by submerging time. The adult emergence was highest (91.1%) in control while lowest (26.7%) for pupae submerged for 4 hrs. However, adult emergence calculated for the pupae submerged for 1,2,3 and 4 hrs were on-significant from each other but significantly lower than the control.

Results regarding post emergence adult mortality, showed that adult mortality was significantly high (4.0%) for pupae submerged for 4 hr and low (1.7%) in control. Similarly, adult emergence from pupae submerged for 1, 2 and 3 hr, and 4 hrs was statistically non-significant from each other but significantly lower than control (no submerging). It is clear that pupal submerging time is directly proportional to the post emergence adult mortality of fruit fly. As the submerging time increased the post emergence adult mortality also increased and vice versa. Almost similar

results were reported by (Liu, 1983) with respect to rainfall and soil moisture content on the development and survival of immature *B. dorsalis* fruit fly. Their populations were considerably depressed and after being subjected to prolong submerging in water and heavy rains for several days.

Table 3: Post emergence adult mortality (%) and emergence of uniform age (24 hrs old) B. cucurbitae pupae affected by submerging time.

Submerging time (hrs)	Adult emer- gence (%)	Post emergence adult mortality (%)
1	33.32 b	2.33 bc
2	35.53 b	2.66 b
3	35.54 b	2.66 b
4	26.66 b	4.00 a
No submerging (control)	91.09 a	1.66 c
LSD (0.05)	14.35	0.93

Means followed by different letters in respective column are significantly different at 5% level of significance followed by LSD test.

Effect of various soil types on uniform age (24 hrs old) pupae of B. cucurbitae

B. cucurbitae percent adult emergence was significantly affected by the soil texture (Table 4). The results from tested soil types showed highest adult emergence (88.9%) in control (pure sand) while the lowest adult emergence (11.1%) was recorded in silt clay loam. The adult's emergence in clay loam and silt clay loam was 17.8% and 11.1% respectively. The adult's emergence in sandy loam soil was significantly higher than the adult's emergence in silt clay loam.

Table 4: Effect of soil type on adult emergence and post emergence adult's mortality of B. cucurbitae.

Soil type	Adult emergence (%)	Adult mortal- ity (%)
Clay loam	17.77 bc	5.88 b
Silt clay loam	11.10 c	11.93 a
Sandy loam	24.43 b	5.44 b
Control (pure sand)	88.86 a	3.15 c
LSD (0.05)	11.43	0.50

Means followed by different letters in respective column are significantly different at 5% level of significance followed by LSD test.

Post emergence adult mortality was also significantly affected by the soil types (Table 4). Highest adult mortality (3.6%) was observed in the pure sand

(control) which was significantly higher than the remaining tested soils types. The lowest fruit fly post emergence adult mortality was observed in the silt clay loam soil i.e. (11.9%). The post emergence adult's mortality in clay loam and sandy loam was non-significant from each other but significantly lower than silt clay loam. Bento et al. (2010) also investigated that the number of emerged D. longicaudata adults was three times higher in sandy loam and lower in a heavy clay soil. The silt clay loam texture reduces the emergence rate of C. capitata but the sandy loam soil favors the pupation Ahmad et al. (2007). Similarly, the pupation depth to which the larva burrows into the soil is also affected by soil texture, moisture, compaction and temperature Dimou et al., 2003.

Conclusions and Recommendations

The present study warrants for fruit fly cultural control practices and indicates that the fruit fly papal emergence and post adult survival is directly affected by the pupal age, submerging time and soil texture classes. Field populations of fruit fly may be depressed considerably if their pupae are subjected to under water submersion from intermittent irrigation or rains for several days. This effect may be more prominent if the standing crop is grown in a clay loam or silt clay loam type soils.

Novelty Statement

This research will provide baseline information about fruit flies, which can further be used to develop management strategy for fruit fly in natural field conditions.

Author's Contribution

IK conceived the idea, wrote the manuscript, Cosupervise the student and critically reviewed the article. RK conducted the experiments, collected data. AU conducted the statistical analysis, wrote the manuscript and supervised the student.

Conflict of interest

The authors have declared no competing interest.

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