Trichogramma yousufi sp. nov. employed for the management of Spodoptera exigua and Spodoptera litura in Indonesia

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Abstract

Trichogramma (Hymenoptera: Trichogrammatidae) are minute polyphagous wasps and endoparasitoids of lepidopteran eggs. The objective of this study was to identify Trichogramma species emerging from the eggs of the Asian corn borer, Ostrinia furnacalis (Guenée) (Lepidoptera: Crambidae) attacking corn, Zea mays L. (Poaceae) in Bunga Raya, Siak, Riau, Sumatra, Indonesia, and individuals from an unidentified species obtained from a commercial laboratory in Jatisari, Karawang, West Java, Java, Indonesia. The emergence rate of Trichogramma yousufi sp. nov. Khan & Ikram (Hymenoptera: Trichogrammatidae) on an infestation containing eggs of both pest species, beet armyworm, Spodoptera exigua (Hübner), and the tobacco cutworm, Spodoptera litura (F.) (both Lepidoptera: Noctuidae), on Acacia crassicarpa A. Cunn ex Benth. (Fabaceae) was compared and separated from its closer species, Trichogramma latipennis Haliday (Hymenoptera: Trichogrammatidae). Trichogramma species, recovered from corn crops and 1 that also was reared in the laboratory, successfully parasitized the eggs of S. exigua and S. litura under controlled conditions. The emergence rate of T. yousufi sp. nov. was recorded at 24.79 to 40.82%, with the highest percentage for more adults of this parasitoid released per m² in the nursery, indicating its potential to be employed as a biological control agent of Spodoptera in natural conditions.

Key Words: new species; Ostrinia furnacalis; parasitism rate

Resumen

Las especies Trichogramma (Hymenoptera: Trichogrammatidae) son avispas polífagas diminutas y endoparasitoides de huevos de lepidópteros. El objetivo de este estudio fue identificar las especies de Trichogramma que emergen de los huevos del barrenador asiático del maíz, Ostrinia furnacalis (Guenée) (Lepidoptera: Crambidae) atacando el maíz, Zea mays L. (Poaceae) en Bunga Raya, Siak, Riau, Sumatra, Indonesia, e individuos de una especie no identificada obtenida de un laboratorio comercial en Jatisari, Karawang, Java Occidental, Java, Indonesia. Se evaluó la tasa de emergencia de Trichogramma yousufi sp. nov. Khan e Ikram (Hymenoptera: Trichogrammatidae) en una infestación que tenía huevos de ambas especies de plagas, el gusano del ejército de remolacha, Spodoptera exigua (Hübner), y el gusano del tabaco, Spodoptera litura (F.) (Lepidoptera: Noctuidae), en Acacia crassicarpa A. Cunn ex Benth. (Fabaceae) también en un vivero en Pangkalang Kerinci, Riau. Las dos especies de Trichogramma, Trichogramma chinonis Ishii (Hymenoptera: Trichogrammatidae), y la nueva especie, T. yousufi sp. nov., fueron recuperados de los huevos de O. furnacalis en Bunga Raya. Trichogramma poliae Nagaraja (Hymenoptera: Trichogrammatidae) fue identificada como la especie criada en el laboratorio en Jatisari. Trichogramma yousufi sp. nov. fue comparada y separada de su especie más cercana, Trichogramma latipennis Haliday (Hymenoptera: Trichogrammatidae). Las especies de Trichogramma, recuperadas de cultivos de maíz, y una criada en el laboratorio, parasitaron con éxito los huevos de S. exigua y S. litura en condiciones controladas. Se registró la tasa de emergencia de T. yousufi sp. nov. en 24.79 a 40.82%, con el porcentaje más alto para adultos de este parasitóide liberado por m² en el vivero, lo que indica su potencial para ser empleado como agente de control biológico de Spodoptera en condiciones naturales.

Palabras Clave: nuevas especies; Ostrinia furnacalis; tasa de parasitismo

The beet armyworm, Spodoptera exigua (Hübner) and the tobacco cutworm, Spodoptera litura (F.) (both Lepidoptera: Noctuidae) are important polyphagous pests with a cosmopolitan distribution (Li et al. 2015). They are recorded as severe defoliating pests of Acacia crassicarpa A. Cunn. ex Benth. (Fabaceae) plantings that are growing in commercial nurseries in Riau, Sumatra, Indonesia (Sulistyono et al. 2020).
Spodoptera exigua and S. litura possibly migrate to A. crassicaarpa from onion, Allium cepa L. (Amaryllidaceae), crops because of the extensive area in which this vegetable is cultivated, which is recorded annually as being infested with these pests in Riau (Sulistyono et al. 2020).

The minute polyphagous wasps, Trichogramma (Hymenoptera: Trichogrammatidae), are endoparasitoids of lepidopteran eggs (Leite et al. 2015, 2017; Pontes et al. 2019). Trichogramma could be recovered locally from crops such as corn, Zea mays L., and sugarcane, Saccharum officinarum L. (both Poaceae), and used in a program to control S. exigua and S. litura on A. crassicaarpa plantings in nurseries in Sumatra. Trichogramma species are not well understood in Indonesia (Yunus 2018). Studies on taxonomy characters and the biology of Trichogramma species on the eggs of alternative and natural hosts in Indonesia are important information for the management of lepidopterans in several crops (Jiang et al. 2019; Khan et al. 2019).

The objectives of this study were undertaken to identify the Trichogramma species recovered from the eggs of the Asian corn borer, Ostrinia furnacalis (Guenée) (Lepidoptera: Crambidae), attacking corn in Bunga Raya, Siak, Riau, Indonesia, and from an established colony in a laboratory in Jatsari, Karawang, West Java, Java, Indonesia. Additionally, to evaluate some biological parameters of these parasitoids on eggs of the rice moth, Corcyra cephalonica (Stainton) (Lepidoptera: Pyralidae) (an alternative host), S. exigua, and S. litura (natural hosts in the laboratory). Finally, we wanted to discover the parasitism rate of Trichogramma yousufi sp. nov. in Balim and Ikram (Hymenoptera: Trichogrammatidae) on eggs of a mixed population of S. exigua and S. litura recovered after a release of this parasitoid in an A. crassicaarpa nursery in Pangkalan Kerinci, Riau.

Materials and Methods

COLLECTION OF TRICHOGRAmma SPECIES

Trichogramma chilonis Ishii (Hymenoptera: Trichogrammatidae) and T. yousufi sp. nov. were reared on eggs of O. furnacalis collected from a corn field during an exploration at Bunga Raya, Indonesia (0.7500°N, 120.0500°E, 15 masl). Trichogramma poliae Nagaraja (Hymenoptera: Trichogrammatidae) was obtained from Belai Besar Peramalan Organisme Pengganggu Tumbuhan, a commercial laboratory in Jatsari, Indonesia (6.3000°S, 107.0000°E, 100 masl), which had been reared for about 5 yr on eggs of C. cephalonica and delivered to farmers to manage lepidopteran pests on sugar cane crops.

LABORATORY REARING

Eggs of O. furnacalis, parasitized by T. chilonis and T. yousufi sp. nov., and of C. cephalonica by T. poliae were taken to the Entomology Laboratory at PT. Riau Andalan Pulp and Paper in Pangkalan Kerinci, Riau, Indonesia, where they were kept at 26 ± 2 °C, 75 ± 5% RH, and a 12:12 h (L:D) photoperiod. Corcyra cephalonica (Cruz 1980) and O. furnacalis (Seo et al. 2014) were identified based on the analysis of the external body morphology of adults. Colonies of these parasitoids were established on C. cephalonica eggs in the Entomology Laboratory.

MAINTENANCE OF TRICHOGRAmma COLONIES

Corcyra cephalonica eggs were obtained from oviposition cages in the laboratory. These cages each consisted of a cylindrical PVC tube (12 cm diam × 30 cm high) with its top opening covered by organza fabric. Newly deposited eggs were separated from impurities using a cotton ball and were glued with diluted Arabic gum (90:10% Arabic gum:distilled water) onto sheets of paperboard (10 cm long × 2 cm wide), totaling around 5,000 C. cephalonica eggs per paperboard sheet. Paperboard sheets then were placed on a plastic tray and exposed to ultraviolet light for 1 h in a chamber (40 cm long × 40 cm high × 40 cm wide) equipped with 2 lamps (Gaxindo®, GX-N093, T5-8W, Jakarta, Indonesia; purple light, lamp length = 29 cm, lamp diam = 1.5 cm, lamp wavelength = 28.5 cm, height from lamp to paperboard sheets = 30 cm) to make the C. cephalonica embryo unviable. After the inviable process, paperboard sheets were exposed individually to a colony of T. chilonis, T. poliae, or T. yousufi sp. nov. adults in a glass jar (12 cm long × 3 cm wide) for 72 h (3,000–3,500 adults per jar). Drops of honey were placed on the inner surface of the jars daily as food for the Trichogramma adults. Steps used to rear the larvae, pupae, and adults of C. cephalonica followed Amadou et al. (2019) with slight modifications.

BIOLOGICAL PARAMETERS OF TRICHOGRAmma PARASITIZING SPODOPTERA EGGS IN THE LABORATORY

Spodoptera exigua and S. litura eggs and larvae were collected manually from an A. crassicaarpa commercial nursery in Pangkalan Kerinci, and taken to the Entomology Laboratory where the larvae were reared on a solid, cube-shaped artificial diet, following fabrication steps described by Tavares et al. (2019). Spodoptera exigua and S. litura newly deposited eggs, laid on paper sheets used as oviposition substrate in cages as previously described, were exposed to ultraviolet light for 1 h in a chamber to make the Spodoptera embryo unviable as previously described. Spodoptera exigua or S. litura paperboard sheets were prepared by cutting the egg masses from the paper sheets leaving 1 mass per sheet, followed by gluing the egg masses onto sheets of paperboards. Each sheet of paperboard was exposed to a colony of T. chilonis, T. poliae, or T. yousufi sp. nov. adults in a jar for 72 h as previously described (each sheet of paperboard had 6 egg masses exposed to 3,000–3,500 adults per jar). One egg mass had an average of 60 and 75 eggs for S. exigua and S. litura, respectively.

After subjection to parasitism, 1 sheet of paperboard was placed into a test tube (2 cm diam × 12 cm high), where it was kept until adult Trichogramma or larval Spodoptera hatched. Individuals of S. exigua and S. litura, from the same laboratory colony, were recently identified through morphology and confirmed via molecular analysis (Sulistyono et al. 2020). The design was completely randomized with 9 treatments (each Trichogramma species parasitizing 1 of Lepidoptera), and with 25 replicates each with a sheet of paperboard. The following parameters were evaluated: incubation period, life cycle, adult longevity (d), female-biased sex ratio (number of females/number of insects), parasitism, and emergence (%). Blackened eggs, 4 d after subjection to parasitism, were classified as parasitized. Males and females of Trichogrammatidae were identified by dimorphism of their antennae, feather in males, and nailed in females (Santos et al. 2020). The data were submitted to Analysis of Variance, and averages were compared by the Tukey range test (Tukey 1949) at 5% probability.

PARASITISM RATE OF SPODOPTERA BY TRICHOGRAmma YOUSUFI SP. NOv. IN THE NURSERY

Trichogramma yousufi sp. nov. sheets of paperboard, consisting of fresh eggs of C. cephalonica subjected to parasitism for 72 h, were taken to a commercial nursery of A. crassicaarpa and were infested by about 95% S. exigua and 5% S. litura in Pangkalan Kerinci. The percentage of Spodoptera species was previously evaluated by collecting the moths using light traps. There were 3 treatments each with 5 replicates: 1, 2, or 5 paperboard sheets stapled per 1, 2, or 3 ground sand beds, respectively. Paperboard sheets were stapled on the abaxial surface of a leaf from the middle plant. Each bed (24 m long × 1 m wide) was
planted with 1,280 plantings. The experimental area was visited every d after the trial was established, and all new Spodoptera egg masses were eliminated. Five d after the paperboard sheets were stapled, 100 Spodoptera egg masses were randomly selected and recovered per treatment by cutting them from the entire leaf using scissors; then they were placed in 500 mL plastic containers (Easiyo®; Pekanbaru, Riau, Indonesia) and taken to the Entomology Laboratory where they were kept under incubation in glass jars (1 mass per jar) until adult Trichogramma or larval Spodoptera hatched. Trichogramma emergence rate was evaluated using the following formula: emergence rate = number of egg masses with adult Trichogramma hatched/number of recovered egg masses × 100. The data were submitted to Analysis of Variance, and averages were compared by the Duncan new multiple range test (Duncan 1955) at 5% probability.

TRICHOG sawma IDENTIFICATION, DESCRIPTION, PHOTOGRAPHY, AND DEPOSIT

Trichogramma species were identified and described after the external and internal morphology analysis of the male. Photographs were taken under a stereo zoom binocular microscope (ARK-zoom Star-Vi, AmScope®; Irvine, California, USA) using a Leitz Labor Lux S (Leica®, Wetzlar, Germany) camera. Body measurements were made with ocularmeter of different magnification powers (10×, 20×, 40×) in the Leitz Labor Lux S. Trichogramma yousufi sp. nov. is deposited under the registration NFIC Accession No.: 22321, Depository: National Forest Insect Collection, Forest Protection Division, Forest Entomology Discipline, Forest Research Institute, Dehradun, Uttarakhand, India 248006.

Results

PARASITISM IN THE LABORATORY

This is the first report of T. chilonis and T. yousufi sp. nov. parasitizing eggs of O. furnacalis. Parasitism of eggs of C. cephalonica, S. exigua, and S. litura was successful using T. chilonis, T. poliae, and T. yousufi sp. nov. The mean incubation period, life cycle, adult longevity, and female-biased sex ratio of Trichogramma were similar on eggs of Spodoptera and C. cephalonica (P < 5%). The parasitism and emergence rates were greater on C. cephalonica eggs than on those of Spodoptera (P > 5%) (Table 1).

PARASITISM IN THE NURSERY

The emergence rate of T. yousufi sp. nov. parasitizing egg masses of a mixed infestation of S. exigua and S. litura in a commercial nursery of A. crassicarpa (Fig. 1A, B) was greater with the density of 1 paperboard sheet stapled per ground sand bed (P > 5%; Table 2).

Table 1. Biological parameters (mean) of Trichogramma chilonis, Trichogramma poliae, and Trichogramma yousufi sp. nov. (Hymenoptera: Trichogrammatidae) parasitizing egg masses of Corcyra cephalonica (CC) (Lepidoptera: Pyralidae), Spodoptera exigua (SE), and Spodoptera litura (SL) (both Lepidoptera: Noctuidae) in the laboratory.

| Parameter               | Trichogramma chilonis | Trichogramma poliae | Trichogramma yousufi sp. nov. |
|-------------------------|-----------------------|---------------------|------------------------------|
|                         | SL        | SE        | CC       | SL        | SE        | CC       | SL        | SE        | CC       |
| Incubation period (d)   | 9 a       | 9 a       | 9 a      | 9 a       | 9 a       | 9 a      | 9 a       | 9 a       | 9 a      |
| Life cycle (d)          | 11 a      | 11 a      | 10.5 a   | 11 a      | 11 a      | 10.5 a   | 11 a      | 11 a      | 10.5 a   |
| Adult longevity (d)     | 4 a       | 4 a       | 4 a      | 4 a       | 4 a       | 4 a      | 4 a       | 4 a       | 4 a      |
| Female-biased sex ratio| 0.7 a     | 0.7 a     | 0.7 a    | 0.7 a     | 0.7 a     | 0.7 a    | 0.7 a     | 0.7 a     | 0.7 a    |
| Parasitism rate (%)     | 75.5 b    | 75.5 b    | 87 a     | 75.5 b    | 75.5 b    | 87 a     | 75.5 b    | 75.5 b    | 87 a     |
| Emergence rate (%)      | 75 b      | 75 b      | 90 a     | 75 b      | 75 b      | 90 a     | 75 b      | 75 b      | 90 a     |
Fig. 1. Adults of *Trichogramma yousufi* sp. nov. (Hymenoptera: Trichogrammatidae) parasitizing an egg mass of *Spodoptera exigua* (Lepidoptera: Noctuidae) (A) and a parasitized egg mass (B) in a commercial nursery of *Acacia crassicarpa* (Fabaceae) in Sumatra, Indonesia.

rows: costal cell narrow; marginal fringe about 1/8th of forewing width (30: 228). FEMALE. Body length 50 mm. Antenna with club more than 2× as long as wide. Ovipositor hidden, slightly shorter than hind tibia.

In *T. latipennis*, male body length 0.42 mm. Antennae with scape, slightly less than 4× as long as broad; pedicel about 2× as long as wide; flagellum 4.7× as long as broad; longest hair of flagellum about 1.5× as long as maximum width of flagellum; flagellum having 41 blunt hairs. Forewings about 2× as long as wide (428: 228) having RS1 4 setae; discal setae arranged in rows; costal cell narrow; marginal fringe about 1/8th of forewing width (30: 228). FEMALE. Body length 41 mm. Antenna with club less than 2× as long as wide. Ovipositor slightly exerted, about as long as hind tibia.

**TRICHOGRAMMA CHILONIS ISHII (FIG. 3A)**

**DIAGNOSIS.** Trichogramma chilonis. Body about 0.45 mm long, honey yellow; male antennae with 35 to 45 long and tapering flagellar hairs, longest hair of flagellum about 2.5× as long as maximum width of flagellum; forewings with fringe on tornus about 1/6th the wing width; male genitalia having DEG with prominent lateral lobes; chelate structure clearly below the level of gonoforceps; median ventral process broad at base; aedeagus longer than apodemes; both together slightly shorter than hind tibia; female with ovipositor as long as hind tibia.

**TRICHOGRAMMA POLIAE NAGARAJA (FIG. 3B)**

**DIAGNOSIS.** Trichogramma poliae. Body slightly more than 0.5 mm long, light brownish yellow; male antennae having flagellum with 30 to 35 long tapering hairs, longest hair about 3× the maximum width of flagellum; forewings with marginal fringe on tornus about 1/6th the wing width; male genitalia having DEG with prominent lateral lobes; CS below the level of GF; MVP large and broad at base; apodemes shorter than aedeagus, both together slightly shorter than hind tibia. Female with ovipositor about as long as hind tibia.

**Discussion**

The similar incubation period of *Trichogramma* shows the eggs of *Spodoptera* and *C. cephalonica* being able to provide adequate nutrients to the larva of this parasitoid. Eggs of *C. cephalonica* (573.5 µm long × 346.1 µm wide, with a volume of 0.036 mm³) and *Spodoptera* (454.9 µm long and 390.2 µm wide, with a volume of 0.036 mm³), similar in size and volume (Cônsoli et al. 1999), are suitable to allow *Trichogramma* to produce large and vigorous adults, resulting in a standard life cycle period and survival rate (Firake & Khan 2014; Edwin et al. 2016). The higher number of females produced with eggs of both host species is beneficial in laboratory rearing for the release of this parasitoid (Brotodjojo & Walter 2006; Oliveira et al. 2017). The lower parasitism and emergence rate of *Trichogramma* on *Spodoptera* is possibly due to the ability of the eggs of this pest to encapsulate the parasitoid larva (Dorémus et al. 2013) and promote physical protection against parasitoids (Sá & Parra 1994). *Spodoptera* eggs are laid in 2 to 3 layers and are protected with hairs deposited by moths, with the parasitoid ovipositor encountering difficulties reaching those in deeper layers (Cônsoli et al. 1999).

The greater emergence rate of *T. yousufi* sp. nov. with a density of 1:1 (paperboard sheet:ground sand bed) was expected because a higher number of adults of this parasitoid was released per m². Higher densities of *Trichogramma* released per m² are more successful in parasitism (Jin et al. 2019; Mohammadpour et al. 2019). The emergence rate of *T. yousufi* sp. nov. in the current study was greater than that of *Trichogramma japonicum* Ashmead (Hymenoptera: Trichogrammatidae) on eggs of the rice yellow stem borer, *Scirpophaga incertulas* (Walker) (Lepidoptera: Crambidae), by 2.05% in Asian rice, *Oryza sativa* L. (Poaceae), cabbage, *Brassica oleracea* L. (Brassicaceae), corn, and tomato, *Solanum lycopersicum* L. (Solanaceae), crops and of *Trichogramma chilotrae* Nagaraja & Nagarkatti (Hymenoptera: Trichogrammatidae) on those of a mixed infestation of the cabbage cluster, *C. cephalonica* (Fabricius) (Lepidoptera: Crambidae), and *S. litura* by 1.67% on cabbage crops in Solok, West Sumatra, Indonesia (Hidrayani et al. 2013).

**Table 2.** Emergence rate (mean) of *Trichogramma yousufi* sp. nov. (Hymenoptera: Trichogrammatidae) parasitizing egg mass of a mixed population of *Spodoptera exigua* and *Spodoptera litura* (both Lepidoptera: Noctuidae) in a commercial nursery of *Acacia crassicarpa* (Fabaceae) in Sumatra, Indonesia.

| Density of paperboards installed per group of ground sand beds | Emergence rate (%) |
|---------------------------------------------------------------|--------------------|
| 1:1                                                           | 40.82 a            |
| 1:2                                                           | 33.34 b            |
| 1:5                                                           | 24.79 c            |
| CV                                                            | 24.32              |

Means followed by different letters differ by the Duncan new multiple range test at 5% probability. CV = Coefficient of variation.
Fig. 2. *Trichogramma yousufi* sp. nov. (Hymenoptera: Trichogrammatidae) adult male (A), adult female (B), fore wing (C), RS1 (D), head with antennae (E), and male genitalia (F).
Fig. 3. Genitalic difference between adults of *Trichogramma chilonis* (A) and *Trichogramma poliae* (B) (Hymenoptera: Trichogrammatidae).
In conclusion, *T. chilonis* and the new species, *T. yousufi* sp. nov., were recovered from *O. furnacalis* eggs during a field exploration in Bunga Raya, whereas *T. poliae* was obtained from *C. cephalonica* eggs in a laboratory in Jatisari. The separation of *T. yousufi* sp. nov. from its closer species, *T. latipennis*, has been discussed in greater detail. The taxonomic characters of all 3 *Trichogramma* species have been given precisely. The *Trichogramma* species, recovered from corn crops and reared in a laboratory, successfully parasitized *S. exigua* and *S. litura* eggs under environmentally controlled conditions, with the parasitism rate of *T. yousufi* sp. nov. ranging from 24.79 to 40.82% on the eggs of *S. exigua* and *S. litura* attacking *A. crassicarpa* plants in a commercial nursery in Pangkalban Kerinci. It therefore appears from this study that *T. yousufi* sp. nov. may be employed as a biological control agent to manage *Spodoptera* in *Acacia* nurseries.

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