Research Article **ISSN: 2393 – 9540**

International Journal of Nutrition and Agriculture Research

Journal home page: www.ijnar.com

EFFECT OF NUTRIENT AND TRIGGERED EGGPLANT DEFENSE SYSTEM BY DIFFERENT PRE-INFESTATION ON THE ACTIVITIES OF WHITEFLY PARASITISM

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ABSTRACT

This study investigated the parasitism activity of a pupal parasitoid, Encarsia hitam (Insecta: Hymenoptera) (Chalcididae: Coccophaginae), on whitefly pupae, Bemisia tabaci (Insecta: Aleyrodidae), infesting eggplants pre-infested with whiteflies and aphids and fertilized with varying levels of nutrient concentrations (50, 150 and 300 ppm). Two-week-old plants without pre-infestation with pests (control) and pest-pre-infested plants (early infestation) were planted in polyethylene bags (30 cm × 30 cm) filled with burnt rice husks and placed under an exposed rainshelter (without walls). This condition enabled the plants to be freely attacked by several eggplant pests, including whiteflies and aphids, as they grew to maturity (bearing fruits). Our findings revealed that Encarsia hitam began to parasitize whitefly pupae as early as vegetative stage (four weeks after transplanting). Parasitizations number were very low on whiteflies infesting control eggplant plants fertilized with nutrient concentrations of 50 and 300 ppm (N1TC and N3TC), whitefly-pre-infested plants receiving 300 ppm of nutrients (N3TW) and aphid-pre-infested plants receiving 150 ppm of nutrients (N2TA). Whitefly- and aphid-pre-infested eggplant plants did not demonstrate significantly increased parasitism of whiteflies by Encarsia hitam, but varying dosages of nutrient and disparities in plant growth exhibited significant effects on parasitism activity. Parasitism on whiteflies declined when the aphids and whiteflies occupied simultaneously on eggplant leaves, which apparently offered better hosts for Encarsia hitam to oviposit in. Whitefly–parasitoid interactions in a multitrophic system generate vital information that improves our understanding of the community ecology, which is useful in the development of effective management techniques for the whitefly and its natural enemy.

KEYWORDS

Bemisia tabaci, *Encarsia hitam*, nutrients concentration, parasitoid and *Solanum melongena*.

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INTRODUCTION

The whitefly, *Bemisia tabaci* Gennadius (Homoptera: Aleyrodidae), is widely known as a detrimental agricultural pest. Several studies have shown that the whitefly species *B. tabaci* (sweet potato whitefly) infests many varieties of plants[1], particularly in lowland agricultural areas in

Malaysia[2]. Chemical treatment of this whitefly is challenging because infestation on the host plants occurs on the undersides of leaves[3], enabling whitefly larvae to easily escape insecticide applications. Additionally, the development of insecticide resistance has led many growers to use mixtures of several insecticides during indiscriminate insecticide treatments, resulting in the development of cross resistances to various insecticide groups, which is detrimental to crop growers[4]. Therefore, in control programs of some whiteflies, such as the silverleaf whitefly (*Bemisia argentifolii*), the citrus whitefly (*Dialeurodes citri*) and the giant whitefly (*Aleurodicus dugesii*), conventional biological controls have been heavily used[5],as these controls are more effective in the field and maintain low populations of these pests. In nature, *B. tabaci* has many natural enemies. Nineteen species of insects from the families Chrysopidae, Miridae, Anthocoridae and Coccinellidae and eleven species of mites from the families Phytoseiidae and Stigmaeidae are recorded as *B. tabaci*'s effective predators[6]. Among these predators are assassin bugs (*Macrolophus caliginosus*), lady beetles (*Delphastus* sp. and *Nephaspis* sp.), green lacewings (*Chrysopa* sp. and *Chrysoperla* sp.), minute pirate bugs (*Orius* sp.), big-eyed bugs (*Geocoris* sp.) and damsel bugs (*Nabis* sp.). The potential predatory behaviors have been studied in *Delphastus catalinae*, *Serangium parcesetosum* (Coccinellidae), *Macrolophus caliginosus* (Miridae), *Chrysopela carnea* and *Chrysoperla rufilabris* (Chrysopidea)[7]; these predators are effective when the density of the pest (whitefly) is low, but severe infestations are often devoid of predators.

A total of 28 insect species parasitize *B. tabaci*. These species include members of Aphelinidae, which consist of one species of *Aphelosoma*, 20 species of *Encarsia* and six species of *Eretmocerus*. In the family Platygasteridae, one species from the genus *Amitus* was recorded as a parasitoid of the whitefly[6,8]. The minute wasps *Eretmocerus* sp. and *Encarsia* sp., each approximately 1 mm long and either yellow, dark brown or bicolored (brown head and yellow body, depending on the species), are active parasitoids of *B. tabaci*. Females of these wasps lay their eggs inside whitefly nymphs. When the eggs hatch, the larvae feed internally on whitefly nymphs, eventually killing the host[5].

Several researchers have discovered that controlling whiteflies using biological control alone is less effective at reducing the population of this pest under field conditions[6-11] due to the daily feeding rate and the very limited searching ability of the parasitoid compared to the rapid reproduction (short life cycle and high fecundity) of whiteflies in warm conditions. However, in combination with other control methods, the parasitoids *Eretmocerus haldemani* (Family: Aphelinidae) and *Eretmocerus mundus* have been shown to be potentially useful against whiteflies in the cotton fields of California, USA, and the cassava fields of Zimbabwe, respectively[12].

Although Coudriet *et al*. (1986), Gerling and Horowitz (1984), Gerling (1986) and Gerling (1990) have found that predators and parasitoids are less effective in reducing *B. tabaci* populations in the field, several parasitoid species have shown considerable parasitic behavior in glasshouses[6-11]. Therefore, the present study was carried out to assess whether variations in the nutrient concentrations applied to the plants as well as exposure of the plants to early infestation (pre-infestation at the seedling stage) would influence the parasitization activity of *Encarsia hitam* on whiteflies infesting eggplant plants.

MATERIAL AND METHODS

Study Area

These experiments were carried out at the Department of Agriculture, Relau, Penang Island. Whiteflies that infested eggplant plants over two cropping periods were investigated. The first cropping began in August 2010 and was completed in October 2010, and the second cropping began in February 2011 and ended in May 2011. All pest analyses were carried out at the Laboratory of Entomology, School of Biological Science, USM Malaysia, in Penang. Penang has a year-round equatorial climate with high rainfall, a relative humidity of 70-85% and a temperature of 30±5ºC.

Eggplant plants were planted under a 60 m × 20 m rain shelter.

Preparation of the Whitefly Stock Culture

Preparation of Eggplant Plants

Selected F1-hybrid eggplant seeds were sown in seedling trays filled with compost in a completely netted nursery to avoid undesirable pest infestation. The plants were watered daily and fertilized weekly with a foliar fertilizer (Byfolan®).

Stock Culture of Whitefly and Aphids

Whitefly and aphid adults were collected from honeydew (*Cucumis melo* var. *cantalupensis*), which was planted under a rain shelter at the Agriculture Centre, Relau, Penang. The insects were cultured in a culture room at a temperature of 24±2ºC, 80±5% relative humidity and a photoperiod of 12:12 (L:D) at the laboratory of School of Biological Sciences, Universiti Sains Malaysia. For the stock culture, two-week-old eggplant seedlings were transplanted into plant pots that measured 12 cm (diameter) × 10 cm. Fifty whitefly adults and several potted eggplant plants were transferred into each of two wooden cages measuring 50 cm × 45 cm × 60 cm. Similarly, 100 aphids were transferred onto the leaves of several eggplant plants and placed in two rearing cages. The cages were fully netted (0.5 mm × 0.5 mm) to allow for light penetration and to provide ventilation as well as to prevent pest escape. All cages were placed on a metal rack with their bases inserted into ant traps filled with liquid detergent to prevent ants or other pests from reaching the cages.

Experimental plot and host plant preparation

A total of 90 five-week old seedlings (eggplant) were transplanted into polyethylene bags (30 cm × 30 cm), three-quarters filled with burnt rice husks. Each group of pre-infestation treatment consisted of ten plants of the following: (1) pre-infested by whitefly, (2) pre-infested by aphids and (3) without pest infestation (control). The polybags were arranged in five blocks that consisted of 18 plants per block and were equipped with a fertigation system. The nutrient mixtures used for fertilization followed the recommendation of the Malaysian Agricultural Research and Development Institute (MARDI)[12] (Manual Highland Tomato, MARDI, 2004).

Treatments

Two treatments were given to the eggplant seedlings: (1) the application of three concentrations of nutrients: N1 = 50 ppm, N2 = 150 ppm, N3 = 300 ppm; and (2) pre-infestation with whiteflies and aphids (Table No.1). Uninfested seedlings were used as controls. All components of the fertigation system, including the piping line, emitters and water pump, were automatically set up at three times of the day, 09:00 am, 12:00 pm and 17:00 pm, for five minutes for each application. Three concentrations of nutrients were prepared weekly and measured daily with a portable DiST®3 tester (HI 98303-Hanna Instruments).

Application of Nutrients

Raw fertilizer was recommended and supplied by the Department of Agriculture (DOA) Malaysia. Nutrient delivery at 150 ppm (N2) was recommended by the Department of Agriculture (DOA) Malaysia and Malaysian Agriculture Research and Development Institute (MARDI). Nutrient solutions were serially diluted with water to the required concentrations according to the methods of Mohd Rasdi *et al.* (2011 and 2005). Two sets of nutrient mixtures were prepared. Set A consisted of 900 g calcium nitrate (Cn-46%) and 41.6 g iron EDTA (Fe-2.15%). Set B consisted of 152 g potassium nitrate (Pn-7.87%), 500 g magnesium sulfate ($MgSO_4$-25.89%), 320 g potassium chloride (16.57%), 13.6 g phosphoric acid (0.7%), 0.1 g zinc sulfate (0.05%), 0.1 g cuprum sulfate (0.05%), 0.4 g ammonium molybdate (0.02%), 2 g manganese sulfate (0.103%) and 1.4 g boric acid (0.072%). Due to their differential solubility in water, each of sets A and B were diluted with 50 liters of water in two small reservoir tanks. Then, sets A and B were mixed and diluted in 1000 liters water. This nutrient mixture was diluted with water in separate tanks to 50 ppm (N1), 150 ppm (N2) and 300 ppm (N3). The pH of the solution was measured to ensure that it ranged between 5.8 and 6.8 for optimum plant growth using a PH METER® (HI-98127-Hanna Instruments).

Pre-infestation of Eggplant Seedlings by Whiteflies

Approximately 100 eggplant seedlings (three weeks old), sown in the seedling tray, were placed in a netted cage (50 × 45 × 60 cm), and approximately 300 whitefly adults from the stock culture were released into the cage, following the technique of Inbar et al. (1999). The plants were gently shaken daily to ensure that whitefly adults moved about and infested all seedlings in the cage. The seedlings were consistently inspected for the presence of immature stages of whitefly on the underside of leaves. After pre-infestation (one week), 15 seedlings were randomly selected and transferred to the experimental plot (under a rain shelter).

Pre-infestation of Eggplant Seedlings by Aphids

Similar procedures as for pre-infestation by whiteflies were followed, but for aphid pre-infestation, ten aphid adults from the mass-rearing cages were introduced manually onto each leaf of the seedlings. After ten days, 15 randomly selected seedlings were transferred to the experimental plot under the rain shelter.

Uninfested (control) Eggplant Seedlings

Fifteen eggplant seedlings in the sowing tray were placed in a cage. The plants were watered daily and transplanted into polybags in the experimental plot. Eggplant seedlings that consisted of eggplants pre-infested by whiteflies (TW), eggplants pre-infested by aphids (TA) and uninfested eggplants (TC) were transplanted into white polybags under a rain shelter. The experiments were arranged in a Randomized Complete Block Design with five replications (blocks) to determine the effect of nitrogen and plant-induced chemical defense (pre-infested plants) or secondary metabolites production by the host plants against the population of pests and natural enemies.

Data Collection

Whiteflies as well as other insects were collected from the second week after transplanting (2 WAT) until the end of harvesting (12 WAT). The samples were collected biweekly in the morning (9.00 am to 10.00 am) from each block, with a total of 45 samples (3 treatments of nitrogen level × 3 treatments of pre-infested plant × 5 replicates per treatment). In each replicate, a leaf sample was collected from the middle stratum of the host plant[14]. The leaf was inserted into a zip-lock plastic bag, its petiole was cut off, and the bag was fastened. All samples were brought back to the Entomology Laboratory. Parasitized immature whiteflies on the eggplant leaves were examined under a stereo microscope, and their numbers were recorded.

Data Analysis

All data were transformed to meet assumptions of normality and homogeneity of variances. Differences in the weekly mean abundance of the parasitized immature whitefly were analyzed using Multivariate Analysis of Variance (MANOVA)[15]. The result indicated the dependency of the two trophic components (parasitoid and whitefly) at different levels of nutrient application and pre-infestation of eggplant plants. Parasitism on whitefly larvae and interactions among factors influencing the parasitism in both cropping periods were analyzed using a multi-way factorial analysis of variance (general linear model)[16]. All values are presented as means (±) standard errors (S.E).

RESULTS AND DISCUSSION

Effect of Different Nutrient and Pre-infestation on Eggplant Plants against Parasitism Activity on Whitefly Pupae by *Encarsia hitam*

The abundance of the whitefly population in the field is partly regulated by natural enemies. Usually, the parasitoid *E. hitam* parasitizes whitefly larvae and pupae for several weeks before symptoms appear. The bodies of parasitized third-instar larvae and the pupae of whiteflies change their color from cream[3-17] to brown and finally to black when they die.

Table No.2 summarizes the whitefly pupal mortality on eggplant leaves after nine different treatments. Symptoms of parasitization were first spotted at 6 WAT. Overall, the total mean number of parasitizations was the highest in N1TA, with 2.25 per leaf, followed by N2TA (1.53/leaf), and the lowest was recorded in N3TW (0.3/leaf). Relatively high pupal parasitism was observed from 6 WAT until 10 WAT. At 6 WAT, the number of parasitized pupae that equaled the number of dead pupae (pupal mortality) was significantly different ($F = 2.187$; df

= 8, 89; P<0.05) among treatments. The highest parasitization was recorded in N1TA (8.1/leaf) and was significantly different (P<0.05) from other treatments, followed by N1TC and N2TC, and the lowest was in N3TW and N2TA. Unlike N1TA, pupal parasitization in these three treatments was not significantly different from other treatments. Decreasing parasitization activity was observed from 8 WAT onward. However, pupal mortality was significantly different among treatments at 8 WAT (F = 2.608; df = 8, 89; P<0.05, with the highest number of parasitized pupae in N2TA, 2.9/leaf). No parasitism was observed in N1TC during this sampling occasion. At 10 WAT and 12 WAT, a relatively high number of pupae were parasitized in N1TW (2.2±1.09) and N2TW (1.7±0.63), respectively. These results suggested that whitefly and aphid pre-infested eggplant plants attracted more parasitoids that parasitized whitefly pupae on the plants.

Data shown in the table are the mean number of larvae and pupae per leaf; WAT-week after transplanting; ANOVA - Analysis of variance. The value in brackets is the percentage of parasitized whitefly (larvae and pupae) over whitefly abundance + parasitized whitefly (larvae and pupae). * Means in the column with the same letters are not significantly different at P= 0.05 based on a Tukeys Multiple Range Test.

Table No.3 summarizes the effects of parasitism on whitefly larvae and pupae in the first cropping period. The effect of parasitization on the larvae and pupae of the whitefly (F = 3.066; df = 2, 144; P = 0.05) was significant on plants to which different levels of nutrients were applied. However, unlike what was observed in the first cropping period, both aphid and whitefly pre-infested plants did not exhibit a significant (P>0.05) increase in parasitism activity. In addition, the age of plants (WAT) strongly affected parasitization by the parasitoids (F=5.818; df =3,144; P<0.01), and the number of parasitized immature whiteflies fluctuated during the study period (Table No.3). Interestingly, there was a change in parasitism activities (F = 2.319; df = 6, 144; P<0.05) among plants receiving different nutrient levels as the plants aged (WAT (time)), showing an interaction between the two factors on the parasitization of whiteflies. The plant age (WAT) however, did not affect whitefly parasitization on pre-infested plants. Finally, no interaction among nutrient level, pre-infestation treatment and plant age (WAT) on parasitism activity was indicated by the results of the statistical analysis.

Table No.4 and Figure No.1 show the parasitism of whiteflies after various treatments of eggplant plants in the first and second crops. In the second cropping period, *E. hitam* exhibited a marked difference in parasitism activities from the first cropping period. Parasitism on whiteflies began earlier, at 4 WAT (N1TC, N3TC, N3TW and N3TA), peaked at 8 WAT and declined gradually until the end of the cropping period (Figure 1b). In general, fewer whiteflies were parasitized in all treatments at all sampling weeks in this cropping period compared to the first cropping period. Among all plants, slightly higher parasitism was observed on control plants receiving different levels of nutrients from 6 WAT to 10 WAT. At 8 WAT, whiteflies on aphid-pre-infested plants applied with 300 ppm nutrients (N3TA) had the highest parasitization among all pest-pre-infested plants in all weeks. There was no significant difference (P>0.05) in the mean number of parasitizations on whitefly pupae for each sampling week from 4 WAT until 12 WAT.

Data shown in the table are the mean number of larvae and pupae per leaf; WAT-week after transplanting; ANOVA – Analysis of variance. The value in brackets is the percentage of parasitized whitefly (larvae and pupae) over whitefly abundance + parasitized whitefly (larvae and pupae). * Means in the column with the same letters are not significantly different at P= 0.05 based on a Tukeys Multiple Range Test.

Except for plant age (WAT), treatments and interactions among treatments and plant age had no significant effect (P>0.05) on the parasitization of whiteflies in the second crop (Table No.5).

In the second cropping period, parasitism activity was influenced by the age of the plant (WAT) (F = 4.854; df = 4, 180; P<0.01), which was related to the abundance of the whiteflies on the plants (Figure No.1). During this cropping period, there was no influence of nutrient level and pre-infested plants on the amount of parasitism on whiteflies. Accordingly, there was no interaction effect among treatments and plant age on the parasitization of whiteflies by the parasitoids in all treated and untreated plants. Distributions of parasitized whiteflies on differently treated plants in both cropping periods are depicted in Figures No.1a and 1b.

Figure No.1 shows the results of the total mean number of parasitization of *E. hitam* against whitefly larvae on the underside of eggplant leaves for the first and second cropping. Symptoms were detected as early as the sixth and fourth WAT in the first cropping and the second cropping periods, respectively. The highest number of parasitizations in treatment N1TA (6.5/leaf) was observed at 6 WAT of the first crop. However, on the following week, treatment N2TA recorded the highest mean number of parasitizations, with 2.9/leaf. The highest mean numbers of parasitizations were found in N1TW and N2TW at 10 and 12 WAT, respectively. The least parasitization symptoms were detected in N3TW, with less than 1.2/leaf for all sampling weeks.

The parasitoid *E. hitam* is commonly manipulated and is used widely in controlling whiteflies in greenhouses or under rain shelters [7]. This species is a solitary, thelytokous (unfertilized eggs produce female offspring) end parasitoid. Females of these tiny parasitic wasps lay their eggs inside whitefly pupae. Once the eggs hatch, the larvae feed internally on the whitefly larvae and pupae, eventually killing the host [5]. In this study, parasitization by *E. hitam* on whitefly larvae and pupae (infesting eggplants grown under a rain shelter) changed color from the cream or transparent body of third instar larvae and pupae[3-18]. It is likely that the larvae were parasitized at least one week before the symptoms appeared because Sanderson (1996) previously reported the appearance of symptoms after ten days of parasitization[19].

Usually, parasitization activity starts just after the whitefly larvae reach the third or fourth instar stages[19] or approximately 10 to 14 days after whiteflies oviposit their eggs on eggplant leaves. During this study, evidence of parasitism (dead pupae) was detected as early as 6 WAT and 4 WAT in the first and second cropping periods, respectively. In the first crop, the parasitoids took some time to locate the whiteflies on the host plants because eggplant was never planted in the area prior to this study. In the second crop, however, parasitization could have taken place at 2 WAT or 3 WAT, as soon as the whiteflies produced their offspring on the plants. Early symptoms of parasitization in the second crop were a result of the availability of the parasitoids in the area due to the presence of host insects established on the eggplant plants during the first cropping period. This finding indicates that the parasitoids have good searching ability and are able to identify and parasitize whitefly larvae on the underside of leaves at the initial stage of plant growth. The females of *E. hitam* prefer to deposit a single egg in the third and fourth instar larvae of whitefly[20-23]. During the oviposition process, the females gain energy and nutrients through feeding on the hemolymph of the host that seeps from the wound[20].

Although earlier parasitization occurred in the second cropping period, the number of parasitized pupae was very low. The composition of other pests may contribute to the variations in parasitism activity on whitefly larvae and pupae. During this cropping period, populations of aphids were much higher than the whitefly populations in all sampling weeks. The presence of aphids provides the parasitoids with choices other than whiteflies because according to Sanderson (1996), *E. hitam* also parasitizes aphids and a few other hosts [19]. Among whitefly species themselves, *E. hitam* prefers *Trialeurodes vaporariorum* to *Bemisia tabaci*[19]. Their females are larger and have more progeny, which developed faster on *T. vaporariorum* than when reared on *B. tabaci*[24]. When the whitefly population was higher than the populations of other pests (e.g., aphids), such as during the first cropping period, the number of parasitized whiteflies was higher. This pattern of parasitism strengthens the hypothesis that *E. hitam* is not host specific. The selection of hosts was based on their availability in the field, which made the parasitoid less effective against whiteflies in the presence of other hosts (pests).

In the first cropping period, higher parasitism occurred in whiteflies infesting low-nutrient, aphid-pre-infested and control plants (N1TA, N1TC and N2TC), leading to a significant difference in the number of parasitized larvae and pupae ($F = 3.066$; $df = 2, 144$; $P = 0.05$) among treatments. The populations of whiteflies on these three treatments were higher than the populations of other hosts of the parasitoids, especially aphids. However, no such difference was observed in the second cropping period. In the second cropping period, a very high number of aphids (more than double the number of whiteflies) infested eggplant leaves. Very low parasitization by *E. hitam* on whitefly larvae and pupae in the second crop was likely related to the low number of whiteflies infesting the leaves. In this situation, the parasitoids had to spend more effort searching for the host insects[12,25] which is one of the decisive factors for successful parasitism. Furthermore, the parasitoids only parasitize third larval instar and pupae. During low populations, the availability of these stages can be very limited. Therefore, the parasitoids shifted their preference for better oviposition opportunities when aphids and other host species were highly available on the leaves. Shifting of parasitism preference has been widely reported in several non-host-specific natural enemies[26,27]. Moreover, the pre-infestation of eggplant plants with whiteflies and aphids did not have any influence on the parasitism activity of *E. hitam* on whitefly larvae in both cropping periods.

The parasitism activity on whitefly larvae and pupae was strongly affected by age of plant (WAT) in both crops. This behavior is greatly influenced by the abundance of whiteflies on the host plants. During the vegetative phase, eggplant leaves harbored high numbers of whiteflies as well as other pests. Accordingly, high numbers of parasitized immature whiteflies were recorded during 6 WAT and 8 WAT in both crops. After 6 WAT, the populations of whiteflies, together with its competitor pests in both crops, declined drastically to very low levels, corroborating high effectiveness of parasitoids to control the pests. High percentages of whitefly parasitism were recorded from 8 WAT onwards, in concordance with the high ratio of parasitized whiteflies to unparasitized whiteflies when very low populations of whiteflies infested the host plant. Based on earlier findings, some host plants were able to produce defensive metabolites to fight the pests that caused sudden a pest population drop after 6 WAT. Reduced leaf vigor or poor nutrient contents of the leaf at such plant ages[28] might also instigate a population drop after 6 WAT. Nevertheless, there has been a tendency to assume that the parasitoids contribute to the effective control of whiteflies as well as other host insects from 6 WAT until the end of the cropping period[7].

In the present study, the effect of nutrient treatments on the host plants of whiteflies on the parasitization of *E. hitam* was not very clear. A significant influence of nutrient concentrations on parasitism activities was observed in the first crop but not in the second crop. Similarly, the interaction effect on the nutrient concentration and time of its application was only observed in the first crop. A larger-scale study may be able to confirm these influences. Pre-infestation of eggplant plants with whiteflies and aphids, a surrogate for the early infestation of pests on the crop in the field, had no effect on the parasitization of *E. hitam*. Gerling *et al.* (2001) reported that several studies have been conducted on natural enemy activity in open fields,[7] in which there was no density-dependent effect demonstrated in untreated plots. However, McAuslane *et al.* (1994) observed an obvious density-dependent reaction when the percentage of parasitism was elevated following an increase in the *B. tabaci* population. [29]No interaction influence among different nutrient treatments and time on parasitism activity was observed in this investigation.

Some infested plants are able to produce chemical compounds or secondary metabolites to attract natural enemies to combat the pests. Alborn *et al.* (1997) identified the compound volicitin as a key component in a chain of chemical signals and biochemical processes that regulate tritrophic interactions among plants, insect herbivores and the natural enemies of the herbivores[30]. The results of the present study showed that the whitefly- and aphid-pre-infested eggplant plants did not produce appropriate chemical compounds or that the compounds produced were insufficient to attract more parasitoids to the eggplant plants, as there was no significant difference in the number of parasitized whiteflies among the pest pre-infested plants and the control plants.

One of the possible explanations for the low activity of parasitism observed in this study is competition among parasitoids and other natural enemies that possibly co-existed in the field. For instance, larvae of the parasitoid *Eretmocerus mundus* attack *Encarsia formosa* larvae for their survival[31]. In contrast, Pang *et al.* (2011) reported lower performances of *E. formosa* and *Encarsia sophia* when they parasitized whiteflies individually[32]. Their performances were much better when they parasitized whiteflies either concurrently or sequentially. However, competition between the two wasps reduced both parasitoids' fecundity. A few other reasons may justify the low parasitization seen in the field: first, the low availability of parasitoids in the field and surrounding area; second, a stronger preference of the parasitoid for larger hosts such as *T. vaporariorum*[32] (Pang *et al.* 2011); and third, a less conducive environment in a monoculture system that lacked a diversity of host plants and herbivores as well as their natural enemies[7] (Gerling *et al.* 2001).

In an Integrated Pest Management (IPM) program, natural enemies are essential to maintain the pest population in balance, below a destructive level (Economic Threshold Level). Although the parasitoid *E. hitam* did not exhibit very high potential for controlling the whitefly population in this study, more effort should be focused on enhancing or maintaining the parasitoid population at an effective level to suppress the pest population. More investigations on natural enemies are required because many previous studies have determined that predators and parasitoids were not effective in reducing *B. tabaci* populations under field conditions[6-11], but were more effective in the glasshouses. Some predators, such as *Delphastus catalinae*, *Serangium parcesetosum* (Coccinellidae), *Macrolophus caliginosus* (Miridae), *Chrysopela carnea* and *Chrysoperla rufilabris* (Chrysopidea)[7], have shown good potential as biological control agents for whiteflies. As reported by a previous study, the combination of two natural enemies, the parasitoid *E. formosa* and the predator *M. caliginosus*, reduced whitefly population effectively[33]. In future investigations, researchers should consider the interactions of several biological control agents in controlling whiteflies. In addition to predators and parasitoids, whiteflies are naturally attacked by insect pathogens such as the entomopathogenic fungi *Beauveria bassiana* and *Verticillium lecanii*. These fungi are useful components of an IPM program as they are relatively host-specific, inexpensive to produce, able to function in a wide range of greenhouse environments and safe for humans[17].

Table No.1: Two treatment (nutrient concentration and pre-infestation of seedlings) combinations used for eggplant plants

N1TC	N1 = 50 ppm; TC = non-pre-infested
N2TC	N2 = 150 ppm; TC = non-pre-infested
N3TC	N3 = 300 ppm; TC = non-pre-infested
N1TW	N1 = 50 ppm; TW = pre-infested by whitefly
N2TW	N2 =150 ppm; TW = pre-infested by whitefly
N3TW	N3 = 300 ppm; TW = pre-infested by whitefly
N1TA	N1 = 50 ppm; TA = pre-infested by aphid
N2TA	N2 = 150 ppm; TA = pre-infested by aphid
N3TA	N3 = 300 ppm; TA = pre-infested by aphid

Table No.2: Activity of parasitism of *Encarsia hitam* on whitefly larvae and pupae (Mean±S.E. and percentage) at every sampling of the first cropping period

S.No		2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT	Mean/ leaf	Total Mean/ 5 leaves
1	N1TC	0.00	0.00	2.8±0.8a (43.75%)	0a (0%)	0.5±0.31a (45.45%)	0.70.33ab (33.33%)	1.00±0.44	4
2	N2TC	0.00	0.00	2.1±0.836a (30.43%)	0.3±0.30a (27.27%)	0.2±0.13a (11.11%)	0.9±0.31ab (36.00%)	0.88±0.33	3.5
3	N3TC	0.00	0.00	1.1±0.314a (7.19%)	0.1±0.10a (5.88%)	0.2±0.13a (10.00%)	1.3±0.79ab (76.47%)	0.68±0.24	2.7
4	N1TW	0.00	0.00	1.2±0.467a (50.00%)	0.2±0.20a (100.00)	2.2±1.09b (36.67%)	0.2±0.20a (4.17%)	0.95±0.36	3.8
5	N2TW	0.00	0.00	1.7±0.651a (28.81%)	0.3±0.30a (23.08%)	1.3±0.54ab (21.31%)	1.7±0.63b (51.52%)	1.25±0.34	5
6	N3TW	0.00	0.00	0.6±0.305a (27.27%)	0.1±0.10a (100%)	0.2±0.13a (7.14%)	0.3±0.30a (12.00%)	0.30±0.09	1.2
7	N1TA	0.00	0.00	6.5±0.337b (21.45%)	0.2±0.20a (33.33%)	0.3±0.21a (17.65%)	1.1±0.31ab (47.83%)	2.03±1.04	8.1
8	N2TA	0.00	0.00	0.6±0.221a (33.33%)	2.9±1.46b (67.44%)	1.2±0.59ab (26.92%)	1.4±0.62ab (36.84%)	1.53±0.45	6.1
9	N3TA	0.00	0.00	1.4±0.618a (6.73%)	0.9±0.71a (21.95%)	0.4±0.40a (18.18%)	0.5±0.34ab (55.56%)	0.80±0.22	3.2
10	ANOVA			F=2.187;df =8,89; P<0.05	F=2.608; df=8,89; P<0.05	F=2.001; df=8,89; P>0.05	F=1.226; df=8,89; P>0.05		

Table No.3: Multiway factorial analysis of variance on the subjects effects between parasitization by *Encarsia hitam*, weeks after transplanting and treatments in the first cropping period.

S.No	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
1	Corrected Model	268.400(a)	35	7.669	2.090	0.001
2	Intercept	259.200	1	259.200	70.637	0.000
3	Treatment1 (Nutrient level)	22.500	2	11.250	3.066	**0.050**
4	Treatment2 (Type of pre-infested)	15.700	2	7.850	2.139	0.121
5	WAT (time)	64.044	3	21.348	5.818	**0.001**
6	Treatment1 * Treatment2	6.800	4	1.700	0.463	0.763
7	Treatment1 * WAT	51.056	6	8.509	2.319	**0.036**
8	Treatment2 * WAT	31.722	6	5.287	1.441	0.203
9	Treatment1 * Treatment2 * WAT	76.578	12	6.381	1.739	0.064
10	Error	528.400	144	3.669		
11	Total	1056.000	180			
12	Corrected Total	796.800	179			

Dependent Variable: Number of Whitefly Larvae –season 1; R Squared = 0.337 (Adjusted R Squared = 0.176)

Table No.4: Activity of parasitism of *Encarsia hitam* on whitefly larvae and pupae (Mean±S.E. and percentage) at every sampling of the second cropping period

S.No		2 WAT	4 WAT	6 WAT	8 WAT	10 WAT	12 WAT	Mean/ leaf	Total Mean/ 5 leaves
1	N1TC	0.00	0.20±0.20 (0.60%)	0.40±0.22 (4.17%)	0.80±0.70 (11.11%)	0.10±0.10 (2.33%)	0.60±0.34 (15.00%)	0.42±0.13	2.1
2	N2TC	0.00	0.00 (0%)	0.70±0.33 (10.45%)	1.60±0.99 (9.64%)	0.8±0.36 (12.50%)	0.40±0.36 (8.33%)	0.70±0.25	3.5
3	N3TC	0.00	0.20±0.20 (0.30%)	0.90±0.60 (9.28%)	0.50±0.40 (6.85%)	0.00 (0%)	0.30±0.21 (5.45%)	0.38±0.14	1.9
4	N1TW	0.00	0.00 (0%)	0.00 (0%)	0.10±0.10 (1.15%)	0.10±0.10 (1.23%)	0.20±0.13 (5.56%)	0.08±0.03	0.4
5	N2TW	0.00	0.00 (0%)	0.10±0.10 (1.33%)	0.70±0.60 (15.56%)	0.20±0.20 (4.00%)	0.20±0.13 (5.56%)	0.24±0.11	1.2
6	N3TW	0.00	0.20±0.20 (0.42%)	0.10±0.10 (1.15%)	0.70±0.50 (5.69%)	0.10±0.10 (14.29%)	0.40±0.40 (6.06%)	0.30±0.11	1.5
7	N1TA	0.00	0.00 (0%)	0.40±0.31 (3.92%)	0.30±0.30 (2.36%)	0.50±0.40 (7.69%)	0.20±0.20 (5.56%)	0.28±0.08	1.4
8	N2TA	0.00	0.10±0.10 (0.78%)	0.30±0.15 (3.90%)	0.30±0.30 (3.37%)	0.20±0.20 (1.89%)	0.30±0.15 (4.92%)	0.24±0.05	1.2
9	N3TA	0.00	0.00 (0%)	0.50±0.27 (2.89%)	1.20±0.63 (4.42%)	0.00 (0%)	0.30±0.15 (4.35%)	0.40±0.19	2
10			F=0.654; df=8,89; P>0.05	F=1.064; df=8,89; P>0.05	F=0.715; df=8,89; P>0.05	F=1.563; df=8,89; P>0.05	F=0.284; df=8,89; P>0.05		

Table No.5: Multiway factorial analysis of variance on the between-subjects effects of parasitization by *Encarsia hitam*, weeks after transplanting (WAT) and treatments in the second cropping period

S.No	Source	Type III Sum of Squares	df	Mean Square	F	Sig.
1	Corrected Model	31.999(a)	44	0.727	1.080	0.354
2	Intercept	41.658	1	41.658	61.877	0.000
3	Treatment1	0.730	2	0.365	0.542	0.583
4	Treatment2	3.545	2	1.773	2.633	0.075
5	WAT	13.072	4	3.268	4.854	**0.001**
6	Treatment1 * Treatment2	1.899	4	0.475	0.705	0.589
7	Treatment1 * WAT	2.901	8	0.363	0.539	0.826
8	Treatment2 * WAT	1.913	8	0.239	0.355	0.942
9	Treatment1 * Treatment2 * WAT	7.532	16	0.471	0.699	0.792
10	Error	121.183	180	0.673		
11	Total	195.000	225			
12	Corrected Total	153.182	224			

Dependent Variable: Number of Whitefly Larval, R Squared = 0.209 (Adjusted R Squared = 0.016)

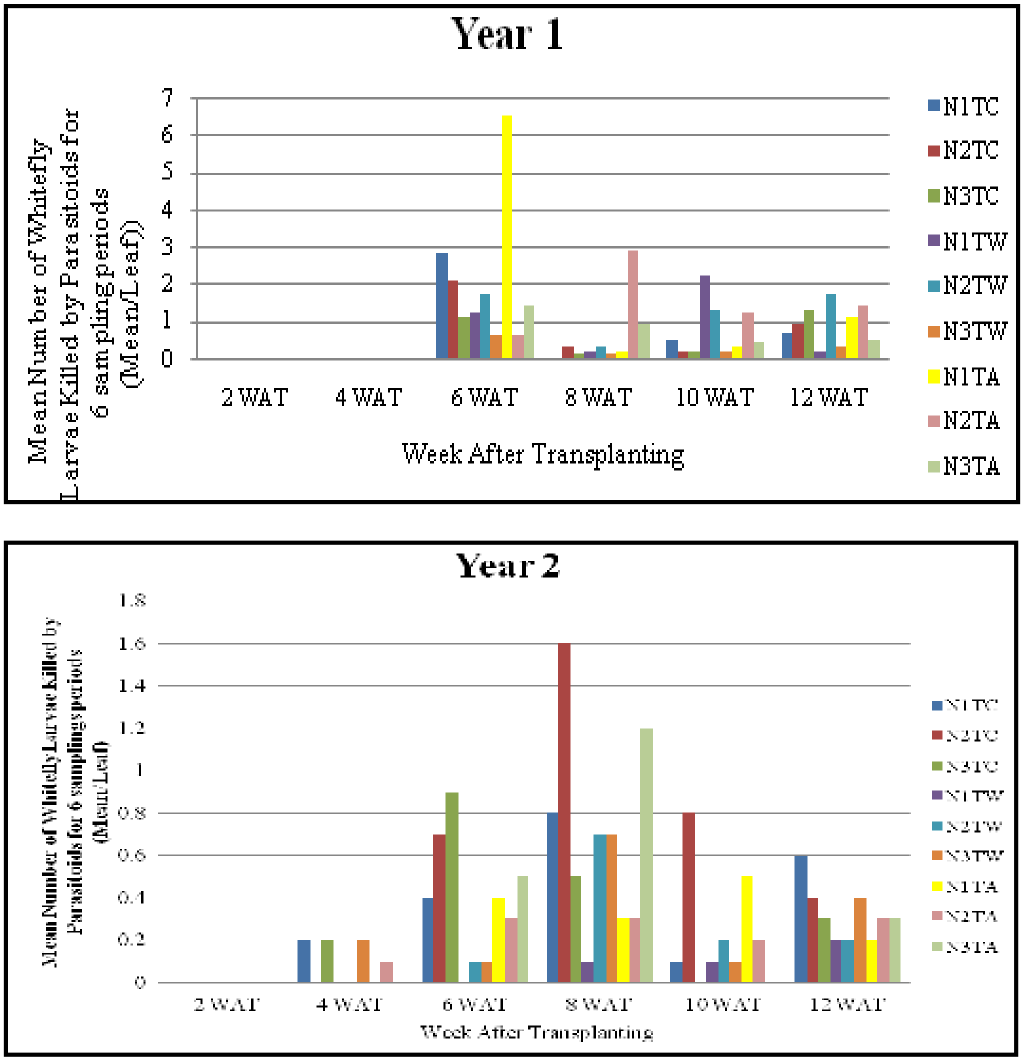

Figure No.1: Mean Number of Whiteflies Killed by their Natural Enemy, *Encarsia hitam* (Parasitization), on the underside of Eggplant Leaves for Both Cropping Periods

CONCLUSION

The findings of this study showed that early infestation (pre-infestation) of eggplant plants did not influence parasitism on the whiteflies infesting these plants, but varying levels of nutrients applied and the age of the plants showed significant effects on the parasitization of the pest. Early establishment of an *E. hitam* population is vital for successful biological control. These parasitoids are readily available in an open rain shelter when highly toxic insecticides were not in use. It is prudent for a biological control program to focus on enhancing the population of this parasitoid and other natural enemies to coincide with the appropriate phase of pest infestations.

ACKNOWLEDGEMENT

We thank the School of Biological Sciences, Universiti Sains Malaysia (USM), for a research grant (1001/PBIOLOGI/844053) and the Agriculture Station in Relau, Penang, for providing field facilities to undertake this study. We are indebted to Hj Hedzir, Siti Khatijah Ghazali and Nur Farhahmizah Askarali for their assistance in the field and in the laboratory. We thank anonymous reviewers for constructive reviews and comments on an earlier draft of this manuscript.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Mohd Rasdi Z *et al*. Effect of nutrient and triggered eggplant defense system by different Pre-infestation on the activities of whitefly parasitism, *International Journal of Nutrition and Agriculture Research*, 3(2), 2016, 67-79.