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Parasitic Hymenoptera in The Population of Two Key Pest of Vegetable Soybean Edamame

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Abstract: Whitefly (*Bemisia tabaci*) and soybean pod borer (*Etiella zinckenella*) are key pest and notorious on edamame soybean. The investation of the two insects pest can cause up to 80% production of productivity of edamame soybean. The chemical pesticides use is the main tactic to control the pests which have negative impact to infarment and develop insect resistance and resurgence. Identification of natural enemy such as Hymenoptera parasitoids are important effort to promote biological control strategy has alternative infarmentfriendly to control the pests. The results show there is one parasitoid Hymenoptera in soybean pod borer and 5 family of Hymenoptera parasitoid and one family of Diptera parasitoid in whitefly.

Keywords: Edamame Soybean; Parasitoids; Soybean Pod Borer; Whitefly

Introduction

Vegetable soybean edamame has began to be cultivated in Jember East Java in 1992 and since 1995 the crops have been exported to Japan with an average annual edamame export capacity of 8000 tons per year in fresh frozen. Edamame soybeans, often called green vegetable soybean is one type of soybean which young pods can be consumed directly as snacks (Khaeruni *et al*., 2008). Therefore, edamame soybean is belong in vegetable type horticultural products, not including legumes and have a larger size of pod than the size of local soybean (Kurniasanti *et al*., 2014). Edamame soybean is the same soybean as local soybean species, but the harvesting time is different (Miles *et al*., 2000). According to Konovsky *et al*. (1994) Edamame soybeans are special soybeans (*Glycine max* (L.) Merr.) that are harvested as vegetables when the pods are immature (R6 stage).

As with any cultivated crop, edamame soybeans are also affected by pests and diseases that can reduce production. The major insect pests reported that attack and devastate edamame soybean are whitefly (*Bemisia tabaci*) and soybean pod borer (*Etiella zinckenella*) (Yuliani *et al*., 2006).

Whitefly is a destructive insect that attack to the lower surface of the leaves. The adult of whitefly is white because clear wing color covered with a layer of starchy wax. Whitefly is polyphagous which can attack various of crops (Ardiansyah, 2014). The nymph and adult stages of whitefly fit on the leaves by sucking plant fluids (Septiatin, 2012). Cause yield losses of up to 80%, and if no control is carried out, there will be crop failure (Marwoto and Inayati, 2011). Soybean pod borer (*E. zinckenella*) can cause damage to pods to reach 80%.

The main tactic to handling and control of whitefly (*B. tabaci*) and soybean pod borer (*E. zinckenella*) still uses chemical insecticides with calendar based spray and broad spectrum insecticides that can develop resistance and resurgence. In addition, the use of pesticides can also cause disadvantages in the form of adverse effects, including: (1) Pesticides negatively affect human health, (2) Pesticides adversely affect the environment, and (3) Pesticides increase the development of populations of plant nuisance organisms (Sunarto, 2020). So, it is

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necessary to find alternative control that more infarmenfriendly.

Based on research conducted by Utami *et al*. (2014) there are 2 families of Hymenoptera parasitoid insects in whitefly population on soybean plants including: *Mymaridae* species *Polynema* sp, and *Aphelinide* two species namely *Eretmocerus* sp. and *Encarsia*. But until now there are still no reports on biological control agent, especially the use of parasitoids carried out to control whitefly and soybean pod borer.

According of personal communication with Joko Ramadhoni (Manager of PT GMIT's edamame soybean estate), soybean pod borer and whitefly is reported to damage edamame soybean. Economic threshold of the soybean pod borer are 2 larvae per 1 kg of harvested soybean pods. This makes soybean pod borer is the most destructive pest besides whitefly in edamame soybean cultivation. If the economic threshold exceeds the limit, edamame soybeans are declared unfit for export. The research studies aimed to identification of Hymenoptera parasitoid in the population of the whitefly and edamame soybean pod borer as potential for promoting biological control agents.

Method

The research procedure was conducted to determine the sampling method and identification of parasitoids of whitefly and edamame soybean pod borer in PT GMIT edamame soybean fields in Jember Regency. Observations were made by observing pod damage and infected leaves by whitefly nymph before harvesting when edamame soybean growth entered the R7 development phase (Phsicologycaly maturity state). This research activity was conducted using purposive sampling method. Sampling was done randomly on edamame soybean pods and infected leaves that indicated the attack of edamame soybean pod borer and whitefly. Sampling was done with a minimum of n = 30 either pods or leaves.

In the mounds during the observation was selected and taken as plant samples that was taken to the laboratory. Pod samples at each field location was taken to the laboratory for dissection and identification of the type of edamame soybean pod borer. Pods and leaves sample was incubated in modified rearing device which in 25cm diameter plastic container with a ventilated lid and then wrapped in a black plastic bag, on which an inverted funnel was placed on of the lid with a 10cm diameter plastic cup so that parasitoids and could collected in the cup (Figure 1). In the parasitoid incubation process, each plastic container contains a maximum of 30 edamame soybean pods and leaves separately tissue is placed inside the container to serve as a pupal chamber. After the parasitoid incubation process, identification of parasitoids of whitefly and edamame soybean pod borers was carried out, followed by making dry specimens of parasitoids.

Figure 1. Rearing (Incubation) Parasitoid of Whitefly and Edamame Soybean Pod Borer

Observation Variable

The observation variables contained in this studies are as follows:

(1) Species and Parasitation Rate of Parasitic Waps

The species observation variable is intended to determine the type of parasitoid found through identification using a microscope which will then be used as a dry specimen. Furthermore, the observation variable of parasitization rate of Hymenoptera parasitoids was calculated using the formula:

$$\%P = \frac{n}{N} x100\% \quad \textbf{(1)}$$

Description:
%P= Parasitation Rate
n= Number of emerging parasitoids
N= Total number of moths appeared + number of emerging parasitoids

(2) Parasitoid Identification

To determine the species of parasitoid that appears, identification is carried out. Identification parasitoid is done by looking at morphology parasitoid using a microscope, the the results of photo images using a microscope was matched with the literature. The literature used to identify is "Hymenoptera of the world: an identification guide to families" (Goulet and Huber, 1993) and other supporting literatures.

(3) Similarity Index

The similarity index (IS) is used to calculate the similarity indec of parasitoids species in a field according to Krebs, 1989; Utami *et al*. (2014): The similarity index is calculated by Formula 2.

$$IS = \frac{2C}{A+B} \quad (2)$$

Description:
IS= Sorensen's Species Similarity Index
A= Number of parasitoid insect species in region 1
B= Number of parasitoid insect species in region 2
C= Number of parasitoid insect species in common in both compared habitats

If the IS value >50%, the similarity of species in a habitat is high, while if the IS value is <50%, the similarity of species in a habitat is low.

Result and Discussion

Table 1. Data on Parasitization rate of Hymenoptera parasitoids

Location	Number of Pods	Emerged imago	Emerged Parasitoid
Salak source, Ledokombo	3	0	0
Sruni, Jenggawah	28	1	1
Salak source, Ledokombo	6	0	0
Mengok, Pujer	158	13	0
Tegal gum, Mrawan, Mayang	72	0	0
Wirowongso, Ajung	32	0	0

According table 1, the percentage of parasitizing Hymenoptera parasitoids on soybean pod borer in 6 locations in Jember Regency was low 0.06%. The factors causes low percentage of Hymenoptera parasitoid parasitation on soybean pod borer because the high spraying of chemical pesticides as a main tactic control for *E. zinckenella*. Chemical pesticides applied during control activities contain several active ingredients such as Cypermethrin, Emamectin Benzoat & L, Imidacloprid & Bifentrin, Methomyl, and Chlorantraniliprole. The doses used of these active ingredients are also quite high and continuous and in close and frequent application intervals. So, it is possible that the low level of parasitization of Hymenoptera parasitoids is due to the intense application of chemical pesticides. The identification of Hymenoptera parasitoid based on wing type was belong family of Braconidae (Figure 2).

Figure 2. Parasitoid of edamame soybean pod borer

Whitefly Parasitoid Identification

The studies of whitefly parasitoid species composition on Edamame soybean crops was conducted in the three areas, namely Kranjingan, Jenggawah, and Cangkring. The leaves sampling in the Kranjingan area consists of Kranjingan 1 (K1), Kranjingan 2 (K2), and Kranjingan 3 (K3). The leaves sampling in the Jenggawah area consists of Jenggawah 1 (J1), Jenggawah 2 (J2), and Jenggawah 3 (J3). Meanwhile, the Cangkring area consists of Cangkring 1 (C1), Cangkring 2 (C2), and Cangkring 3 (C3). The results of the identification of morphological characters are obtained in Table 2.

Table 2. Identification results of whitefly parasitoid species (*B. tabaci*) on edamame plants

Family	Genus	K1	K2	K3	J1	J2	J3	C1	C2	C3	Total
Aphelinidae	*Encarsia* sp.	2	7	2	105	102	112	62	69	33	494
Braconidea	*Microplitis* sp.	1	1	1							3
	Apanteles sp.						1				1
Cecidomyiidae	*Lestremia* sp.			1							1
Eulophidae	*Elachertus* sp.				1						1
Mymaridae	*Cleruchus* sp.				3	2		1			6
Trichogrammatidae						1					1
	Total	3	8	4	109	105		113	63	69	33

Based on the parasitoid collection process that has been carried out in the three sides, it has been obtained 507 parasitoid individuals belonging to the Hymenoptera and Diptera order.

Key to determination of Hymenoptera parasitoids of whitefly (B. tabaci)

It is known that determining the superfamily, family, genus and species of insects requires detailed knowledge of the morphology and characteristics of the insects.

1. Forewings have normal venation and have indistinct or even absent costal cells, veins C, Sc, R and Rs merge between wing base and pretostigma. Long threadlike (filiform) antennae with 18 or more segments. In females the ovipositor is generally clearly visible (Erniwati and Ubaidillah, 2011)……..(Ichneumonoidea) 2

The forewings have abnormal or reduced venation and are transparent, lacking costal veins. Generally have dark, yellow and some metallic colors (Lee, 2009). The anther is generally angled and never more than 13 segments in males and 14 segments in females. The hind limbs are enlarged (Erniwati and Ubaidillah, 2011)……(Chalcidoidea) 3

2. Forewing wing with rs+m venation visible, 2m- cu venation not visible (Erniwati and Ubaidillah, 2011). The hind wing with venation is well developed, with ir-m venation separating the R1 and rs venation (Goulet and Huber, 1993)…….....(Microplitis sp.) 9

3. The forewings have distinctive venation with long marginal veins, short stigma veins and do not appear to be clearly distinct from the wing . Setae on the wing are almost absent, generally surrounding the wing or present only at a few points or in a line. Antennae 8 segments or less with visible funicel segments. Mesosoma and metasoma broadly fused. Color yellowish brown, pale yellow to white sometimes with dark markings or dark throughout but not metallic. Maximum body size 1 mm. (Handbook Of Nearctic Chalcidoidea)……..(Aphelinidae) 4

The forewing is membrane smooth, has linear or highly reduced venation and ends at one third of the wing, no stigma or postmarginal venation. hind wing is elongated and dangling. The elongated metasoma is fused with the mesosoma. In females the antennae are dangling and in males they are filiform. Body size 0.2-1mm (Handbook of Nearctic Chalcidoidea)……(Mymaridae) 8

Forewings lack postmarginal venation, stigma venation is elongated, setae on wings are in regular rows. Antennae short with 0 - 4 segments, funicle 1-2 segments. Color whitish pale yellow, dark marked but never metallic. Body size 0.3 - 1.2 mm (Handbook of Nearctic Chalcidoidea)……(Trichogrammatidae) 9

The hind wings have no visible setae widened, venation on forewing ending beyond basal 1/3, postmarginal visible, elongated stigma without pedicels. Antennae 5-8 segments including pedicle and club. Body length generally 1 mm or more, color dark, often metallic. Metasoma slightly joined to propodeum (Handbook Of Nearctic Chalcidoidea). Mid-tibia tapered, short and slender (Ferriere and Kerrich, 1958)…….(Eulophidae) 6

4. Tarsi 5 segments, club antennae usually 2-3 segments, rarely full. Forewings lack speculum, with submarginal vein not enlarged, no postmarginal, rarely developed (Shafee and Rizvi, 1990)……..(coccophaginae foerster, 1878) 5

5. The antennae are distinct and have various shapes, the club is generally distinct from the funicle. Marginal veins generally longer than costal sell (Shafee and Rizvi, 1990)……...(Encarsia Foerster, 1878)

6. Forewings usually with evenly distributed setae from posterior marginal vein, without row of setae, rarely with "line of setae" radiating from stigma, but when visible usually only a line, postmarginal vein visible. (Handbook of Nearctic Chalcidoidea)……(Eulophinae) 7

7. Antennal funicles 4 segments, head and thorax entirely colored, propodeum without lateral median teeth, forewings with small stigma (Askew, 1968)…….(Elachertus)

8. Forewings with nearly parallel sides, hardly broader near the apex. Tarsi 4 segments, antennal funicles usually 4-6 segments (Lin *et al.*, 2007). Gastral petiole indistinct, gaster broadly joined to mesosoma, petiole dilated (Beardsley, 2000)…….(Cleruchus)

9. Antenna 16 segments, forewing generally (80%) without 1Rs cells or if 1Rs cells are present, then cells are not very broad, Rs venation does not reach wing margin as tubular venation. On the hind wing almost always without venation 2m- cu (Goulet and Huber, 1993)……...(Microgastrinae) 10

10. The front wing has an aerolet with a triangular or rectangular shape (Figure 4.1.h) and a short ovipositor (Fakhrudin and Inayatullah, 2015)……(Microplitis Forester)

11. Forewings without aerolets, vannal lobe on hind wings concave and ovipositor sheaths all hair (Fakhrudin and Inayatullah, 2015)……..(Apanteles Forester)

Key to the determination of Diptera parasitoids of whitefly (Bemisia tabaci)

1. Wings with Rs venation are more obvious or equal to other venation, wings with m venation are distinct, sometimes faint. Tarsi on segments 1 is longer than the tarsi of segment 2, tarsi less than 5 segments.

Ovipositor lamellae 1-3 segments. Veins M1+2 branched with further branched portions of the trunk, veins cu separate (Hackston, 2013a)……(Lestremiinae) 2

2. Venation of M1-M2 forms a fork, M3+4 does not form a fork, both r-s and sub brief r-m equal length M3+4 free of M. ocelli 2 or absent, center of fork longer than stem (Yukawa, 1971)……(Lestremiini) 3
3. Flagellum antennae 14 segments (Yukawa, 1971). In males the antennae are 16 segments, on the 3rd segment backwards with a clear narrow section between segments, female antennae 11-13 segments. Antennae have simple sensory hairs on the 3rd segment onwards. Wings with Cu1 venation extending back to wing base, at least up to the r-s vein (Hackston, 2013b)……...(Lestremia)

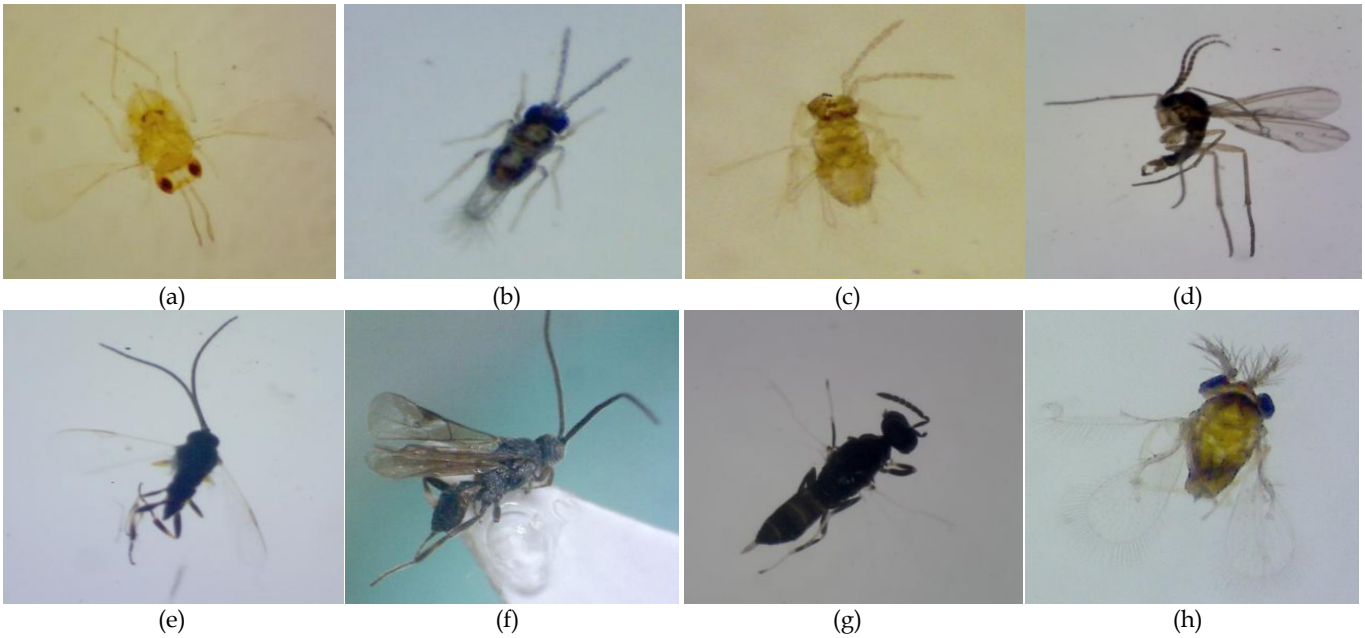

Figure 3. Parasitoids obtained *(a) Encarsia* sp., (b) and (c) *Cleruchus* sp., (d) *Lestremia* sp., (e) *Apanteles* sp., (f) *Microplitis* sp., (h) aerolet on the wing of *Microplitis* sp., (i) *Elachertus* sp., (j) *Trichogrammatidae*

Species diversity

Table 3. Species diversity of whitefly parasitoids (*Bemisia tabaci*) on edamame plants

Region	Diversity species (H')	Category
Kranjingan 1	0,03	Low
Kranjingan 2	0,07	Low
Kranjingan 3	0,04	Low
Jenggawah 1	0,33	Low
Jenggawah 2	0,33	Low
Jenggawah 3	0,33	Low
Cangkring 1	0,26	Low
Cangkring 2	0,27	Low
Cangkring 3	0,18	Low

Based on the research that has been done, species diversity in the areas that have been sampled shows low diversity. Because in all areas shows the value of H' <1 this can be shown in table 3. Low species diversity indicates that only a small number of parasitoid species were found in the area. The lowest diversity was shown in Kranjingan 1 with an H' value of 0.03, followed by Kranjingan 3, and Kranjingan 2 with H' values of 0.04 and 0.07, respectively. While the highest diversity value is shown in Jenggawah 1, Jenggawah 2 and Jenggawah 1 with the same H' value of 0.33 although it is still categorized as low species diversity.

Species abundance

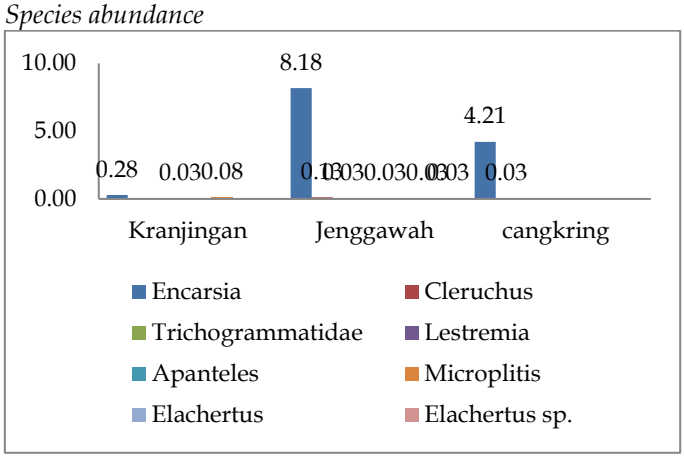

Figure 4. Graph of the abundance of whitefly parasitoid species (*Bemisia tabaci*) on edamame plants

Based on Figure 4 the abundance of parasitoid species *Encarsia* sp. looks evenly distributed in all sampling areas, although in the Kranjingan area the population of parasitoid can be seen very abundant in the field. While in other parasitoid species such as *Cleruchus* sp., *Trichogrammatidae*, *Lestremia* sp., *Apanteles* sp., *Microplitis* sp. and *Elachertus* sp. the abundance of species can be said to be low. The abundance of species obtained in *Encarsia* sp. in the Kranjingan, Jenggawah and Cangkring areas was 0.28; 8.18 and 4.21, respectively. In *Cleruchus* sp. is 0; 0.13 and 0.03. In *Trichogrammatidae* is 0; 0.03 and 0. In *Lestremia* sp. were 0.03; 0 and 0. In *Apanteles* sp. are 0' 0.03 and 0. On *Microplitis* sp. are 0.08; 0 and 0. In *Elachertus* sp. were 0; 0.03 and 0. But overall the highest species abundance was shown in the Jenggawah collection area.

Index of Similarity

The similarity index can be used to determine the level of similarity of parasitoid species in two different regions. So that it can be known in both regions, which species appear a lot. From research which has been done, index similitude parasitoids that the highest is in the area comparison between Jenggawah and Cangkring with the percentage value of the similarity index >50%, which is 57%. While the lowest parasitoid similarity index in the comparison of Kranjingan area with Jenggawah is 25% which shows the percentage of similarity index <50%, so it can be categorized as low.

Table 4. Similarity index of whitefly parasitoids (*Bemisia tabaci*) between communities/regions in edamame crops

Region		Similarity Index of whitefly parasitoids (%)	Category
Kranjingan Jenggawah	-	25	Low
Jenggawah Cangkring	-	57	High
Cangkring Kranjingan	-	40	Low

Parasitation Percentage

The percentage of parasitation can be used to determine the high and low attack of parasitoids on their host insects in an area. Based on the research that has been done, the parasitoid parasitation rate in one region with other regions looks different. The highest parasitation percentage occurred in the Jenggawah area with a total parasitation percentage of 69.29%, precisely in Jenggawah 1 (J1) which is with a parasitation percentage of 28.61%. Followed by Jenggawah 3 (J3) 21.52% and Jenggawah 2 (J2) 19.16%. This shows that in the Jenggawah area the level of parasitoid attack can be said to be high compared to other areas. While the area with the lowest parasitation rate is in the Kranjingan area with a total parasitation percentage of 0.23%, namely in Kranjingan 1 (K1) 0.05%, Kranjingan 2 (K2) 0.11%, and Kranjingan 3 (K3) 0.07%, so it can be said that the level of parasitoid attack on whitefly (*B. tabaci*) in the Kranjingan area is low.

Table 5. Total population of whitefly (*Bemisia tabaci*) and parasitoids obtained during rearing

Region	Number of whitefly (*Bemisia tabaci*)	Number of Parasitoids
Kranjingan 1	6.049	3
Kranjingan 2	7.140	8
Kranjingan 3	7.217	4
Jenggawah 1	381	109
Jenggawah 2	574	105
Jenggawah 3	446	113
Cangkring 1	2.939	63
Cangkring 2	2.905	69
Cangkring 3	1.578	33
Total	29.229	507

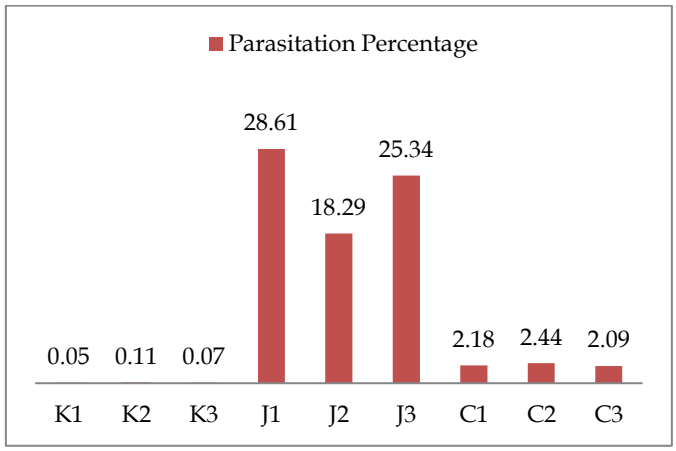

Figure 5. Graph of parasitation percentage of whitefly parasitoid (*B. tabaci*) on edamame plants

Based on the Table 5, it is known that parasitoids were found in all sampling areas. However, the highest parasitoid population was obtained in the Jenggawah sampling area, followed by Cangkring and the last in the Kranjingan sampling area with the number of parasitoid individuals 327, 175, and 15, respectively. High and low populations can be influenced by abiotic factors such as temperature, rainfall and altitude. From the table above, it can be seen that the Kranjingan sampling area has moderate rain intensity and the Jenggawah and Cangkring areas have light rain intensity. Because according to BMKG, (2016) monthly rainfall < 300 mm = light rain intensity, monthly rainfall 300-500 mm= moderate-heavy rain intensity, and monthly rainfall > 500 mm = very heavy.

Conclusion

This study identified parasitoid species associated with two key pests of edamame soybean: *Bemisia tabaci* and *Etiella zinckenella*. A single Hymenoptera parasitoid (Braconidae) was found in soybean pod borers with a low parasitization rate (0.06%), likely due to intensive pesticide use. In contrast, whitefly populations yielded 507 parasitoid individuals from six families of Hymenoptera and one family of Diptera, with Encarsia sp. (Aphelinidae) being the most abundant. Despite high parasitization rates in Jenggawah (up to 69.29%), overall species diversity remained low. These highlight the potential of parasitoids as biological control agents and the need to reduce chemical pesticide reliance to enhance sustainable pest management in edamame cultivation.

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Author Contributions
Hari Purnomo conceptualized and supervised the research. Intan Nur Rohma and Wildan Muhlison conducted field sampling and laboratory analysis. Irwanto Sucipto performed data processing and statistical analysis. All authors contributed to writing and reviewing and the manuscript and approved the final version.

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Conflicts of Interests
The authors declare no conflict of interest.

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