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Diversity and Low Parasitism of Egg Parasitoids Associated with Maize (*Zea mays*) in Madura, Indonesia

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ABSTRACT

This study aimed to explore and identify egg parasitoids associated with maize (Zea mays) in Keleyan Village, Bangkalan Regency, Madura. Field observations were conducted during both vegetative and generative growth stages using a direct survey method. Eggs of lepidopteran pests were collected and reared under laboratory conditions to observe the emergence of adult parasitoids. The results showed that only one genus of egg parasitoid was found, namely Trichogramma spp., which parasitized the eggs of Spodoptera frugiperda at a parasitism rate of 17.3%. This rate was low for a biological control program. These findings indicate that the presence of natural parasitoids in the study area was not sufficiently effective in suppressing pest populations under natural conditions. Nevertheless, Trichogramma spp. still shows potential as a biological control agent, particularly if supported by augmentative or conservation-based approaches. This results can be used as foundation for the development of ecological-based environmentally friendly, and sustainable pest control strategies in lowland maize cultivation systems, especially in the Madura region.

1. INTRODUCTION

As a carbohydrate rich commodity, maize has the potential to serve as an alternative staple food to rice. In addition to its role as a rice substitute, maize also offers a variety of other benefits, including being processed into food products, used as seed, fulfilling feed requirements, and serving as raw material for various industrial purposes. Maize is generally cultivated in dryland areas and more frequently grown during the dry season since it requires relatively little water. It also thrives in areas with environmental conditions favorable for its growth (Dewi *et al*., 2018). In Madura Island, particularly in Bangkalan Regency, maize is an important crop cultivated predominantly in dryland farming systems. These agroecosystems provide favorable conditions for both pest development and the activity of natural enemies.

One of the major factors contributing to yield reduction in maize is the emergence of invasive pests, such as the fall armyworm *Spodoptera frugiperda* (Smith) (*Lepidoptera*: *Noctuidae*). The fall armyworm has emerged as a major invasive pest threatening maize production worldwide, including in Indonesia. This pest is highly destructive due to its wide host range and rapid infestation rate, causing significant yield losses and economic damage. The impact of *S. frugiperda* infestation is highly detrimental. This pest has caused global maize yield losses ranging from 8.3 to 20.6 million tons per year (21–53% of production), with estimated economic damages reaching US$ 2.5 to 6.2 billion annually (Abrahams *et al*., 2017; Day *et al*., 2017). Widely known as the fall armyworm, this pest exhibits a high infestation rate across various crop species, with a host range covering 353 species from 76 plant families, particularly Poaceae, Asteraceae, and Fabaceae (Montezona *et al*., 2018).

Biological control is a pest management method that utilizes natural enemies such as predators, parasitoids, and entomopathogens to suppress pest populations below economic thresholds. Egg parasitoids are among the most commonly used biological agents in pest suppression. Field research conducted by Otim *et al*. (2021) demonstrated that natural enemies were able to destroy up to 64% of *S. frugiperda* eggs, highlighting the important role of parasitoids in pest regulation. Given the significant contribution of parasitoids in suppressing pest populations, the exploration and identification of egg parasitoids attacking maize pests, particularly in the lowland areas of Bangkalan, Madura, is an essential step. However, information on the diversity and effectiveness of egg parasitoids associated with maize in Madura remains limited. In particular, the natural parasitism rate and its potential contribution to pest suppression under local field conditions have not been well documented. Therefore, this study aimed to identify egg parasitoids associated with maize and to evaluate their parasitism rate against *S. frugiperda* in Bangkalan, Madura. This research not only support environmentally friendly pest control efforts but also contributes to enriching knowledge of parasitoid diversity in maize ecosystems and their potential application in biological control strategies. The findings are expected to provide baseline information for the development of ecologically based pest management strategies.

2. MATERIALS AND METHODS

This research was conducted during the period of February to April 2025. Sampling was carried out in maize cultivation areas located in Bangkalan Regency, Madura. Bangkalan is recognized as the second largest maize producing region in Madura after Sumenep Regency. This corresponds to the soil characteristics in Madura Island, particularly in Bangkalan Regency, where most land consists of dry fields. One of the villages in Socah District that serves as a key maize production center is Keleyan Village.

The rearing and identification of suspected parasitized eggs were conducted at the Plant Health Laboratory, Universitas Pembangunan Nasional "Veteran" Jawa Timur. The materials used in this study included maize plants, suspected parasitized eggs, labels, and 70% alcohol. The equipment used in this research consisted of scissors, Petri dishes, test tubes, a binocular microscope, collection bottles, and a camera. In addition, the identification process was assisted by the iNaturalist application and the Bugguide.net website (https://bugguide.net). Reference literature used included Grissell & Schauff (1990), Goulet & Huber (1993), and Shepard *et al*. (1995).

2.1. Research Methods

1. Observation and Sampling

This study was conducted in an exploratory manner using a comprehensive survey method, in which the entire maize field was observed without applying specific sampling techniques. Egg sampling of pests was carried out in two growth stages of the crop, namely the vegetative phase (28–42 days after planting) and the generative phase (49 days after planting). Egg collection was performed by examining plant parts that are potential oviposition sites, such as the underside of leaves, leaf surfaces, young leaves (particularly the top three leaves), the plant base, and maize silks.

Egg masses that were found were transferred into plastic Petri dishes. The eggs were then examined to confirm indications of parasitism. Each Petri dish was properly labeled and stored until parasitoid adults emerged from the eggs. Suspected parasitized eggs were reared at the Plant Health Laboratory, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" Jawa Timur. Emerged parasitoid adults were preserved in collection vials containing 70% alcohol. Individuals emerging from the same egg mass were stored in the same vial. The vials were labeled according to the sampling location, egg cluster, and collection time, following a modified method from Hendrival *et al*. (2022).

2. Identification of Egg Parasitoids

Observations were conducted using a binocular microscope, referring to several taxonomic determination keys. Identification was primarily conducted using morphological keys. Digital platforms such as iNaturalist and the Bugguide were used as supporting tools for preliminary comparison. Identification was carried out to the morphospecies level by adapting determination keys developed by Grissell & Schauff (1990), Goulet & Huber (1993), and Shepard *et al*. (1995). The identification aimed to describe the types of egg parasitoids found in maize cultivation areas (Hendrival *et al*., 2022). The identification process of parasitoids involved the following steps:

1. Observing morphological characteristics of the insects under the microscope.
2. Identification using the iNaturalist application (https://www.inaturalist.org/). Photos of parasitoids were uploaded to the application, which then suggested the genus, species, and provided information on the top 10 species with morphological features similar to the uploaded photos for comparison (Argiyanti *et al*., 2022).
3. Comparing parasitoid morphological characteristics and iNaturalist results with data available on the Bugguide.net website (https://bugguide.net), following the approach of Windriyanti *et al*. (2020).
4. Cross-checking with reference literature, including Grissell & Schauff (1990), Goulet & Huber (1993), and Shepard *et al*. (1995).

2.2. Observation Variables

1. Morphological characteristics: This parameter included observations of parasitoid morphological traits, such as body size, coloration, antennal shape, wing structure, and ovipositor type. Identification was carried out using a microscope and reference guides for parasitoid insect identification.
2. Attack frequency level: The attack frequency level was calculated by comparing the number of plants that were infested with the total number of observed plants. The value was then expressed as a percentage (%) using the following formula (Tribowo *et al*., 2014):

$$FS = \frac{A}{B} \times 100\ \% \quad (1)$$

where FS is attack frequency (%), A is number of infested plants, and B is total number of observed plants

3. Parasitism rate (PR): The rate of parasitized eggs was calculated using formula adapted from Ulya *et al*. (2024):

$$PR = \frac{P}{T} \times 100\ \% \quad (2)$$

where PR is parasitism rate (%), P is number of parasitized eggs, and T is total number of observed eggs.

2.3. Data Analysis

Data were analyzed descriptively and presented as percentages, tables, and graphs using GraphPad Prism. The observed parameters included parasitoid morphological characteristics, pest attack frequency on maize plants, and egg parasitism rate. No inferential statistical analysis was applied due to the exploratory nature of the study.

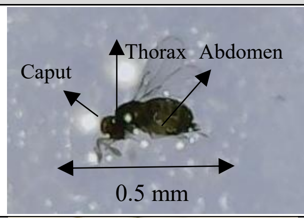
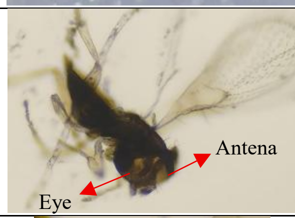
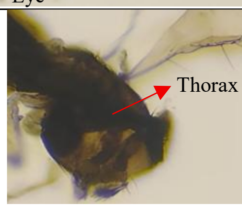
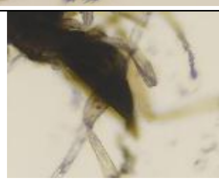
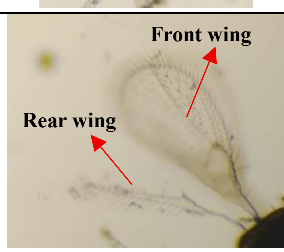
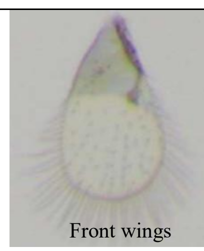
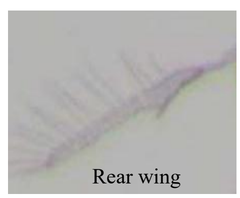
3. RESULTS AND DISCUSSION

3.1. Research Location Conditions

The research site was a large maize cultivation area managed by local farmers under an intercropping system, in which maize was planted simultaneously with groundnut (*Arachis hypogaea*). The maize seeds used in this system were hybrid varieties, which have advantages in terms of yield potential, uniform growth, and tolerance to environmental stress. Throughout the cropping period until harvest, farmers did not apply chemical pest control. Interestingly, despite the absence of pest control interventions, the plants continued to grow well and appeared healthy, indicating the presence of a stable natural ecosystem that supported crop growth and allowed the persistence of natural enemies such as parasitoids. The microhabitat conditions favorable for the survival of *Trichogramma* spp. were particularly associated with the maize–groundnut intercropping system, which created a more stable environment and provided alternative resources for these parasitoids.

Climatologically, the study area had relatively homogeneous temperatures due to its elevation, which did not vary significantly. Temperature differences in a region are generally influenced by altitude, where air temperature decreases by approximately 0.6 °C for every 100-meter increase in elevation (Istiqomah & Purnomo, 2019). This characteristic reflects the condition of the tropospheric layer, which supports temperature stability in the cultivation area. The stability contributed to balanced populations of organisms in the field, including both pests and their natural enemies, thereby maintaining a sustainable agroecosystem.

Table 1. Identification of *Trichogramma* spp. parasitoids [Ref: Wahyudin *et al.* (2024); Maramis *et al.* (2013); Hakim *et al.* (2014)]

Body part	Illustration	Reference	Summary description
Whole body			Body size very small, usually <1 mm; body color pale yellow; lacking metallic sheen; body short and compact.
Caput (Head)			Yellow head, with large compound eyes that appear bright red when alive.
Thorax			Legs attached to the thorax, with three-segmented tarsi on all legs; thorax coloration similar to the body, non-metallic.
Abdomen			Broadly attached to the thorax, lacking a petiole, and oval in shape.
Wings		 	Forewings: Broad and rounded in shape; post-marginal vein almost indistinct; setae (fine hairs) arranged in a characteristic pattern. Hindwings: Narrower and elongated, with long marginal hairs; wings appear hair-like or as thin ribbons.
Whole body			The body is yellowish-brown with red eyes. Male body length ranges from 0.57–0.79 mm, while females measure 0.73–0.79 mm.
Abdomen			Broadly attached to the thorax without a petiole, oval in shape.
Wings			The wings are oval in shape and covered with fine setae, giving them a distinctly fringed appearance. The marginal fringes are particularly noticeable, with the longest hairs located along the outer edges of the wings

In addition to physical factors, the preference of *Trichogramma* spp. for *Spodoptera frugiperda* eggs may also be influenced by chemical signals released either by host eggs or by oviposited plants. Semiochemicals such as kairomones play a role in enabling parasitoids to detect host presence from a certain distance. However, since this study was limited to morphological observations and preliminary identification, more in-depth research on the chemical and ecological behavior of *Trichogramma* spp. toward *S. frugiperda* is still required.

3.2. Egg Parasitoids Identified

Identification based on the keys of Goulet & Huber (1993) revealed that the parasitoids had black-colored bodies and were extremely small, ranging from 0.3 to 1 mm in length. Their antennae consisted of six segments, with the terminal segment bearing short setae. The wings appeared short and broad, with the margins covered by fine setae, which are characteristic features of this genus. The marginal setae were relatively longer, and the tarsi of the legs were divided into three segments. The body morphology was compact and somewhat rounded, with a prominent abdomen (Table 1). These findings are consistent with the observations of Waruwu *et al*. (2023), who described similar morphological traits of *Trichogramma* sp. collected from maize fields in North Sumatra.

During field explorations, several types of parasitoids were discovered, but all belonged to the same genus. Within the genus *Trichogramma*, more than one morphospecies was identified. This variation was evident from distinct morphological traits, such as body coloration: some individuals exhibited a dark black body, while others had a yellowish-brown coloration resembling that of small wasps. Such color variation provides an initial indication of different morphospecies, although further confirmation requires morphometric or molecular analyses.

In general, the genus *Trichogramma* consists of numerous species widely distributed across various regions, each with different capabilities in parasitizing host eggs. Commonly reported species include *Trichogramma chilonis*, *Trichogramma japonicum*, *Trichogramma evanescens*, *Trichogramma pretiosum*, and *Trichogramma dendrolimi*. Each species displays specific host preferences and varying responses to environmental conditions. Therefore, identifying the dominant species within a particular agroecosystem is essential for understanding their ecological role and potential application in biological control programs.

3.3. Parasitism Effectiveness of *Trichogramma* spp.

In general, healthy eggs of *Spodoptera frugiperda* are characterized by relatively bright colors, such as yellowish-white or pale green when newly laid, which gradually darken as the embryo develops inside (Sumaryati *et al*., 2023). The eggs of *S. frugiperda* are dome-shaped with a diameter of approximately 0.51 mm (Figure 1). At the time of oviposition, the eggs may exhibit a variety of colors, including greenish-white, green, cream, and pink. Subsequently, the egg color changes to brown and eventually turns black as hatching approaches. In contrast to healthy eggs, parasitized eggs typically show distinctive changes in both color and structure. The egg color may become darker, dull, or even brownish (Figure 1). In addition to color alteration, parasitized eggs usually fail to hatch into pest larvae; instead, they release parasitoid adults after completing their life cycle within the host egg. In some cases, parasitized eggs may also appear slightly shrunken or hardened, depending on the developmental stage of the parasitoid inside.

The number of *Spodoptera frugiperda* eggs in the field increased markedly from the third to the fifth week, with the peak observed in the fourth week. Most of the eggs collected were healthy, whereas parasitized eggs accounted for only a small proportion of the total. After the fifth week, the number of eggs declined sharply, and by the seventh to

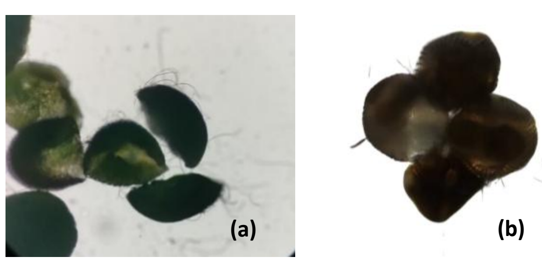


Figure 1. Eggs of *Spodoptera frugiperda*: (a) Healthy, (b) Parasitized

ninth week, they were nearly absent (Table 2). Eggs exhibiting signs of parasitism were first recorded in the third week week, when maize plants were at 15–21 days after planting (DAP). These parasitized eggs displayed characteristic discoloration, ranging from pale to darkened tones. By the sixth week, no egg masses of *S. frugiperda* were found. This phenomenon is likely attributable to the preference of adult moths for oviposition on younger maize leaves (Wayan Supartha *et al*., 2021). The population dynamics of moths in maize fields are influenced by volatile chemical compounds released by young plants. Maize is known to produce phenolic compounds, including vanillic acid, which function as attractants for gravid females in selecting oviposition sites (Horvat *et al*., 2020).

Table 2. Number of healthy and parasitized eggs of *Spodoptera frugiperda* observed in maize fields

Observation Period	Total Healthy Egg Masses	Count of Parasitized Egg Masses
M1 (1-7 DAP)	0	0
M2 (8-14 DAP)	2	0
M3 (15-21 DAP)	12	2
M4 (22-28 DAP)	10	3
M5 (29-35 DAP)	4	1
M6 (36-42 DAP)	0	0
M7 (43-49 DAP)	0	0
M8 (50-56 DAP)	0	0
M9 (>56 DAP)	0	0

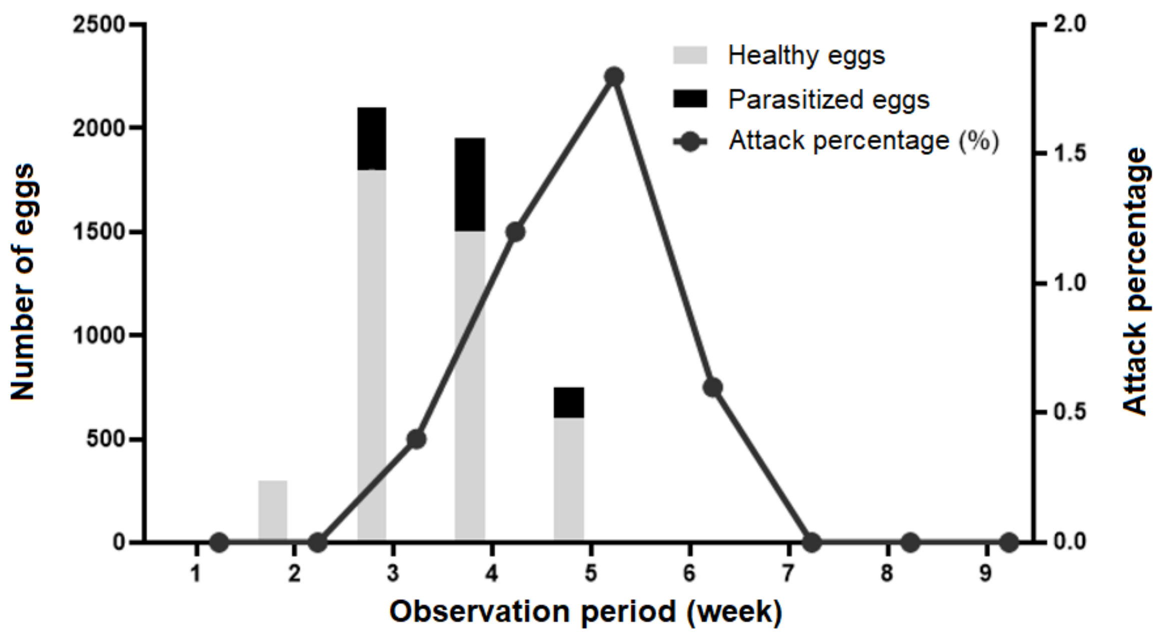


Figure 2. Number of *Spodoptera frugiperda* eggs (healthy and parasitized) and percentage of larval infestation per week during the observation period

The percentage of pest infestation increased in parallel with the rising number of eggs, with the peak infestation observed in the fifth week. Thereafter, the infestation rate declined sharply, corresponding with the decrease in egg numbers (Figure 2). This pattern indicates that the level of larval infestation by *Spodoptera frugiperda* is strongly influenced by the dynamics of egg populations. However, despite the increase in egg numbers from the third to the fifth week, the parasitism rate remained relatively low. This suggests that the effectiveness of parasitoids in regulating egg populations was not yet optimal during this period. Larvae of *S. frugiperda* were generally found in the whorl of the plant. Infested whorls exhibited multiple feeding holes and larval frass while the leaves were still folded. Once the leaves had unfolded, visible damage appeared in the form of tears and perforations caused by larval feeding activity.

The development of *S. frugiperda* is influenced by host plant quality and age. At 50 days after planting (DAP), maize leaves undergo senescence, leading to a decline in nutritional content and consequently reducing their ability to support optimal larval growth and development (Subiono, 2019).

Female adults of *Trichogramma* spp. are capable of producing 50–200 eggs during their lifetime, with oviposition activity being most intense within the first 2–3 days after mating. The number of eggs laid is influenced by species, age, environmental conditions, nutritional status during the larval stage, as well as the availability and size of host eggs. Oviposition efficiency serves as a critical indicator for evaluating the potential of *Trichogramma* as a biological control agent. Based on the observations, the parasitism rate of *Trichogramma* spp. on *Spodoptera frugiperda* eggs was recorded at 17.3% (Table 3). These finding is within the values reported by Wahyuningsih *et al*. (2022) in the range of 15.6 to 52.5% for three different corn ecosystems (agroforestry, rice field, and rainfed field) in East Java. The rate, however, is almost double than the parasitism rate of 9.2% reported by Otim *et al*. (2023) in Uganda. The parasitism rate of 17.3% observed in this study is relatively low compared to previous reports, which often exceed 40% under favorable conditions. This indicates that natural populations of *Trichogramma* spp. in the study area are not sufficient to effectively suppress *S. frugiperda* populations.

The parasitism ability of *Trichogramma* spp. has been reported to be lower than that of *Telenomus remus*, particularly on eggs covered with thick scales (Wahyuningsih *et al*., 2022), as these layers act as mechanical barriers during oviposition. In contrast, eggs without scales or with only a thin layer of scales are more easily parasitized. Several ecological factors may contribute to this limitation, including predation on host eggs, concealed oviposition behavior of the pest, and the limited dispersal ability of *Trichogramma* spp. Additionally, the presence of intercropping systems may create a complex habitat that influences host–parasitoid interactions. The relatively small body size of *Trichogramma* spp. further limits their dispersal ability and capacity to penetrate the egg chorion, thereby reducing their parasitism effectiveness.

Table 3. Parasitism rate of *Trichogramma* spp. on *Spodoptera frugiperda*

Egg masses observed	28 egg masses
Number of eggs per egg mass	100-200 eggs (average 150)
Number of healthy eggs	5200
Number of parasitized eggs	900
Parasitism rate	$\frac{900}{5200} \times 100\% = 17.3\ \%$

The low parasitism rate observed in the field is presumed to be closely associated with the presence of predators such as *Cheilomenes* sp. and *Paederus* sp., which are commonly found in maize–groundnut intercropping systems. These predators are capable of preying upon eggs before they can be parasitized by *Trichogramma* spp., thereby reducing the likelihood of parasitoids locating suitable hosts. Furthermore, the host-searching period of *Trichogramma* spp. is generally longer compared to *Telenomus remus*, resulting in a greater window of vulnerability of host eggs to predation. Predators are also opportunistic, often destroying egg masses regardless of whether they have been parasitized. Collectively, these conditions diminish the overall effectiveness and efficiency of parasitism within agroecosystems rich in natural enemies (Fanani *et al*., 2023).

3. CONCLUSION

This study revealed that egg parasitoids associated with maize in Bangkalan, Madura were dominated by *Trichogramma* spp., with no other genera observed. Despite their presence, the parasitism rate on *Spodoptera frugiperda* eggs was relatively low (17.3%), indicating limited effectiveness of natural biological control under field conditions. These findings suggest that the existing parasitoid population alone is insufficient to suppress pest populations, highlighting the need for improved biological control strategies. Enhancing the effectiveness of *Trichogramma* spp. through augmentative release or habitat management may be necessary to optimize their role in sustainable maize pest management systems.

AUTHOR CONTRIBUTION STATEMENT

Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
PAV	✓	✓	✓			✓			✓	✓	✓		✓	
WW				✓			✓	✓				✓		
RMK				✓	✓			✓			✓	✓		

C: Conceptualization	Fo: Formal Analysis	O: Writing - Original Draft	Fu: Funding Acquisition
M: Methodology	I: Investigation	E: Writing - Review & Editing	P: Project Administration
So: Software	D: Data Curation	Vi: Visualization	
Va: Validation	R: Resources	Su: Supervision	

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