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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.

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),

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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):

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

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