CHAPTER 6: ENVIRONMENTAL FACTORS AFFECTING THE IMMATURE MORTALITY OF *BACTROCERA PAPAYAE* IN THE SOIL

6.1 Introduction

Bactrocera papayae immatures in the fruits are exposed to various natural hazards such as attacked by braconid parasitoids and pathogenic micro-organism before the fruit ripen or dropped. These caused immature mortalities. The pathogenic microorganisms and saprophytic fungi will effect the immature as long as the immatures remains in fruits. However, the parasitoids only killed the immatures at the pupal stage. Dropped fruits are affected by other predators. Ants and bird were the main predators of *B. papayae* immatures in the University Putra Malaysia's (UPM) fruit farm in Serdang and University Malaya's (UM) farm in the campus.

The immatures inside the fruits find refuge from physical and climatic factors outside. When outside the fruits they become vulnerable to the effects of extreme heat and dryness, drowning, toxic chemicals and pathogenic organisms and their toxin that are associated with soil.

Fitt(1981), Liu (1982), Bagle and Prasad (1983), Miller *et al.*, (1993), and Keng Hong et *al.*, (1994) found that the amount the rainfall showed a significant effect on immatures.

Under the subtropical condition (such as in Hawaii, Taiwan and Northern India) both *B. papayae* populations and its hosts fluctuate with weather. Bee and Haramote (1961), Newel and Haramote (1980 & 1981), Liu (1982), Varga *et al.*, (1990), Bagle & Prasad (1983), Messino (1993), found that 75% to 85 % of larvae present in fruits at time the fruits fell to the ground did not produce adult flies or parasites even in absence of predator due to the fruit decay caused by fungi.

In orchards, the spraying of insecticide may cause a high mortality to pupating larvae and pupae in soil.

The aim of this study was to evaluate the contribution of factors mentioned to the mortalities of immatures of *B. papayae*. Many environmental factors were involved. Some were interdependent to one another and this made analyses and interpretation difficult.

6.2 Materials and methods

This field study was conducted in Serdang (UPM farm) and Kuala Lumpur (UM farm) and was carried out into two stages. In the first stage, damaged ripen papaya fruits in various types of cages were exposed to the wet and dry seasons of the year to assess the effects of biotic factors and weather on the immature mortalities of *B. papayae*. In the second stage, the immature mortality in the soil was evaluated.

6.2.1 Cages:

The cages used in the experiment were made of square grids from wood of thickness 2.5cm and a dimension 40×40cm^2 square x 12.0cm high. Cage 1 (plate 3) was prepared from two grids, one as cover and the other as base.

Three types of cages (i.e., 2, 3, 4) were prepared. For Cage 2 (plate 4), the cover and base were separate in order to facilitate opening. A fine copper mesh with the diameter of 0.5mm was greased and nailed at all sides. For Cage 3 (plate 5), the cover and base were not separate but fixed with a gap of 3.0cm in between. The gap was covered completely with a fine mesh with the diameter of 1.0mm and was nailed at all sides. In this case, the mesh was nailed to cover at two sides (one side for each cover and base) where the grease was not used. The other sides of mesh were flexible and were reinforced with a thicker wire (diameter 2.0mm). The flexible sides were used as the opening. For Cage 4 (plate 6) the cover and base were not fixed to one another,

therefore, separable. A fine copper mesh (diameter 1.0mm) was nailed at all sides to cover where the grease was not used.

The purpose of using the copper mesh were (i) it helped in ventilation and at the same time allowed free interaction between the biotic and a biotic factors from outside and the larvae inside the cage, (ii) it helped to prevent the larvae, adults flies and parasitoids in cage from escaping and (iii) the fine mesh (i.e., with diameter 0.5mm) was used to isolate ants from the cage while the thicker mesh (i.e., with diameter 1.0mm) allowed ants to enter the cage.

6.2.2 Treatments:

In the first stage of the study, four treatment were employed; 1 (a control), 2 (using Type 2 cages to isolate all predators from larvae), 3 (using Type 3 cages to allow only the ants to act on the larvae), and 4 (using type 4 cages to expose larvae to all predators). Three replications were carried out.

The papaya fruits of the same ripeness and size were collected from the trees and distributed at random in the four treatments of the experimental set. A total of three papaya fruits were used for each treatment.

Three replicates of experimental set were placed at each site (i.e., UM and UPM farms experiments). Each cage was dug into the soil cover just the base portion of the cage, exposing only its cover. The soil in enclosed by each cage was filtered to eliminate live larvae and pupae in it. For the soil in Type 2 cages, the ants were being eliminated. All covers were placed at their respective position except for the cage Type 4, which was left uncovered. In addition, the contact points between the base and cover for Type 2

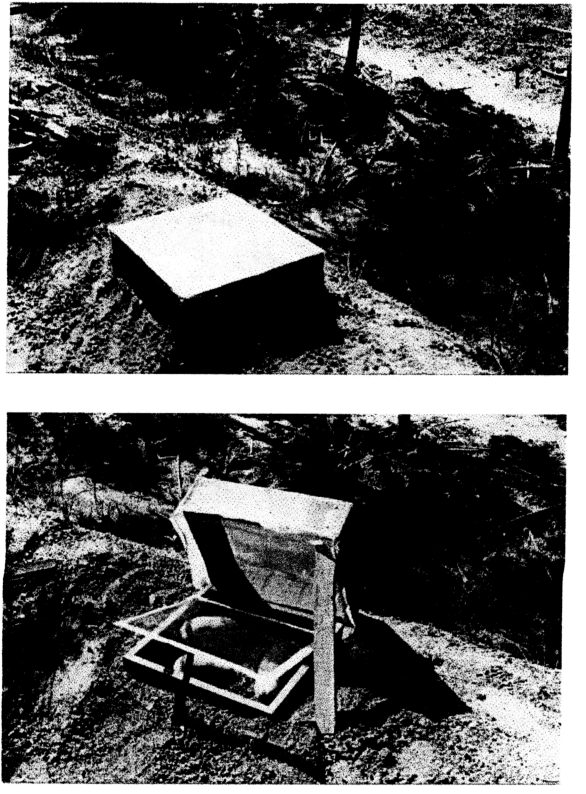

Plate 4: Type B cage 2, which used to isolate all predators from *Bactrocera papayae* immatures in papaya fruit (top) at starting the experiment (bottom) show placement of one sticky trap into the cage

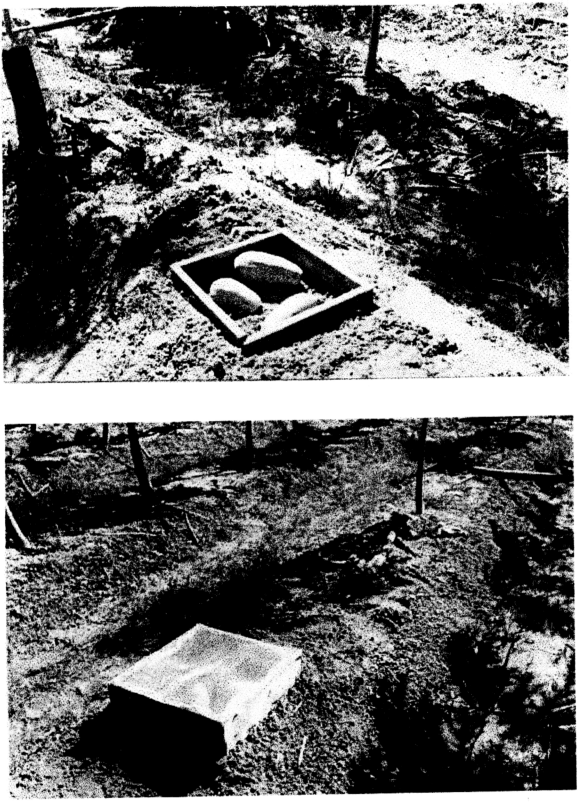

Plate 5: Type C cage 3 which was used to expose *Bactrocera papaye* immatures in papaya fruit at starting the experiment (top) and the cage covered after three days (bottom)

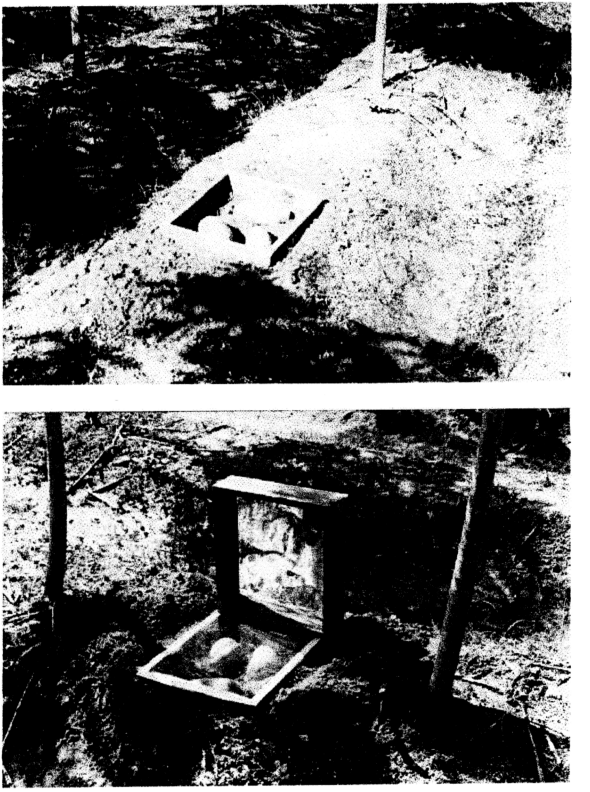

Plate 6: Type D cage 4 which was used to exposed *Bactrocera papayae* immature in the papaya fruit to all predators (top) at starting of the experiment (middle) shows placement of one sticky trap into the cage (bottom) at of the experiment

cages were greased to prevent reinvasion by ants. Concurrent to this preparation, which was followed by a testing period, the soil was allowed to return to its normal conditions for 2 - 3 days before papaya fruits where placed into each cage.

After placing papaya fruits into all the cages, Type 2 cages were greased at the points of contact between their base and cover. The cover of Type 2 cages were bound with a fine wire to their base. For Type 3 cages, the flexible sides of copper mesh were secured with a fine wire to cover. Type 4 cages were uncovered for three days to allow predators (including livestock and ants) to feed on the fruit and larvae. During the three days of the exposure, the cages and the fruits were examined or inspected twice a day to ensure that the fruits were fed by the predator. Throughout the experiment, if any of the Type 2, 3, and 4 cages were reinvaded by ants, they were either repaired or replaced or repositioned but within the limits of the experimental spot.

After the three days of complete exposure Type 4 cages were covered for three days. During, this period the larvae escaped from the fruits to pupate. Covering was necessary to prevent the pupating larvae from escaping. After three days of covering one fruit per cage from Type 4 cages was examined for the presence of larvae. Types 2 and 3 cages were not examined for the presence of larvae because they were covered throughout the experiment. If larvae were present, the cage would remain covered until all the larvae inside the fruits had escaped. This normally took 5 to 7 days from the first day of exposure. In the absence of larvae in fruits, the Type 4 cages were uncovered for two days so that larvae and pupae would be exposed to predators again. During this period Type 4 as well as Type 2 and 3 cages were examined on alternate days.

In the laboratory, 18 sticky traps of size 39.5 × 39.5 cm were prepared. Each trap was prepared by spreading a thin layer of sticky material on both sides of transparent plastic sheet of thickness 0.05 mm. At beginning of the second week of fruit

exposure, one stick trap was put into each Type 2,3, and 4 cages. It was used to trap adult flies and parasitoids during emergence. At the end of the second week the traps were inspected on alternate day. The total adult flies and parasitoids were recorded and removed from the traps (parasitoids identified to their species with the help of a magnifying glass). Trap inspection continued for about another week. Therefore, one experiment would last between 3 to 4 weeks.

Fruit used for the control treatments were brought to the laboratory to be cultured. They were maintained under the laboratory condition (i.e., temperature 25-27 ^{0}C and 79 ± 85 % RH) separately in plastic container (35 × 20 × 28 cm). When the matured larvae came out from the fruits they were transferred into small plastic container (10 × 10 × 10 cm) that contained clear sterilized sand for pupation. After about a week of culture the fruits were dissected to look for the presence of live and dead larvae. In the absence of live larvae, the fruits were discarded. The total number of mature larvae and pupae died and total number of adult flies and parasitoids emerged from the pupae were recorded for each fruit.

The experiment was repeated three times in the wet season (i.e., from September to November 1997) and 3 times in dry season (i.e., from mid of December 1997 to mid of March 1998). In the field, for every experiment, the spots were shifted within a season. However the same spots used for experiments were also used in the other season, though the position of cages within a spot were never repeated throughout the all the six experiments.

The second stage of the study started a month after the completion of the first stage. In the second stage, the soil from each experimental spot of the first stage were collected to the depth of about 3 cm. The soil was brought to laboratory for the following experiment. Under the treatment, two one half portion of the soil was

autoclaved at 120 °C for 30 minutes. Autoclaving helped to reduce microorganism and denatured their toxins. After autoclaving the soil was left overnight under the laboratory conditions before used. The other half of the soil was not autoclaved and was used untreated soil (i.e., Treatment 3). For the control (i.e., Treatment 1), fine sand taken from a clean area (i.e., free contamination by any insecticides and human waste) was used. This sand was soaked overnight in distilled water and was repeated three times. This was done to reduce possible precence toxic substance in the soil. After soaking the sand was autoclaved at 120 °C for 30 minutes.

For each treatment, 9 plastic container (10 × 10 × 10 cm) were used. The containers contained clean sand (6-cm depth) and were moistened with sterilised distilled water. Twenty matured *B. papayae* larvae from a laboratory culture were placed into each container. When the larvae entered the soil the container was covered (i.e., the cover with window 4 × 4-cm from copper mesh 0.5 mm) until the adult emergence.

When all the adults emerged from the soil, adult flies were counted and the soils were sieved to search for dead larvae and pupae. In this experiment it was difficult to find the exact number of dead larvae in the soil because dead larvae would turn black and disintegrate. However dead pupae and pupal cases were easily observed. The total dead larvae were taken as the difference between the total placed and the sum of dead pupae and pupal cases in container. Total adults died half-emerged (which considered as pupal mortality) and total adult that emerged from pupae but died before escaping from the soil were also counted. The experiment was repeated twice each time using different soil samples.

6.3 Results

6.3.1 Larval pupal mortality due to fruit factors

The total number of *B. papayae* larvae in Treatment I (i.e., 3 papaya fruits) varied from 57 to 80 and 80 to 99 during wet and dry seasons, respectively. The means of seasons were 80 ± 2.50 and 98 ± 10.57 respectively (Table 11). Appendix T tests (LSD) for variable larva 183 and 185 - T 1.

Under Treatment I, the means of total adult flies and parasitoids emerged: from the 80 larvae (under the Treatment I) during the wet season were 60±2.35 and 33.3±0.5 respectively. ANOVA showed that the mean was significantly different between seasons. During the dry seasons, the means of total adult flies and parasitoids emerged from 98 larvae were 70.3± 5.8 and 17.5± 1.2 respectively (Table 11) .The means of total adult flies and parasitiods emerged were significantly different for both seasons. The means of total adult flies and parasitiods emerged during the wet season were significantly higher than during the dry season. (Appendix dependent adult and parasitoids 183and 185)

The means of the overall percentage larval pupal mortality (i.e., sum of larval and pupal mortalities) under Treatment I were 19.7 and 21.9 during the wet and dry seasons, respectively, and the mean for the dry season was significantly higher than that during wet season.

Natural larval pupal mortality in the soil was 5.0 % subtract the larval and pupal mortality from the overall mortality under treatment I. The remaining 15.8 % was therefore contributed by larval mortality in the fruits. In this study, the agent causing larval mortality in the fruits (collectively termed as the fruit factors) was mainly those that were associated with toxic substances. The percentage of larvae that survived

Table 11 . Predation of *B.papayae* (means + s.e) in Petaling jaya (UM) and Serdang (upm)

Seasons	Treatments#	Mean Larvae##	Adult files	Parasitoids	% Larval - pupal mortality
Wet	I	80 ± 2.9 a	60.0± 2.6 b	33.3 ± 0.8 a	19.7 ± 2.6 d
	II	68 ± 1.6 b	22.3± 0.3 cd	12.0 ± 0.7 c	64.9 ± 1.7 c
	III	66 ± 0.4 b	19.1± 0.2 cd	7.0 ± 0.4 d	71.2 ± 0.9 f
	IV	65 ± 1.9 b	5.0± 0.6 e	5.0 ± 0.4 e	92.2 ± 3.4 a
Dry	I	98 ±10.6 a	70.3 ± 2.1 a	17.6 ± 1.2 b	21.9 ± 2.1 e
	II	78 ± 1.9 b	25.0 ± 2.9 c	7.0 ± 0.6 d	67.7 ± 2.5 c
	II	78.3 ±9.6b	15.0 ± 2.9 d	4.6 ± 0.6 d	80.4 ± 2.8 b
	IV	70 ± 2.9 b	5.0 ± 0.6 e	1.7 ± 0.5 e	92.8 ± 1.1 a

I = control , II = Absence of ants and fowls , III = prsence of ants alone , And IV = Presence of both ants and fowls and other soil fauna .

M ean larvae per treatment and each treatment was replicated 18 times per Season , total larvae in treatment I , II , III and IV were considered equal. Same Alphabet in a column were not significantly different at P = o.o5 .

through the fruit factors was 84.2 %. The surviving larvae matured and left the fruits for pupation in the soil.

6.3.2 Natural larval pupal mortality in the soil.

In the second phase of the study, for every 20 matured larvae used under Treatment I, 0.5 (2.5 %) of them died at larval stages while 0.6 (3.0 %) died at pupal stage. The larval to pupal mortality was therefore 1.1 (5.5 %). The mean of total adult flies emerged under Treatment I was 19 (95.0 %) (Table 12). The mean of larval and pupal mortalities and total adult flies emerged were significantly different. (Appendix 186 dependent variable: larva and pupa).

This finding shows that 5.0 % of mature larvae in the soil failed to form adult flies even in absence of the physical factors, biotic factors and the extreme weather conditions of the soil. This percentage killed is therefore due to the natural mortality in the soil. In the presence of the natural mortality and fruit factors, only 95 % of the larvae in the fruits could survive to adult flies and parasitiods.

6.3.3 Larval pupal mortality due to soil factors.

The overall contribution of soil factors towards the immature mortality is currently derived from the differences in the percentage larval and pupal mortalities between Treatment I and II (Table 11). Under, Treatments 1, 2 and 3 (Table 12), natural mortality as well as the physical and biotic factors of the Kuala Lumpur and Serdang soil were assessed separately.

Under Treatment II, the means of total adult flies and parasitiods that emerged during the wet season were 22.3 and 12 respectively. During the dry season, the means were 25 and 7 respectively. The means in two seasons were not significantly different

Table 12 . Larval and pupal mortalities (means + s.e) * of *B . papayae* in soil in Petaling Jaya and Serdang

Soil # Treatments	Total ## larvae	Larval Mortality	Pupal mortality	Adult flies	% Larvae Pupal mortality
1	20	0.5 ± 0.2 b	0.6 ± 0.2 b	19.0 ± 0.2 a	5.0 ± 0.1 b
2	20	0.8 ± 0.2 b	0.8 ± 0.2 b	18.7 ± 0.2 a	7.5 ± 0.1 b
3	20	1.0 ± 0.3 b	2.3 ± 0.6 a	16.7 ± 0.5 b	1 6.5± 0.7 a

* Means with similler alphabet were insignificantly different at P = o.o5

# 1 = control treatment (clean soil , autoclaved) .
2 = Autoclaved soil .
3 = Untreament soil .

Total larvae per treatment and each treatment was replicated 9 times .

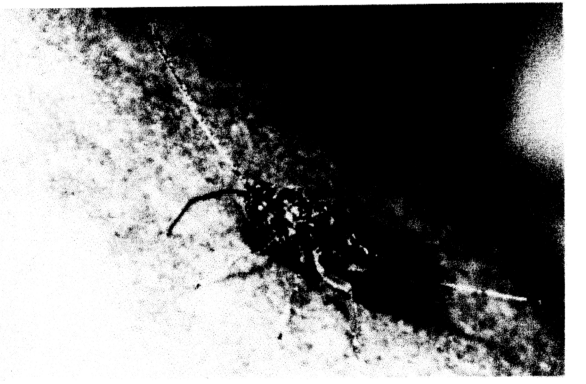

Plate 7: *Biosters arisanus* (Sonaus) parasitoid of *Bactrocera papayae* eggs

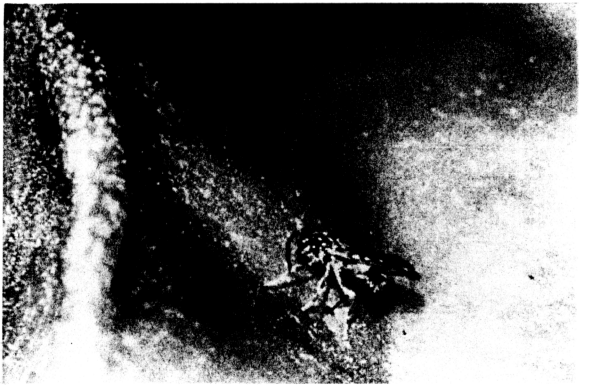

Plate 8: *Biosteres Vandenboschi* (Fullaway) parasitoid of the first instar larvae of *Bactrocera papayae*

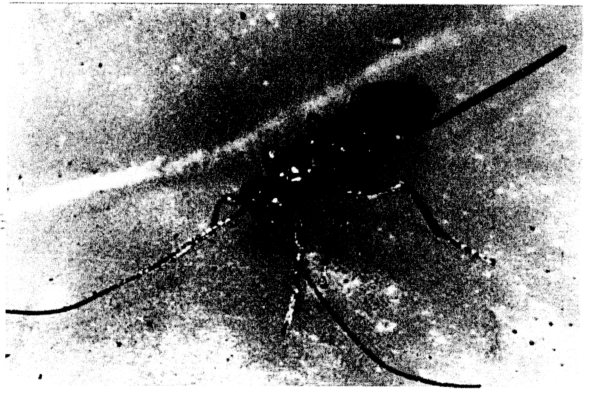

Plate 9: *Biosteres longicaudatus* (Silvestri) parasitoid of *Bactrocera papayae* Larvae (attack all stage of larvae)

(Table 11). The means of total adult flies and parasitiods that emerged for the two seasons were also not significantly different. However, the means for Treatments I and II were significantly different in both seasons (Appendix 186 dependent variable: adult).

Assuming that total larvae in Treatment II, III and IV were similar to that of Treatment I, the means percentage larval pupal mortalities under Treatment II were 64.9 ± 1.67 and 67.7 ± 2.50 for the wet and dry seasons, respectively. The means were not significantly different (Appendix, 184 and 186 T tests (LSD) percentage T2). The difference in the percentage survivals for Treatment I and II was 81.9. The agents causing this mortality are collectively termed the soil factors, which included the biotic factors and extreme weather conditions of the soil. Since the mean percentages of larval pupal mortality for Treatment I and II were significantly different for both seasons, the overall contribution of the soil factors towards larval pupal mortalities in Kuala Lumpur (UM farm) and Serdang (UPM farm) were therefore significant.

6.3.4 Physical factors of the soil.

Under Treatment 2 (Table 12), the means of larval pupal mortalities were 0.8 ± 0.2 (4.0%) and 0.8 ± 0.2 (4.0 %), respectively. Larval pupal mortality was 1.6 (8.0 %). Therefore 15.0 % of larval pupal mortality in the soil occurred in the larval stages while the remaining 85.0 % occurred in pupal stage. The mean of total adult flies emerged under Treatment 2 was 18.7 (93.5 %) (Table12). This result show that 8.0 % of matured larvae failed to form adult flies when they were allowed to pupate in autoclaved experiments soil (UM, Kuala Lumpur and UPM, Serdang). However the means of larval pupal mortalities under Treatment 2 were not significantly different ((Table 11). The means of total adult flies and parasitiods that emerged for the two seasons were also not significantly different (Appendix 186 dependent variable: adult).

The difference in the percentage larval and pupal mortalities between Treatment 1 and 2 was 1.5 and 1.0 respectively. Therefore, percentage larval pupal mortalites was 2.5. The agent causing this mortality is collectively termed the physical factors of the soil which include mainly the soil texture and its toxic substances or chemicals, the weather conditions of places of experiments soil not included in the physical factors and therefore non climatic. Since all differences in larval and pupal mortalities as well as in the percentage larval pupal mortalities between 1and 2 were not significant the contribution of physical factors towards the immature mortalities in these places was not significant at. ((Table 11). The means of total adult flies and parasitiods that emerged for the two seasons were also not significantly different (Appendix, 186 and 187 T tests (LSD) for variable: larval and pupa- T1 and T2).

6.3.5 Biotic factors of the soil.

The means of larval and pupal insulate mortalities under Treatment 3 (Table 12) were 1.0±0.3 (5.0 %) and 2.3± 0.6 (11.5 %) respectively. The larval pupa mortality was 3.3 (16.5%). Therefore, 11.5 % of the larval pupa mortality in the soil occurred in the larval stages while the remaining 88.5 % occurred during pupal stage. The mean of total adult flies that emerged was 16.7 (83.5 %)(Table 12). The means of larval pupal mortalities were significantly different.

The difference in the percentage larval pupal mortalities between 2 and 3 (Table 12) was 1.0 and 7.5 respectively. The difference in percentage larval pupal mortalities between the two treatments was 9.2. The agents causing the mortalities are collectively termed as the biotic factors of the soil. They are mainly micro-organisms and their heat resistant toxin. Since all the difference in the larval and pupal mortalities as well as in the larval pupal mortalities between 2 and 3 were significant the contribution of the biotic factor of the soil towards the immature mortalities was said to be significant

different (Appendix, 186 and 187 T tests (LSD) for variable: larval and pupa- T1 and T3).

6.3.6 Larval pupal mortality due to predators.

The overall contribution of predators towards the immature mortalities of *B. papayae* in the area (Petaling jaya & Kuala Lumpur) of the experiment was derived from the differences in the larval pupal mortalities between II, II and IV. This was due to the predacious ant species *Camponotus* sp., *Leptogenys* sp. and *Dolichoderus* sp.

During the wet season, the means of the total adult flies and parasitoids emerged under Treatment III 19.1± 0.2 and 7.0 ± 0.4 respectively. The means were not significantly different. During the dry season, the means were 15.0± 2.9 and 4.6± 0.6 respectively (Table 11) .The means were not significantly different. The means of total adult flies and parasitoids that emerged for two seasons were not significantly different under Treatment III (Appendix, 183 and 185 T tests (LSD) for variable: adult T3) .

The means of total adult flies and parasitoids emerged under Treatment IV (Table 11) were not significantly different during the wet season were 5.0 ± 0.6 and 5.0 ± 0.6 respectively.. During the dry season, the means were 5.0±0.6 and 1.7± 1.5 respectively (Table 11). The means were not significantly different. The means of total adult flies and parasitoid emerged for two seasons were also not significantly different under Treatment IV. The means of total adult flies and parasitoids between Treatment III and IV were not significantly different for both seasons (Appendix 183 and 185 T tests (LSD) for variable: adult T4).

Under Treatment III the percentage larval pupal mortalities 71.2± 0.9 and 80.4± 2.8 during the wet and dry seasons respectively, the average for two seasons 75.8. The means were significantly differently different. Percentage larval pupal mortality under Treatment IV for wet and dry seasons were 92.3 ± 3.4 and 92.8± 1.1 respectively and

the average for 2 seasons being 92.6 .The means were not significantly different. On overall, the difference in the percentage of the larval pupal mortalities between Treatment II and III as well as between III and IV were significantly different for both seasons (Appendix 184 and 185 T tests (LSD) for variable: percent T3).

6.3.7 Seasonal differences in the larval pupal mortality.

The last 3 experiments during of the wet season of the year were conducted between September and November 1997. During this period, it rained almost every day. Means of daily rainfalls during the first second and third experiments were 7.0 ± 3.2, 13 ±2.1 and 12.3 ± 3.4 mm, respectively. Daily air temperatures fluctuated between 23.1^{0}C and 32.6 ^{0}C while relative humidity fluctuated between 91and 100 %. The means daily air temperatures and relative humidity were 27.9 ± 4.8 ^{0}C and 95.6 ± 4.0 %.

The last 3 experiments during the dry season of year were conducted from mid December 1997 to mid March 1998). Daily air temperature and relatively humidity, on the other hand, did not fluctuate very much. The highest and lowest daily air temperatures recorded during the experiments were 34.2 ^{0}C and 23.6 ^{0}C with mean of 28.9 ± 4.3. The highest and lowest daily relative humidity were 100 and 80.1 % with mean of 88.4 ± 1.3.

The difference in the effect of wet and dry seasons on the immature mortalities *B. papayae* in Kuala Lumpur (UM) and Serdang (UPM) was derived from the differences in the percentages larval pupa mortalities during the different seasons for the same treatment. Under Treatment I, the difference in the percentage larval pupa mortalities between the two seasons was 2.2. Therefore, the increase in the percentage larval pupa mortality from wet to dry season was 12.2 %.

In the field, the different in the percentage larval pupa mortalities between the two seasons under Treatments II, III and IV were 2.8, 9.2 and 0.6 respectively.

Therefore, the average difference in the percentage larval pupa mortalities in the field between the 2 seasons were 4.2 % while the average increase was 5.9 %.

This study showed that all the percentage larval pupa mortalities were higher during the dry season. However, the overall differences in the larval pupa mortalities for the two seasons were not significant and that the effects of wet and dry seasons on larval pupa mortalities in Kuala Lumpur (UM) and Serdang (UPM) were therefore similar (Appendix 183 and 185 T tests (LSD) for variable: larva T1, T2, T3 and T4).

6.4 Discussion

Inherent difficulties encountered in this study were many and they imposed serious problem on the reliability of the result obtained. Firstly, *B. papayae* is a multi-voltine species and its generations are overlapping. Since it was impossible to evaluate the immature mortalities under the condition of overlapping generations, the first step in mortality assessment was to isolate on cohort of the same stage of development (in this study, the larvae) from the rest.

Isolation of larvae was easily done through fruit sampling, which contains eggs and larvae of *B. papayae* and through brief confinement of the fruits in cages, and all eggs would hatched into larvae. Therefore, in due time, immatures in the fruits were comprised only of various stages. This step follows by a series of evaluations on larval pupa mortality in cages. However, the act of isolating larvae from other immatures is in itself a weakness in which the element of bias was incorporated into the study. This is so because in the process, the multiple overlapping generations of *B. papayae* were conditioned into a single, non-overlapping one. The extent of bias involved in the study was immeasurable. Therefore, the larval pupa mortalities under the conditions of a non overlapping and overlapping generations had to assumed similar.

Secondly, environmental factors interacting with *B. papayae* in the field are many and most are closely interdependent, only a few could be evaluated separately. For instance larval and pupal mortalities in the soil could not be evaluated separately but combined as a larval mortality that was derived from the difference between total in the control (i.e., Treatment I) and total adult flies and parasitoids that emerged subsequently. Again to assume that total larvae in the field for Treatment II, III and IV as equal to that in the control (Treatment I) was also unrealistic (and therefore a bias). It was also impractical to count total larvae in the field treatments (i.e., an absolute counting) without interfering with their survival because in doing so. Fruits had to be dissected and this would cause desiccation of fruits and larvae inside. Because larval mortality involved in absolute counting can cause greater error compared to that in the larval estimation, the former is therefore preferred to the later. The basis for the above assumption was the random sampling in the distribution in to various treatments in each experimental set.

Lastly, to evaluate the effect of factor or a group of factors, a proper control treatment was needed as a contrast. However, control treatments were not always feasible. In the order to evaluate their effects, fruits should be sterilized as control treatment. To sterilize fruit without interfering with the larval survival inside was not feasible. Newell and Haramoto, (1968) sterilized guavas by dipping them in copper salt solution but the technique could not disinfect the fruits internally and therefore the fruits were not fully sterilized. There are numerous lesions on the fruits that could contain bacteria and fungus spores, and these would not be affected to any extent if disinfection was only externally done. Bacteria and fungi could be also enter fruits by *B. papayae* females during oviposition and by parasitoids when they attack *B. papayae* in the fruits.

Various species of *Mucor* are common components of soil flora, which are known to attack a variety of vegetables and fruits. These fungi cause a rapid breakdown of fruits, which was proven detrimental to the developing larvae inside (Newell and Haramoto, 1968). In the field as a result of dropping, the internal flesh of fruits was loosened and this facilitates fruit decay. However, a direct mortality on larvae by *Mucor* species could also be due to toxic by products of fruit decomposition which include the succinic, lactic and oxalic acid, ethyl alcohol and ammonia (Newell and Harmoto, 1968). Microorganisms in the fruits which include the bacterium, *Serratia marcescens* Bizio, and two other fungi *Penicillium* sp. and *Aspergillus* sp. had only been reported to cause *B. arisanus* (< biblio >) injured mortality to eggs.

Newell and Haramoto, (1968) suggested that 75 to 85 % of larvae in the fruits failed to form adult flies or parasitoids in the field, even in the absence of predators. They also suggested that about 80% of this mortality were due directly or indirectly to actions of *Mucor* spp. on the fruits. The present study did not support this finding since the percentage of larvae died in the unsterilized papaya fruits was only 14.7 %.

In soil the mature larvae and pupae were exposed to natural mortality. The larval pupa mortality in the clean sterilized soil was 5.0 % (Table 2 treatment 1). In this soil, toxic substance was absent or minimal and living micoorganisms were previously killed by autoclaving. Therefore, the percentage of larval pupal mortality in this section of study could well be considered as due to the natural mortality in the soil. If the soil factors and predators were eliminated (i.e., under Treatment I) fruit factors and natural mortality alone could reduce the percentage of adult flies and parasitoids surviving from the total larvae in the fruits to 75 %.

The matured larvae in the soil were not only exposed to the natural, but also variety of factors that reduce their survival. This group of factors, which caused

immature mortality in the soil, is termed as the soil factors, which minimally include soil texture, toxic substance and weather conditions. However only a limited number of these factors could be evaluated (Table 12). The overall effect of the soil factors accounted for 45.5% of the immature mortality, and together with fruit factors and natural mortality, their combined effects reducing the percentage of the adults flies and parasitoids surviving from the total larvae in the fruits to 33.3 % .

In the autoclaved soil larval pupal mortality was 7.5 %. If it could be assumed that all the living microorganisms were killed by autoclaving soil of the experiments soil should contain only the heat resistant toxic substances or chemicals (which were collectively termed the physical factors).

When the natural mortality was subtracted from overall mortality in the autoclaved soil, the remainder (which is 2.5 %) should give the total mortality due to physical factors of the soil. However physical factors do not include weather conditions of the soil since weather was not simulated in the laboratory. Since the percentage larval pupal mortalities in Treatment 1 (i.e., natural mortality) and 2 (i.e., autoclaved soil) were not significantly different at $P = 0.05$, the effect of the physical factors of the soil on larval pupal mortality in the Kuala Lumpur (UM) and Serding (UPM) were not significant.

Further observation showed that an appreciable number of pupae died half emerged when trapped in soil lumps. The matured larvae when they bored through the soil to pupate penetrate in all directions. But if they penetrate into the soil lump and pupate there the adult will not be fully emerge. However, in Treatment 2, most of the pupal mortalities (which may be due to the physical factors) were actually contributed by this type of pupal death.

In the untreated experimental soil, the larval pupal mortality was only 16.5 %. This mortality undoubtedly includes the natural mortality and mortalities due to the soil physical and biotic factors. If the natural mortality and mortality due to the soil physical factors were subtracted from the total mortality in treated soil, the remainder (which is 9.0 %) should give the mortality due to soil biotic factors.

One common observation amongst Treatments 1, 2, and 3 (Table 12) was that most of the matured larvae manage to pupate in the soil. Even in the untreated soil, there was 95.0 % of the matured larvae pupated and most immature mortality in the soil occurred in the pupal stage. Most mature larvae that dropped to ground were able to pupate naturally in the field conditions.

If the mortalities due to soil physical and biotic factors are added together, they contributed 29.0 % to the mortality induced by soil factors. This mean that 71.0 % of the mortality due to soil factors remained unaccounted for by 2 factors (Table 12), however factors responsible for this mortality were not immediately known, principally because they could not be evaluated. However, it is necessary to suggest some of the important factors that could have played significant role in it because the same soil under field conditions could cause a higher mortality when used in the laboratory.

One major difference between the laboratory and field conditions was that weather (which was the inherent part of the field conditions) was not simulated in the laboratory. However it was not the only possible factor involved since there is also a possibility that the physical and biotic factors of the soil could become more detriment to larvae and pupae under the field conditions. The unaccounted mortality could also be due to some form of interactions between various factors such interactions could produce a stronger influence on the larval pupal mortality in the field. There may be

others contributing factors but the following evidence strongly suggest that weather was main factor.

The first phase of this study was carried out during the extreme wet and dry seasons of the year. When the first 3 experiments were carried out, heavy rains fell almost every day. Under this condition the mature larvae and pupae in the soil were mostly submerged in the water which caused drowning of larvae and pupae. Also a constant wetness of the soil could lead to a more rapid decomposition of fruits.

The last three experiments were carried out during the extreme dry season of the year with little rain. The larval and pupal were never exposed to rain since it rained at the end of the experiments (i.e., when emergence was almost completed). The matured larvae that dropped to the ground to pupate were immediately exposed to shocks caused by extreme heat and low soil humidity. These shocks could cause desication of larvae and pupae in the soil. Larvae might die or at most if pupate, they would not form healthy pupae which died subsequently.

The present study showed larval pupal mortalities during extreme wet and dry seasons in the tropic were equally detrimental to larvae and pupae in the soil. This could well be considered as the major factor causing the mortality and accounted for physical (non climatic) and biotic factors of the soil.

At this juncture, it is necessary to point out that the difference in the larval pupal mortalities between Treatment I for wet and dry seasons was not possibly due to weather since experiment were both carried out under laboratory conditions. However, the difference could have been caused by competition amongst larval abundance in the fruits during the dry season. Suitable host fruits during the dry seasons were few in number and more females had to oviposit on the fruit. These increases in larval abundance in the fruits during dry seasons.

The percentage of individuals surviving from the fruit and soil factors was 18.9, and this portion was predated upon by ant) (*Camponotus., Leptogenys* and *Dolicholderus* sp.*)* which are common predator on larvae and pupae of *B. papayae* .

Newell and Haramoto (1968) found that predators killed 40 to 60 % of the individuals that survived through fruit and soil factors. Therefore, the present study also proved predators are an important group affecting *B. papayae* survival in the field. In the field the ants were frequently carrying dead larvae and pupae. The mature larvae, as they came out from fruits would immediately jump up many times before they finally entered soil to pupate. Jumping could help them to escape from higher density of the predating ants. Sometimes, larvae could shake off some ants from their body by vigorous jumping. But, when caught, one ant would be sufficient to kill a larva.

Among the 3 major group of environmental factors affecting the survival of the soil associated immatures of *B.papayae*, soil factors played the most important role. This factors reduced the larval survival higher in dry than wet season (Table 11). The mean of larval and pupal mortality were higher in dry season. Within the soil factors, weather condition are probably the most important. Other important groups of environmental factors were the predators and fruit factors were more affected on dry than wet season.