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Effects of temperature and nonionizing ultraviolet radiation treatments of eggs of five host insects on production of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) for biological control applications

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ABSTRACT

Trichogramma species are used worldwide as biological control agents. A particularly important application is mass-rearing and release for management of field-crop and warehouse insect pests. Eggs of commonly available hosts, *Spodoptera litura*, *Corcyra cephalonica*, *Plutella xylostella* and *Helicoverpa armigera,* were exposed to different temperature and nonionizing ultraviolet (UV) radiation treatments to consider whether particular combinations of treatments positively affected *T. chilonis* development. The treatments had different effects on three measures of parasitoid production: the rate of parasitization, adult emergence, and adult viability. At constant temperature (24, 28, 32 °C), the mean percentage of egg parasitization was greatest on treatments of *S. litura* eggs. However, the mean percentage of adult emergence was significantly greater from *C. cephalonica* eggs at 28 °C than from eggs in other treatments. The mean percentage of adult viability was found to be 83.9% from *C. cephalonica* eggs at 28 °C. Ultraviolet radiation treatments (3, 6, or 9 min at 254 nm) significantly increased the mean percentage parasitization over that of the non-UV treatments. Also, the mean percentage of adult parasitoid emergence and viability were greater from *C. cephalonica* eggs exposed to non-ionizing UV radiation than from eggs of other hosts in all other treatments. This information can be used by managers of mass-rearing programs to increase the effectiveness of *T. chilonis* production for biological control of pest insects.

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Introduction

Trichogramma parasitoids are used worldwide as biological control agents (Kuske et al., 2003; Senthil-Nathan et al., 2006), and attack the eggs of over 200 insect species (Boo and Yang, 1998; McGregor et al., 1998; Orr et al., 2000; Wright et al., 2002; Mansfield and Mills, 2004). Mass rearing of *Trichogramma* spp. is an economically feasible methodology for control of many Lepidopteran pests (Wang et al., 2012). *Spodoptera* species, for example, are important Lepidopteran pests of agricultural crops in Asia. This polyphagous genus attacking about 150 host species (Rao et al., 1993; Gothama et al., 1995; Ferry et al., 2004; Senthil-Nathan and Kalaivani, 2005, 2006; Capinera, 1999, 2014) is economically important in many countries and has become resistant to

many chemical insecticides (Zhou and Huang, 2002; Hu et al., 2007; Senthil-Nathan, 2013, 2015).

The rice-moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) is a serious pest of stored cereals and cereal commodities in India as well as in other tropical and subtropical regions of the world (Shukla and Tiwari, 2011). The larval stages cause the most serious damage to rice, gram, sorghum, maize, groundnut, raisins, nutmeg, chocolates and milled products, *etc.* (Madhavi and Raja, 2012).

The diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae), is one of the most notorious pests of cruciferous vegetables in the world (Talekar and Shelton, 1993; Wang and Wu, 2012; Xia et al., 2014), damaging cabbage with losses as much as 100% (Castelo-Branco and Gatehouse, 2001). It has developed resistance against the synthetic and biologically based insecticides, including toxins produced by *Bacillus thuringiensis* (Sun, 1992; Talekar and Shelton, 1993; Tabashnik, 1994), pyrethroids (Shelton and Wyman, 1992; Liang et al., 2003), spinosad, avermectins (abamectin and emamectin benzoate),

indoxacarb and chlorantraniliprole (Sayyed and Wright, 2006; Pu et al., 2010; Wang and Wu, 2012; Xia et al., 2014).

American bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is a polyphagous and serious pest damaging over 181 species of host plants belonging to 45 families (Zalucki et al., 1986; Srivastava et al., 2005; Ahmad et al., 2013). It feeds primarily on nitrogen-rich pods, leaves, buds and flowers, causing losses up to 70–80% (Prakash et al., 2007; Ahmad et al., 2013).

Trichogramma species parasitize eggs of Lepidoptera by laying one or more eggs inside the eggs of the host insects. This study considers potential improvements in the suitability of host eggs for mass-rearing of *T. chilonis* obtainable through pre-exposure of eggs to temperature (Gandhi et al., 2005; Perveen et al., 2012) and radiation (Tuncbilek et al., 2012) treatments found previously to improve parasitoid production. Suitability for mass-rearing (production performance) was assessed by comparisons of percentage parasitization, adult emergence, and adult viability among treatments.

Materials and methods

Trichogramma chilonis colony

The *T. chilonis* used for parasitization studies were obtained from Project Directorate of Biological Control, Bangalore, and Karnataka, India. The colony was maintained in the biopesticide and environmental toxicology laboratory (BETL), MS University, Alwarkurichi, Tamil Nadu, India without exposure to any insecticides or treatment since 2007. The colony was reared on eggs of *C. cephalonica*, a colony of which was maintained also at BETL. The *C. cephalonica* eggs were sterilized under an UV lamp for 0.5 h and attached to paper cards coated with locally obtained 10% *Acacia arabica* (Lam.) Willd gum. Egg cards were placed in containers (30 × 10 cm diam.) and maintained at 27 ± 1 °C, 70–80% RH, and L 16:8 D photoperiod. Male and female adult parasitoids emerged within the bags mated within the first 24 h after emergence began. The adults were fed with 10% honey-water solution.

S. litura colony

A colony of *S. litura* was maintained under the procedure developed by Senthil-Nathan et al. (2005). *S. litura* larvae were reared in the laboratory on castor leaves. The castor plants were harvested 1.5–2 months after planting. To obtain sufficient specimens, we used mature leaves (75–125 cm^2) that were removed from the upper third of the plants. Pre-pupae were separated and provided with vermiculture clay as pupation sites. Emerging adult moths were transferred to cages and fed on a 10% sucrose solution. Moths were transferred at a ratio of 1 male: 2 females to oviposition cages containing castor leaves and covered with sterilized muslin cloth for egg laying. The muslin cloths containing eggs were removed daily and eggs present were surface sterilized (to prevent entry of pathogen) *in situ* by dipping in 10% formaldehyde solution for about 2–5 min, then washing with distilled water for 2 min. The muslin cloths containing eggs were collected and stuck to white hard card with glue. These white cards containing eggs were used for further experiments.

C. cephalonica colony

Eggs were obtained from naturally infested grain stored in a local storage warehouse in Ambasamudram and were maintained at 28 ± 2 °C, 65% RH, with a 14:10 light: dark cycle in plastic troughs (30 × 10 cm diam.) on soft white wheat, *Triticum aestivum* L., bought from a local market (Senthil-Nathan et al., 2006). To obtain eggs for developmental experiments, adults were transferred into plastic jars and fed with 10% sucrose solution and libitum. After three days, eggs deposited in the jars were brushed out and collected. The collected eggs were attached to cards with glue.

P. xylostella colony

P. xylostella pupae were obtained from National Bureau of Agricultural Insects Resources (ICAR) Bangalore and reared in the laboratory at 24 ± 2 °C and 60 ± 2% relative humidity. Emerged moths were fed on 10% honey solution in culture cages (50 × 30 cm diam.). Moths were reared in cages each containing a piece of seal film as a substrate for eggs. The eggs deposited on the film were brushed on to cards and used for parasitization.

H. armigera colony

The *H. armigera* adults used for the experiment were taken from a colony maintained at BETL under 26 °C and 60–70% humidity. The oviposition cage for *H. armigera* was of cylindrical frame (30 × 10 cm diam.) with a cylindrical type plastic mesh positioned 5 cm above the base of the frame for support. A white cotton cloth was wrapped on the frame. Pupae were kept in Petri dishes by wrapping them in a muslin cloth, and were maintained under laboratory conditions of 26 °C and 60–70% humidity. A 10% honey-water solution was provided to feed emerging adults. The eggs laid on the cloth were collected and later sterilized using 0.05% sodium hypochlorite followed by two water rinses. Eggs were then fixed on cards for parasitization.

Egg treatments with constant temperatures and non-ionizing (UV) radiation

Development of *T. chilonis* in different host eggs was monitored at constant temperatures (24, 28 and 32 °C ± 1 °C) at 16:8 light: dark period in a growth chamber. Fresh eggs of *C. cephalonica, S. litura, P. xylostella* and *H. armigera* were obtained from culture stock. A total of 100 eggs were stuck to white hard card (1.5 × 3 cm) with glue and placed in transparent glass jars (10 × 3 cm diam.) for 24 h. Each jar contained 20 mated one-day-old female parasitoids. This experiment was replicated 5 times.

For UV radiation tests, host eggs were collected onto cards and exposed to UV-rays of 254 nm wavelength at 3, 6 and 9 min durations. An UV lamp (Mineralight Lamp, shortwave UV, 254 nm 215–250 V, 56–60 Hz, 0.12 A) was used. For irradiation, the eggs were kept in a 10 × 3 cm box placed on the surface 5 cm away from the lamp. The UV treated eggs then were subjected to parasitization by *T. chilonis*.

Measures of T. chilonis production performance

The parasitization rate of *T. chilonis* was measured using treated and freshly laid (untreated control) host eggs. Approximately 80–100 host eggs were used in each experiment. The eggs glued to a card were introduced into a glass vial (10 × 3 cm), where the male and female adult parasitoids were allowed to mate earlier. The adults were fed with 5% honey solution and were allowed to parasitize the eggs. After four days, the egg cards were taken out and parasitized eggs, which turn black, were counted under a microscope. The per cent parasitization was calculated based on the observation on number of black eggs to the total number of eggs on 7th day after exposure.

The percentages of adults emerged were calculated according to Eq. (1):

$$\text{Percentage of emergence} = \frac{\text{Number of } T.chilonis \text{ emerged}}{\text{Number of parasitized eggs}} \times 100 \tag{1}$$

The percentage of viability of emerged adults was observed in two different experiments. The adults emerged from different temperatures were maintained at same temperature and the viability of adults was noted. The adults emerged from different durations of nonionizing UV treated eggs were observed for viability in laboratory conditions. The

adults were fed with 5% honey-water solution. The percentage of adult viability was calculated similarly as in Eq. (1).

Statistical analysis

The data obtained from measurements of egg parasitization, adult emergence, and adult viability were subjected to analysis of variance (ANOVA of arcsine, logarithmic and square root transformed percentages) (SAS Institute, 2001) and data were expressed as a mean of five replicates. Significant differences among treatment groups were analysed by Tukey's multiple range test (significance at $P < 0.05$) using the Minitab®17 programme.

Results

Effects of constant temperatures on T. chilonis production measures

The production of *T. chilonis* on host eggs at different temperatures is shown in Table 1. At 24 °C and 32 °C, a significantly greater mean parasitism rate was observed on *S. litura* eggs compared to means in all other hosts. The mean percentage parasitization was significantly greater on *S. litura* eggs than on *P. xylostella* and *C. cephalonica* at 28 °C. The mean percentage of adult emergence was significantly greater from *C. cephalonica* eggs than from *P. xylostella* and *S. litura* eggs at 24 and 28 °C and was significantly greater than from all other host eggs at 32 °C. The mean percentage viability was significantly greater from *C. cephalonica* eggs than from all other host eggs at all three tested temperatures. The highest values of the three *T. chilonis* production measures in the temperature treatments occurred for all hosts eggs at 28 °C.

Effects of non-ionizing (UV) radiation on T. chilonis production measures

The duration of non-ionizing UV radiation significantly affected *T. chilonis* production measures (Table 2). The mean parasitization rate increased with increasing exposure duration in all host eggs, and was significantly greater in *S. litura* host eggs than in all other hosts at 3 and 6 min durations. There were no significant differences among mean parasitization rates of different host eggs in the 9-min-exposure treatment. The mean percentage of adult emergence of *T. chilonis* was greater from *C. cephalonica* eggs than from *S. litura* eggs at all tested exposure durations. The mean percentage viability was greater from *C. cephalonica* eggs than from eggs of all other hosts at all tested exposure durations.

Table 1
Measurements of parasitoid production from eggs of different hosts in temperature treatments. Percentages are based on mean (±SEM) number of eggs parasitized, adults emerged from host egg samples, and viability of adult *T. chilonis* per 100-egg sample (Eq. (1)). Temperature treatment means followed by the same letters are not significantly different according to a Tukey's test ($P < 0.05$).

Temperature/species	% Parasitization Mean ± SE	% Emergence Mean ± SE	% Viability Mean ± SE
$T = 24$ °C	$F_{3, 16} = 6.31$	$F_{3, 16} = 4.23$	$F_{3, 16} = 9.77$
C. cephalonica	60.4 ± 6.62[b]	80.3 ± 8.56[a]	81.02 ± 4.02[a]
H. armigera	62.7 ± 3.65[b]	73.7 ± 8.30[ab]	52.90 ± 2.46[b]
P. xylostella	61.9 ± 4.72[b]	65.0 ± 9.96[b]	43.30 ± 3.1[c]
S. litura	74.0 ± 6.63[a]	64.0 ± 6.33[b]	31.6 ± 4.92[d]
$T = 28$ °C	$F_{3, 16} = 6.52$	$F_{3, 16} = 5.42$	$F_{3, 16} = 8.88$
C. cephalonica	63.70 ± 7.45[b]	85.7 ± 7.20[a]	83.9 ± 12.16[a]
H. armigera	68.54 ± 11.84[ab]	80.7 ± 2.31[ab]	65.7 ± 7.54[b]
P. xylostella	67.50 ± 4.82[b]	77.8 ± 2.59[b]	58.2 ± 10.81[b]
S. litura	84.26 ± 9.26[a]	74.1 ± 3.11[b]	53.8 ± 8.67[b]
$T = 32$ °C	$F_{3, 16} = 1.60$	$F_{3, 16} = 4.60$	$F_{3, 16} = 9.71$
C. cephalonica	53.2 ± 7.46[b]	60.8 ± 3.03[a]	65.1 ± 6.24[a]
H. armigera	56.2 ± 7.29[b]	55.1 ± 6.28[b]	45.2 ± 9.94[b]
P. xylostella	54.2 ± 7.16[b]	52.3 ± 4.31[b]	36.5 ± 8.90[b]
S. litura	62.8 ± 8.56[a]	50.7 ± 4.31[b]	32.7 ± 9.25[b]

Table 2
Measurements of parasitoid production from eggs of different hosts in non-ionizing UV radiation (254 nm) treatments of different durations. Percentages are based on mean (±SEM) number of eggs parasitized, adults emerged from host egg samples, and viability of adult *T. chilonis* per 100-egg sample. UV treatment means followed by the same letters are not significantly different according to a Tukey's test ($P < 0.05$).

Radiation/species	% Parasitization Mean ± SE	% Emergence Mean ± SE	% Viability Mean ± SE
3 min	$F_{3, 16} = 4.91$	$F_{3, 16} = 7.85$	$F_{3, 16} = 12.3$
C. cephalonica	63.00 ± 5.18[b]	85.2 ± 7.95[a]	82.7 ± 6.58[a]
H. armigera	66.98 ± 7.93[b]	68.8 ± 8.09[b]	55.2 ± 2.56[b]
P. xylostella	64.50 ± 5.82[b]	65.0 ± 7.62[b]	39.7 ± 3.34[c]
S. litura	74.0 ± 6.63[a]	64.4 ± 7.70[b]	34.0 ± 2.78[c]
6 min	$F_{3, 16} = 4.15$	$F_{3, 16} = 7.88$	$F_{3, 16} = 15.6$
C. cephalonica	76.00 ± 7.20[b]	91.8 ± 5.26[a]	90.6 ± 4.27[a]
H. armigera	73.84 ± 9.53[b]	83.4 ± 7.40[ab]	58.0 ± 1.17[b]
P. xylostella	74.40 ± 7.66[b]	74.6 ± 5.54[b]	45.0 ± 3.89[c]
S. litura	91.90 ± 2.58[a]	72.4 ± 4.80[b]	35.4 ± 3.64[d]
9 min	$F_{3, 16} = 2.73$	$F_{3, 16} = 3.98$	$F_{3,16} = 20.03$
C. cephalonica	84.1 ± 6.68[a]	94.92 ± 4.37[a]	91.5 ± 5.55[a]
H. armigera	91.9 ± 5.62[a]	91.92 ± 5.02[a]	62.4 ± 8.36[b]
P. xylostella	89.0 ± 5.76[a]	89.25 ± 5.18[a]	54.8 ± 9.12[c]
S. litura	94.4 ± 3.84[a]	84.50 ± 6.06[b]	46.4 ± 3.66[d]

Comparisons of effects of temperature (28 °C) and non-ionizing UV radiation (9-min) treatments on T. chilonis production measures

The temperature and non-ionizing UV radiation treatments above that resulted in the highest values of *T. chilonis* production measures, 28 °C and 9-min-duration of exposure, respectively, are compared statistically in Figs. 1-3. The mean rates of parasitization were significantly greater on *C. cephalonica*, *H. armigera* and *P. xylostella* eggs in the 9-min UV radiation than in the 28 °C treatment but were not significantly different on *S. litura* eggs. The highest mean emergence rate was observed from *C. cephalonica* followed by *S. litura* eggs in the UV exposure treatment but none of the comparisons were significantly different between the two treatments (Fig. 2). The mean percentage viability from *C. cephalonica* eggs in the 9-min UV radiation treatment was significantly greater than the means from eggs of all other hosts at 28 °C.

Comparing all the data, *C. cephalonica* eggs provided greater levels of emergence and viability although the percentage of parasitization was greater on *S. litura* eggs. The parasitization effects of different host eggs in temperature and UV treatment was presented in Fig. 4.

Discussion

Trichogrammatid parasitoids are common natural enemies used in the world against eggs of many lepidopterous pests (Wajnberg et al., 2003, Tezze and Botto, 2004). Prior studies (Singh et al., 1994,
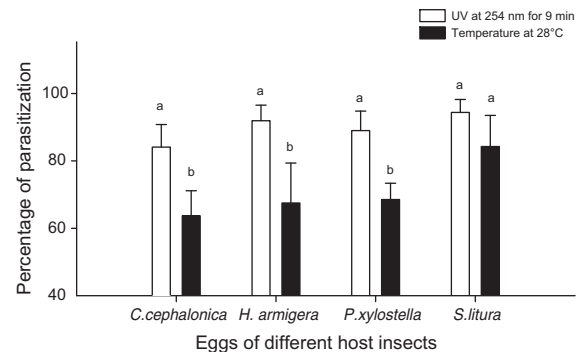

Fig. 1. Comparison of effects of 28 °C temperature and 9-min. UV treatments on parasitization. Means (± (SE) standard error) followed by the same letters above bars indicate no significant difference between means in each host egg treatment in a Tukey's test ($P < 0.05$).
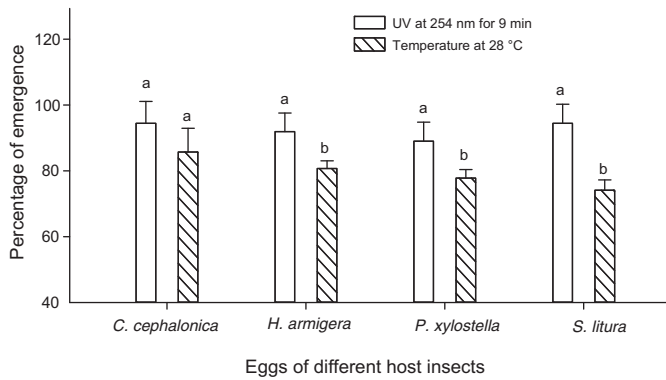
Fig. 2. Comparison of effects of 28 °C temperature and 9-min. UV treatments on adult emergence. Means (± (SE) standard error) followed by the same letters above bars indicate no significant difference in a Tukey's test (*P* < 0.05).

Vasquez et al., 1997) on the production and use of the egg parasitoid *Trichogramma* revealed their importance in biological control programs (Smith, 1996; Jeong et al., 2010; Karimi et al., 2012). Our study provides improved understanding about favorable conditions for mass-rearing of *T. chilonis*.

There were significant differences in mean percentage parasitization, emergence and viability at 24 °C, 28 °C and 32 °C. Results similar to ours were reported by Perveen et al. (2012) found 28 °C was the most favorable temperature for parasitization and adult emergence of *T. chilonis* from *S. cerealella* when compared to 24 and 32 °C. The parasitization rate was minimal at 32 °C and there was no significant difference in mean parasitization rates on *C. cephalonica*, *H. armigera* and *P. xylostella*. In contrast, Bueno et al. (2012) found *T. pretiosum* had the ability to parasitize the eggs of *Pseudoplusia includens* and *Anticarsia gemmatalis* even at extreme temperatures of 18 and 32 °C.

Effect of temperature on emergence

The emergence rate of *T. chilonis* has been reported to vary with the size of the egg, number of parasitoids that developed per egg, development period in host eggs, and temperature (Doyon and Boivin, 2005; Pratissoli et al., 2005). The greatest percentage of emergence was observed in *C. cephalonica* eggs at 28 °C followed by *P. xylostella*, *H. armigera* and *S. litura*. There was a significant difference in emergence of adults from the eggs of *C. cephalonica* and *P. xylostella* treated at 28 °C. At 24 °C, greater emergence rates (85.2%) were observed in *C. cephalonica* while a lower emergence rate was observed in *S. litura* (3.4%). At 32 °C, the maximum percentage emergence was observed
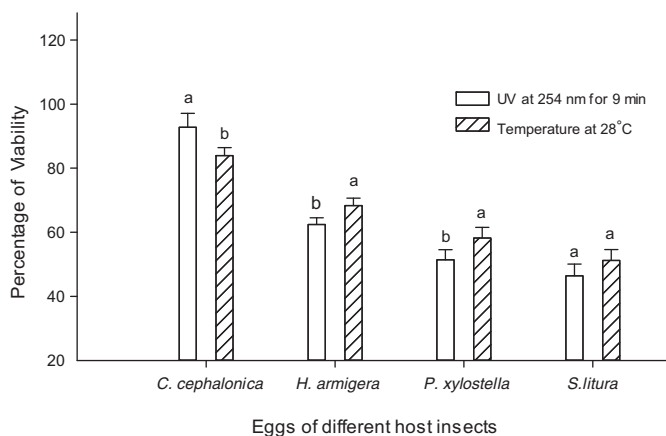

Fig. 3. Comparison of effects of 28 °C temperature and 9-min. UV treatments on adult viability. Means (± (SE) standard error) above bars indicate no significant difference in a Tukey's test (P < 0.05).

in *C. cephalonica* eggs follows by the *P. xylostella* and *H. armigera* and minimal emergence resulted in *S. litura* eggs treated with 32 °C. This decrease in the emergence rate possibly was due to high temperature, infertile eggs of host insects, and not being able to overcome the host embryo as it developed.

Viability rate on temperature

The success of biological control practices using parasitoids is determined mainly by their longevity and reproductive success (Strand, 1986; Zaviezo and Mills, 2001; Meyhöfer and Klug, 2002; Begum et al., 2006; Díaz et al., 2012). Adult longevity of parasitoid was decreased from the temperature 20–30 °C (Park et al., 2000; Shirazi, 2006). The viability rate of *T. chilonis* was greater in *C. cephalonica* eggs when compared to eggs of other hosts tested at all temperatures. Miura and Kobayashi (1995) reported the longevity rate of *T. chilonis* adults emerging from *H. armigera* eggs was reduced as the temperature increased from 24 to 32 °C. Our results on longevity of *H. armigera* are in correlation with his findings. Bueno et al. (2012) found the longevity was reduced in *T. pretiosum* at higher temperature in *Pseudoplusia includens* and *Anticarsia gemmatalis* eggs. The viability rate was higher (85.6% at 28 °C) in *C. cephalonica* eggs compared to a lower rate (5.2% at 28 °C) in *S. litura* eggs. These data indicate significant difference in the viability rate among different host eggs. The viability rate was decreased due to low nutrient quantity in parasitized eggs, more number of adults emerged from a single egg and adult cannot survive at high temperature. Large eggs potentially offer more food and nutrients to support progeny development for longer viability (Brotodjojo and Walter, 2006). Less preference by *T. chilonis* on high temperature treated host eggs might be due to the desiccation of eggs to loose moisture and nutrient contents coupled with misshaping of eggs through compression dorso-ventrally or laterally, besides making the eggs less suitable for oviposition and further development of parasitoids.

Non-ionizing radiation improved the rate of parasitization, emergence and viability. Tuncbilek et al. (2012) studied the effect of ionizing (gamma) and non-ionizing (UV) radiation on the development of *T. euproctidis* in *Ephestia kuehniella* eggs and found that an increase in exposure time to UV rays caused a gradual decrease in the percentage of hatching eggs. The UV radiation damaged the eggs by preventing further development and it made the *T. chilonis* parasitization more successful.

Non-ionizing radiation on parasitization

The mean percentage parasitization was greater in *C. cephalonica* eggs in treatments with non-ionizing radiation. This was due to smaller size of the eggs and thin outer layer. The UV rays used had damaged the host embryo. The damaged embryo could not develop into next stage. At the same time, damaged embryo had helped the *T. chilonis* for successful parasitization. Embryo damage level was previously studied in *H. armigera* eggs by *T. australicum* (Jarjees and Merritt, 2003).

Emergence and viability on UV radiation

The emergence rate was greater in *C. cephalonica* eggs treated with UV radiation possibly due to the small size of the eggs. When the host eggs size was larger, more than one adult may develop within the host egg. This would affect the immunity as the nutrient content is low. So, if two or more adult parasitoids emerged from single host egg, they may not be viable for longer time periods.

In comparisons of eggs treated with different temperatures and UV radiation, the non-ionizing radiation showed higher mean rates of parasitization, emergence and viability. Twenty eight degrees was the optimum temperature for *T. chilonis* that developed on the eggs of *P. xylostella* (Miura and Kobayashi, 1993), *Sitotroga cerealella* and *Corcyra cephalonica* Perveen et al. (2012). Similarly, 28 °C was the optimum
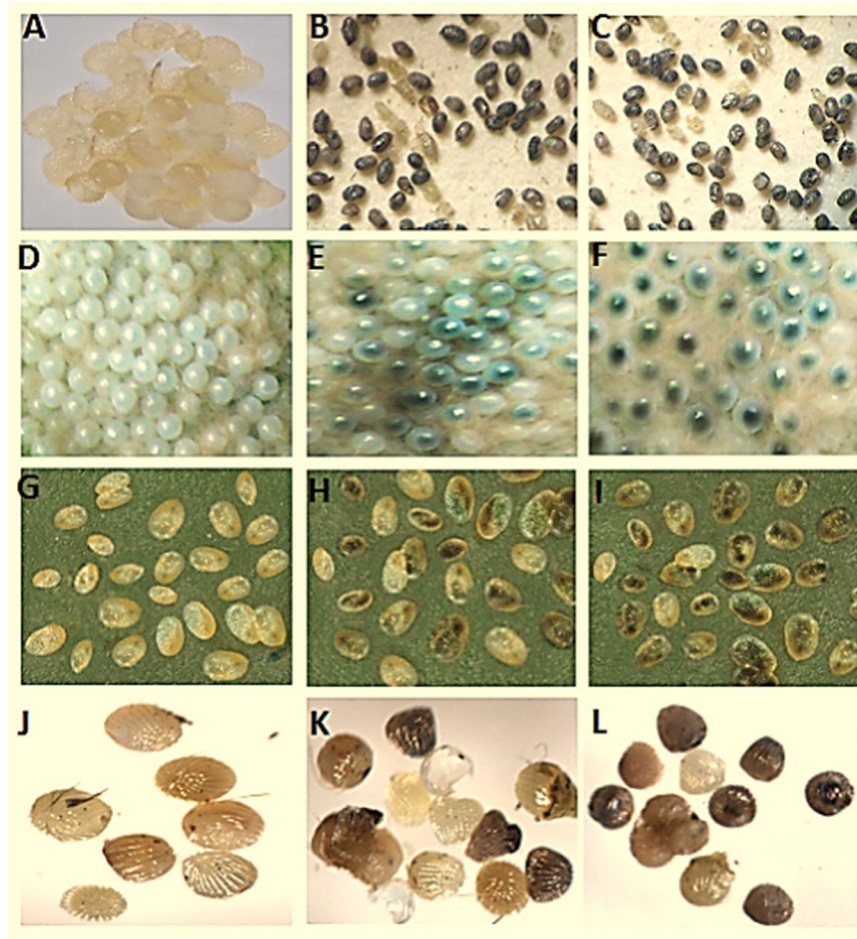
Fig. 4. Microscopic observation of eggs (A) Control eggs of *C. cephalonica* (B) Parasitized eggs of *C. cephalonica* at the temperature of 28 °C (C) Parasitized eggs of *C. cephalonica* in UV treatment at 9 min exposure (D) Control eggs of *S. litura* (E) Parasitized eggs of *S. litura* at the temperature of 28 °C (F) Parasitized eggs of *S. litura* in UV treatment at 9 min exposure (G) Control eggs of *P. xylostella* (H) Parasitized eggs of *P. xylostella* at the temperature of 28 °C (I) Parasitized eggs of *P. xylostella* in UV treatment at 9 min exposure (J) Control eggs of *H. armigera* (K) Parasitized eggs of *H. armigera* at the temperature of 28 °C (L) Parasitized eggs of *H. armigera* in UV treatment at 9 min exposure.

temperature for *T. chilonis* development in *S. litura* and *H. armigera* eggs (Edwin and Senthil-Nathan, unpublished data).

Trichogramma species face challenges in parasitizing eggs in normal conditions due to natural resistance mechanisms evolved by the host. In this study the percentage of parasitization, emergence and viability was enhanced by inducing stress factors such as temperature and UV radiation to the eggs. These stress factors helps to increase in parasitization, emergence and viability rates in *C. cephalonica* eggs. These eggs can be used to successfully rear *T. chilonis* and for use as a biocontrol agent for lepidopteron pest.

From our findings, *T. chilonis* has an ability to parasitize many lepidopteran pests and control their populations in their earlier stage of development. The stress factor, temperature, clearly affects the activity and sustainability of egg parasitoid in different environmental conditions. The UV radiation also increased the parasitization rate. Although a higher rate of parasitization occurs with this method, it should not be used in an open environmental condition; however, it is highly recommended for most of the lepidopteran pest of grains at warehouse. Advantages of this method are, the radiation helps the parasitoid to parasitize eggs without any difficulty and if the eggs escape from the parasitoid, the radiation can damage the host eggs.

Further research work is necessary on vitality of *T. chilonis* in agriculture fields at different climatic conditions and various stress factors such as chemical pesticide and non-target insect damage, which impact the parasitoid in the environment, to enhance the pest management by biological methods.

References

Ahmad, S., Ansari, M.S., Moraiet, M.A., 2013. Demographic changes in *Helicoverpa armigera* after exposure to neemazal (1% EC azadirachtin). Crop. Prot. 50, 30–36.

Begum, M., Gurr, G.M., Wratten, S.D., Hedberg, P.R., Nicol, H.I., 2006. Using selective food plants to maximize biological control of vineyard pests. J. Apl. Ecol. 43, 547–554.

Boo, K.S., Yang, J.P., 1998. Olfactory response of *Trichogramma chilonis* to *Capsicum annuum*. J. Asia-Pacific Entomol. 1 (2), 123–129.

Brotodjojo, R.R.R., Walter, G.H., 2006. Oviposition and reproductive performance of a generalist parasitoid (*Trichogramma pretiosum*) exposed to host species that differ in their physical characteristics. Biol. Control 39, 300–312.

Bueno, R.C.O.F., Parra, J.R.P., Bueno, A.F., 2012. *Trichogramma pretiosum* parasitism of *Pseudoplusia* includes and *Anticarsia gemmatalis* eggs at different temperatures. Biol. Control 60, 154–162.

Capinera, J.L., 1999. Beet Armyworm, *Spodoptera exigua* (Hubner) (Insecta: Lepidoptera: Noctuidae). University of Florida Cooperative Extension Service. Institute of Food and Agricultural Sciences, EDIS.

Capinera, J.L., 2014. Fall Armyworm, *Spodoptera frugiperda* (J. Smith) (Insecta: Lepidoptera: Noctuidae). http://entnemdept.ufl.edu/creatures/veg/leaf/beetarmyworm.html.

Castelo-Branco, M., Gatehouse, A.G., 2001. Survey of insecticide susceptibility in *Plutella xylostella* (L) (Lep, Yponomeutidae) in the Federal District. Brazil. Neotrop. Entomol. 30, 327–332.

Díaz, M.F., Ramírez, A., Poveda, K., 2012. Efficiency of different egg parasitoids and increased floral diversity for the biological control of noctuid pests. Biol. Control 60, 182–191.

Doyon, J., Boivin, G., 2005. The effect of development time on the fitness of female *Trichogramma evanescens*. J. Insect Sci. 54, 1–5.

Ferry, N., Edwards, M.G., Gatehouse, J.A., Gatehouse, A.M.R., 2004. Plant-insect interaction, molecular approaches to insect resistance (edited by Sasaki T, Christou P). Curr. Opin. Biotechnol. 15, 155–161.

Gandhi, P.I., Gunasekaran, K., Poonguzhali, S., Anandham, R., Kim, G.H., Chung, K.Y., Sa, T., 2005. Laboratory evaluation of relative toxicities of some insecticides against Trichogramma chilonis (Hymenoptera: Trichogrammatidae) and Chrysoperla carnea (Neuroptera: Chrysopidae). J. Asia-Pacific Entomol. 8 (4), 381–386.

Gothama, A., Silkorowski, P.P., Lawrence, G.W., 1995. Interactive effects of Steinernema carpocapsae and Spodoptera exigua nuclear polyhedrosis virus on Spodoptera exigua larvae. J. Invertebr. Pathol. 86, 270–276.

Hu, Q.B., Ren, S.Z., An, Z.C., Qian, M.H., 2007. Insecticidal activity influence of destruxins on the pathogenicity of Paecilomyces javanicus against Spodoptera litura. J. Appl. Entomol. 131, 262–268.

Jarjees, E.A., Merritt, J.A., 2003. Structure of the gut contents of Trichogramma australicum Girault (Hymenoptera: Trichogrammatidae) larvae fixed in situ in eggs of its host Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae). Aust. J. Entomol. 42, 203–209.

Jeong, G., Kim, H., Choi, Y., Kim, W., Park, K., Bae, S., Kim, J., Choi, J., 2010. Molecular identification of two Trichogramma species (Hymenoptera: Trichogrammatidae) in Korea. J. Asia-Pacific Entomol. 13 (1), 41–44.

Karimi, J., Darsouei, R., Hosseini, M., Stouthamer, R., 2012. Molecular characterization of Iranian trichogrammatids (Hymenoptera: Trichogrammatidae) and their Wolbachia endosymbiont. J. Asia-Pacific Entomol. 15 (1), 73–77.

Kuske, S., Widmer, F., Edwards, P.J., Turlings, T.C.J., Babendreier, D., Bigler, F., 2003. Dispersal and persistence of mass released Trichogramma brassicae (Hymenoptera, Trichogrammatidae) in non-target habitats. Biol. Control 27, 181–193.

Liang, G., Chen, W., Liu, T.X., 2003. Effects of three neem-based insecticides on diamondback moth (Lepidoptera, Plutellidae). Crop. Prot. 22, 333–340.

Madhavi, M., Raja, S.S., 2012. Influence of Vitex negundo on the morphogenesis of Corcyra cephalonica. J. Pharmac. 3, 136–137.

Mansfield, S., Mills, N.J., 2004. A comparison of methanologies for the assessment of host performance of the gregarious egg parasitoid Trichogramma platneri. Biol. Control 29, 332–340.

McGregor, R., Hueppelsheuser, T., Luczynski, A., Henderson, D., 1998. Collection and evaluation of Trichogramma species (Hymenoptera, Trichogrammatidae) as biological controls of the oblique-banded leafroller Choristoneura arosaceana (Harris) (Lepidoptera, Tortricidae) in raspberries and blueberries. Biol. Control 11, 38–42.

Meyhöfer, R., Klug, T., 2002. Intraguild predation on the aphid parasitoid Lysiphlebus fabarum (Marshall) (Hymenoptera: Aphidiidae): mortality risks and behavioral decisions made under the threats of predation. Biol. Control 25, 239–248.

Miura, K., Kobayashi, M., 1993. Effect of temperature on the development of Trichogramma chilonis Ishii (Hymenoptera, Trichogrammatidae), an egg parasitoid of the diamondback moth. J. Appl. Entomol. 28, 393–396.

Orr, D.B., Salazar, C.G., Landis, D.A., 2000. Trichogramma Non Target Impacts, a Method for Biological Control Risk Assessment. In: Follett, P.A., Duan, J.J. (Eds.), Non-Target Effects of Biological Control. Kluwer Academic Publishers, Boston, MA, pp. 111–125.

Park, Y.K., Lee, H.P., Lee, K.S., 2000. Effect of temperature on the biology of Trichogramma dendrolimi (Hymenoptera: Trichogrammatidae) reared on a factitious host, Antheraea pernyi (Lepidoptera: Saturniidae) egg. J. Asia-Pacific Entomol. 3 (2), 65–70.

Perveen, F., Sultan, R., Ul-Haque, E., 2012. Role of temperature and hosts (Sitotroga cerealella and Corcyra cephalonica) egg age on the quality production of Trichogramma chilonis. Arthropods 1, 144–150.

Prakash, M.R., Ram, U., Tariq, A., 2007. Evaluation of chickpea (Cicer arietinum L.) germplasm for the resistance to gram pod borer, Helicoverpa armigera Hubner (Lepidoptera, Noctuidae). Entomol. Res. 31, 215–218.

Pratissoli, D., Zanuncio, J.C., Vianna, U.R., Andrade, J.S., Pinon, T.B.M., 2005. Biological characteristics of Trichogramma pretiosum and Trichogramma acacioi (Hym, Trichogrammatidae), parasitoids of the avocado defoliator Nipteria panacea (Lep., Geometridae), on eggs of Anagasta kuehniella (Lep., Pyralidae). Braz. Arch. Biol. Technol. 48, 7–13.

Pu, X., Yang, Y., Wu, S., Wu, Y., 2010. Characterization of abamectin resistance in a field-evolved multi-resistant population of Plutella xylostella. Pest Manag. Sci. 66, 371–378.

Rao, G.V.R., Wightman, J.A., Ranga-Rao, D.V., 1993. World review of the natural enemies and diseases of Spodoptera litura (F.) (Lepidoptera, Noctuidae). Insect Sci Appl. 14, 273–284.

SAS Institute, 2001. The SAS System for Windows, release 8.1 Cary, NC.

Sayyed, A.H., Wright, D.J., 2006. Genetics and evidence for an esterase-associated mechanism of resistance to indoxacarb in a field population of diamondback moth (Lepidoptera, Plutellidae). Pest Manag. Sci. 62, 1045–1051.

Senthil-Nathan, S., 2013. Physiological and biochemical effect of neem and other Meliaceae plants secondary metabolites against lepidopteran insects. Front. Physiol. 4, 1–17.

Senthil-Nathan, S., 2015. A Review of Biopesticides and their Mode of Action against Insect pest's. Environmental Sustainability- Role of Green Technologies, Springer-Verlag, In, pp. 49–63.

Senthil-Nathan, S., Kalaivani, K., 2005. Efficacy of nucleopolyhedrovirus (NPV) and azadirachtin on Spodoptera litura Fab. (Lepidoptera: Noctuidae). Biol. Control 34, 93–98.

Senthil-Nathan, S., Kalaivani, K., 2006. Combined effects of azadirachtin and nucleopolyhedro virus (SpltNPV) on Spodoptera litura Fab (Lepidoptera: Noctuidae) larvae. Biol. Control 39, 9–104.

Senthil-Nathan, S., Kalaivani, K., Chung, P.G., 2005. The effects of azadirachtin and nucleopolyhedro virus on midgut enzymatic profile of Spodoptera litura Fab. (Lepidoptera, Noctuidae). Pestic. Biochem. Physiol. 83, 46–57.

Senthil-Nathan, S., Kalaivani, K., Mankin, R.W., Murugan, K., 2006. Effects of millet, wheat, Rice, and sorghum diets on development of Corcyra cephalonica (Stainton) (Lepidoptera, Galleriidae) and its suitability as a host for Trichogramma chilonis Ishii (Hymenoptera, Trichogrammatidae). Environ. Entomol. 35, 784–788.

Shelton, A.M., Wyman, J.A., 1992. Insecticide resistance for diamondback moth in North America. In: Talekar, N·S. (Ed.), Diamondback Moth and Other Crucifer Pests. Proceedings of the Second International Workshop, Tainan, Taiwan, 10–14 December 1990. 92, pp. 447–454 Asian Veg. Res. Dev. Control Publ.

Shirazi, J., 2006. Effect of temperature and photoperiod on the biological characters of parasitoid, Trichogramma chilonis Ishii (Hymenoptera, Trichogrammatidae). Pak. J. Biol. Sci. 9, 820–824.

Shukla, S., Tiwari, S.K., 2011. Insecticidal activity of Dryopteris filix-mas (Linn.) Schott ethanolic extract against Corcyra cephalonica Staint. (Lepidoptera, Pyralidae). J. Biopest. 4, 138–143.

Singh, S.P., Jalali, S.K., Bhumannavar, B.S., Bakthavatsalam, N., Pushpalatha, N.A., 1994. Production and use of Trichogrammatid egg parasitoids. Tech Bull. Project Directorate of Biological Control, ICAR. 11, 1–17.

Smith, S.M., 1996. Biological control with Trichogramma: advances, successes, and potential of their use. Annu. Rev. Entomol. 41, 375–406.

Srivastava, C.P., Ahmad, R., Ujagir, R., Das, S.B., 2005. Helicoverpa armigera management in pulses present scenario and future strategies. Proceedings of Indian Society of Pulses Research and Development, Recent Advances in Helicoverpa armigera Management, pp. 265–286 Kanpur, India.

Strand, M.R., 1986. The Physiological Interactions of Parasitoids with their Host and their Influence on Reproductive Strategies. In: Waage, J., Greathead, D. (Eds.), Insect Parasitoids. Academic Press, London, pp. 97–136.

Sun, C., 1992. Insecticide Resistance in Diamondback Moth. In: Talekar, S. (Ed.), "Diamondback Moth and Other Crucifer Pests, Proc. 2nd Int. Workshop" (N. AVRDC, Shanhua, Taiwan, pp. 419–426.

Tabashnik, B.E., 1994. Evolution of resistance to Bacillus thuringiensis. Annu. Rev. Entomol. 39, 47–79.

Talekar, N.S., Shelton, A.M., 1993. Biology, ecology, and management of the diamondback moth. Annu. Rev. Entomol. 38, 275–301.

Tezze, A.A., Botto, E.N., 2004. Effect of cold storage on the quality of Trichogramma nerudai (Hymenoptera, Trichogrammatidae). Biol. Control 30, 11–16.

Tuncbilek, A.S., Ercan, F.S., Canpolat, U., 2012. Effect of ionizing (gamma) and non-ionizing (uv) radiation on the development of Trichogramma euproctidis (Hymenoptera: Trichogrammatidae). Arch. Biol. Sci. 64, 287–295.

Vasquez, L.A., Shelton, A.M., Hoffmann, M.P., Roush, R.T., 1997. Laboratory evaluation of commercial Trichogrammatid products for potential use against Plutella xylostella (L.) (Lepidoptera, Plutellidae). Biol. Control 9, 143–148.

Wajnberg, E., Gonsard, P.A., Tabone, E., Curty, C., Lezcano, N., Colazza, S., 2003. A comparative analysis of patch-leaving decision rules in a parasitoid family. J. Anim. Ecol. 72, 618–626.

Wang, X.L., Wu, Y.D., 2012. High levels of resistance to chlorantraniliprole evolved in field populations of Plutella xylostella. J. Econ. Entomol. 105, 1019–1023.

Wang, Y., Yu, R., Zhao, X., Chen, L., Wu, C., Chang, T., Wang, Q., 2012. Susceptibility of adult Trichogramma nubilale (Hymenoptera, Trichogrammatidae) to selected insecticides with different modes of action. Crop. Prot. 34, 76–82.

Wright, M.G., Kuhar, T.P., Hoffmann, M.P., Chenus, S.A., 2002. Effect of inoculative releases of Trichogramma ostriniae on populations of Ostrinia nubilalis and damage to sweet corn and field corn. Biol. Control 23, 149–155.

Xia, Y., Lu, Y., Shen, J., Gao, X., Qiu, H., Li, J., 2014. Resistance monitoring for eight insecticides in Plutella xylostella in central China. Crop. Prot. 63, 1–7.

Zalucki, M.P., Daglish, G., Firempong, S., Twine, P.H., 1986. The biology and ecology of Heliothis armigera (Hübner) and H. punctigera Wallengren (Lepidoptera, Noctuidae) in Australia, what do we know? Aust. J. Zool. 34, 779–814.

Zaviezo, T., Mills, N., 2001. The response of Hyssopus pallidus to hosts previously parasitized by Ascogaster quadridentata: hetero specific discrimination and host quality. Ecol. Entomol. 26, 91–99.

Zhou, X.M., Huang, B.Q., 2002. Insecticide resistance of the common cutworm (Spodoptera litura) and its control strategies. Entomol. Knowl. (In Chinese) 39, 98–102.