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Egg Parasitoid Complex *of Helicoverpha armigera* (Hübner) and *Conopomorpha cramrella* Snellen in North Sulawesi

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ABSTRACT

Backround: *Helicoverpa armigera* is major maize pest, while *Conopomorpha cramerella* is cocoa is main insect pest. Their larvaes lie in corn cob and cocoa pulp which can be controlled using egg parasitoids.

Methods: Exploring *H. armigera* eggs on female flowers on a corn plant along the 50 m line at 10 points with a 4 m distance to obtain parasitoids. *C. cramrella*. Egg parasitoids were obtained by taking eggs from cacao pod, while *Corcyra cephalonica* from egg traps along a straight line with 10 points with 3 m distance.

Result: *H. armigera* egg parasitoids include *Trichogramma* sp. and *Trichogrammatoidea armigera*. Corn grown in between coconut palms has higher parasitism than without coconut palm shade and highest parasitism was in Bolaang (60.91±2.52 %). Parasitoids of *Trichogaramma* sp. produce unrearable female offsprings in *C. cephalonica* eggs. Dominant *Trichogrammatoidea* sp. were found in egg traps while only two *C. cramerella* parasitized eggs were found in cacao pod. Dominant *Trichogaramma* sp. Parasitoids in egg traps were found in Tombariri. *C. cephalonica* egg trap is the latest method for monitoring *Trichogrammatoidea* sp parasitoid The parasitism of *Trichogrammatoidea* sp. on *C. cramerella* eggs and *C. cephalonica* egg traps is low.

Key words: *Conopomorpha cramerella, Helicoverpha armigera*, Parasitism, Parasitoid.

INTRODUCTION

Helicoverpa armigera (Lepidoptera: Noctuidae) is an important pest in crops in Asia, Europe, Africa and Australia, with an estimated damage of billions of dollars per year (Tay, 2013). Corncob borer, *H. armigera* is still a major pest in corn plants in North Sulawesi. This pest caused 51.9-53.4% decrease in harvest in Sulawesi Island (Karim *et al*., 2013). Insecticides were inadequate in controlling *H. armigera* because the larvae are in corn cobs. Egg parasitoids can be alternative control against this pest. There are nine types of egg parasitoids infecting *H. armigera* including *Trichogrammatoidea armigera*, *Trichogramma chilonis* and *Trichogramma ochaeae* (Alba, 1988; Reddy and Manjunatha, 2000). *Trichogramma* sp has shown promising results; however, *H. armigera* parasitoids distribution in North Sulawesi is unknown.

Indonesia is the world's third-biggest cocoa producers after Ghana and Ivory Coast. However, the production is still low of below 1.0 ton/ha mainly due to cocoa pod borer, *Conopomorpha cramerella* (Lepidoptera: Gracillariidae) (Anonim, 2016). *C. cram*erella in cocoa plantations in North Sulawesi causes damage with an intensity of up to 40% (Kandowangko *et al*., 2015). The distribution of parasitoids in North Sulawesi must be indentified prior to the initial stage of control.

In Indonesia, egg parasitoids of corn cobs and cocoa pod borer have not been reported. Hawkins *et al*. (1997) stated that parasitoids were studied and showed good results in pest control compared to predatory insects. Information about egg parasitoids that parasitize pest eggs on crops is still very limited. Meanwhile, agricultural fields often use synthetic pesticides that cause parasitoid extinction. Thus, it is necessary to study the parasitoids of *H. armigera* and *C. cramerella* for biological control programs.

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MATERIALS AND METHODS

Location description

The egg parasitoids survey of *H. armigera* of corn (*Zea mays* L) and *C. cramerella* on the cocoa plant (*Theobroma cacao* L) was carried out in North Sulawesi (Table's 1 and 2). The survey was conducted from April to August 2018.

H. armigera egg sampling procedure

Egg collection from the female flowers of corn was carried out in a straight line along 50 m at 10 points with a 4 m distance. For egg collection, two corn plants with 80-90% of pink female flowers and the age of 45 days after planting (DAP) were taken at every point. Followed at the age of 55 DAP that generally female flowers are brownish. In each location, four plant plots were determined to obtain the eggs of *H. armigera*. There were two locations in each district and each location sized ± 1.0 ha. In Bolaang, Poigar and Bolaang, the collection of *H. armigera* eggs was carried out to the corn plan with and without coconut shade. The study locations belong to the farmer and was not given insecticide.

H.armigera egg parasitoid

Eggs were put into test tubes (1.5 cm × 15 cm) until they hatch into parasitoids. In each test tube, 3-5 eggs were put and bred for 10 days at morning temperatures 07.00 WITA (central Indonesian time) of 26,71±0,611°C and during the day 14.00 WITA of 28,05±0,86°C. The observations were made every day to determine changes in parasitized eggs and healthy unhatched eggs. The parasitoid obtained from the eggs were collected for identification.

$$\frac{\text{Parasitism: Number of parasitized eggs}}{\text{number of eggs}} \times 100\%$$

Rearing parasitoid

The rearing of *Trichogramma* sp. parasitoid and *C. cephalonica* in *C. cephalonica* was done by putting 30 eggs aged 0-24 hours per test tube and simultaneously putting two male individuals and three female individuals of *T. armigera* parasitoid and only female *Trichogramma* sp parasitoid. This experiment was repeated three times. Observations were made every day to find out parasitoid reproduction in the laboratory.

The use of _C. cephalonica_ eggs as a trap

C. cephalonica eggs were irradiated with ultraviolet for 45 minutes to inhibit the development of the embryo not to hatch into larvae until they are bred in the laboratory. *C. cepahlonica* eggs are glued to a green plastic card (2 cm × 3 cm) to be placed on the cacao plant. Eggs are used as parasitoid traps from 0 to 24 hours.

A sampling of _C. cramerella_ eggs and egg traps

Survey of *C. cramerella* egg parasitoids was carried out in two ways, namely taking cocoa fruit with 10 cm long and *C. cephalonica* egg traps. In each district, one location was determined for collecting cocoa and egg traps at 10 points in a straight line with 3 m distance between points. If the length was insufficient, it was deflected back to the original distance with a distance of one line of cacao tree. Two plots of the cocoa and egg traps were determined in the research location. There were 2-3 cocoas picked per tree. *C. cramerella* eggs were taken with the help of a binocular microscope. Eggs were bred in the laboratory to obtain parasitoids. The cocoa plant used was 5-6 years old. The parasitoid survey was carried out 4 times at intervals of 2 weeks.

For the egg trap, 25 plastic cards containing 20 eggs of *C. cephalonica* were put in each location. 2-3 plastic cards were put in each point on the cocoa plant. A plastic card was stuck into the cocoa fruit with 10 cm long. The edge of the card was smeared with lime to avoid ants. After four days, the plastic card was taken back and incubated for 10 days and observed every day to find out the changes in parasitized eggs.

$$\frac{\text{Parasitism: Number of cards containing parasitized eggs}}{\text{number of cards containing eggs}} \times 100\%$$

Table 1: Survey area description of egg parasitoids of *H.armigera.*

Province	Regency/City	District	Coordinate	Altitude
North Sulawesi	South Minahasa	Motoling	06°64′20.1″N 01°02′02.2″E	421 m
	Minahasa	Kakas	01°13′11.6″N 124°12′50.5″E	546 m
	Kota Tomohon	Tomohon Timur	07°05′79.9″N 01°48′62.6″E	779 m
	Bolaang Mongondow	Poigar	00°98′97.2″N 124°26′79.8″E	17 m
		Bolaang	00°86′63.4″N 124°12′33.2″E	26 m
		Dumoga Timur	00°57′96.6″N 124°01′05.4″E	159 m

Tabel 2: Survey description of egg parasitoid of *C. cram*erella.

Province	Regency	District	Coordinate	Altitude
North Sulawesi	Bolaang Mongondow	Passi	00°75′58.9″N 124°29′99.9″E	133 m
		Poigar	06°39′00.3″N 01°07′81.6″E	21 m
	Minahasa	Tombariri	06°79′232″N 01°55′08.1″E	54 m

Parasitoid identification and statistical analysis

The identification of parasitoids was based on morphological characters using insect identification keys. The parasitism of egg parasitoid was analyzed descriptively and Chi-square test performed by SPSS version 23.

RESULTS AND DISCUSSION

***H.armigera* egg parasitoid**

H. armigera egg parasitoids consist of *Trichogramma* sp. and *T. armigera. Trichogramma* sp. parasitoid is a solitary parasitoid, whereas *T. armigera* is a solitary and gregarious one. *Trichogramma* sp. produces female offspring while *T. armigera* produces male and female ones. *Trichogramma* parasitoids were not found Kakas, Tomohon Timur and, Motoling. Conversely, *T. armigera* was found in all locations. *Trichogramma* sp. was not parasitized *C. cephalonica* host eggs, however, the parasitoid *T. armigera* can parasitize *C. cephalonica*. *Trichogramma* sp. parasitoid is included in Thelytokous parthenogenesis. Huigens and Stouthamer (2003) stated that Wolbachia is common in female insects, female parasitoids infected with Wolbachia happen to the thelytoky parasitoid and female offspring. Hoffmann *et al.* (2001) added that females infected with Wolbachia bacteria produce fewer female offspring than uninfected parasitoids. The population of *T. kaykai* parasitoids infected with Wolbachia is low (Russel *et al*., 2018).

The population of *Trichogramma* sp. was 2-8 individuals in each egg sampling, while more than 10 individuals of *T. armigera* were taken. *T. armigera* parasitosoid is a reddish-brown parasite with the dark brown middle coxa. Its thorax is dark brown with a darker abdomen. Its antenna is yellow with the last club-shaped segment, the front wing has a slight trichia and long fringe setae. Its female length is 0.42 mm± 0.02 mm and 0.39 mm±0.02 mm for the male. *Trichogramma* sp. yellowish color, head, thorax, yellow antenna and the last segment is club-shaped. The abdomen has a brown transverse strip and a brown abdomen tip. The front wing has many trichia and fringe setae rather long and the length of the female (0.49 mm±0.02 mm). Parasitic eggs turn black, while healthy eggs turn yellowish-white to hatch into larvae. The Chi-square analysis of parasitism for those without coconut palm shade was not significantly different from 45 DAP (÷2 =9.600; p>0,087), but 55 DAP was different (÷2 =15.222; p < 0,009) (Table 3).

Parasitism data provided a significant influence for corn cobs borer since, during the research, severe damage to the cobs had not been found. Parasitoid surveys without shading had a higher tendency of parasitism at 45 DAP. 55 DAP parasitism was low, female flowers might have 40-50 % dry and turned brownish to black. Thus, it might not be attractive for parasitoids to find the eggs. Around the study location, corn plants with red female flowers were found, which easily attracted the parasitoids. Red female flowers in *H. armigera* population tends to be higher than brown female flowers.

The highest parasitism without coconut shade in Bolaang (36.46±5.18 %). *H.armigera* population in Bolaang is relatively high compared to other locations. Thus, it is concluded from this experiment that parasitism correlated with the number of host eggs. Population density of the host is an important aspect that influences the high parasitism (Montoya *et al*., 2000; Jones *et al*., 2003). Romeis *et al.* (1999) said that *Trichogramma* sp. parasitism to *H.armigera* eggs depends on the location and number of host eggs. Generalist parasitoid is higher in polyculture plantations compared to monocultures (Menalled *et al*., 1999). Unlike in Kakas, the number of eggs tends to be high, but the parasitism of *T. armigera* is not maximal. The laboratory experiment revealed that parasitoid efficiency decreases with a decrease in temperature (Mills and Getz, 1996). Besides egg density, the difference in parasitism is also influenced by microclimate, geographical location and plant cultivation practice. The Results of parasitism indicated that changes in weather factors as a result of climate change would have considerable influence on survival and development of parasitoids (Kuzhandhaivel *et al*., 2016).

Parasitism for those with coconut palm shade is higher than without those shade. Parasitism with the highest shade is in Bolaang (60.91±2.52%) (Table 4). In Bolaang, in addition to coconut plants, banana and mango trees are found in maize plantations that also affect microclimate. Liu *et al.* (2016) stated that heterogeneous landscapes could effectively increase *H. armigera* parasitoids in cotton plants. Data obtained suggest that the ecological influence for biological control through conservation is needed for non-corn plants. In Kakas, Tomohon and Motoling, corn was planted without shade, because of the lack of coconut plantations. The Chi-square analysis showed significant differences in parasitism of the 45 DAP (÷2 =20.397; p< 0,000, 55 DAP ÷2 =10.047; p<0,007) (Table 4).

Table 3: The parasitism *H.armigera* egg in the corn plant without coconut palm shade.

Location	Parasitism (%)	
	45 DAP	55 DAP
Poigar	17.09 ±1.72	9.79±0.81
Dumoga Timur	20.46 ±3.23	16.78±6.49
Bolaang	36.46 ±5.18	29.60±3.25
Motoling	30.11±14.29	22.28±9.74
Kakas	23.72±9.97	17.92±6.43
Tomohon Timur	23.45±4.06	10.84±5.90

Table 4: Parasitism of *H.armigera* eggs in corn plants with coconut palm shade.

Location	Parasitism (%)	
	45 HST	55 HST
Poigar	23.45±3.81	22.55±1.01
Dumoga Timur	31.86±3.23	19.97±1.41
Bolaang	60.91±2.52	42.47±10.82

Table 5: Eggs are parasitized on plastic cards and parasitized eggs.

Location	Plastic cards containing parasitized eggs (%) (observation)				Parasitized eggs (%) (observation)			
	I	II	III	IV	I	II	III	IV
Passi	0	4	0	0	0	1.6	0	0
Poigar	0	0	8	0	0	0	2.6	0
Tombariri	8	0	4	8	3.2	0	0.8	2.4

The shade density of coconut palm plants in Bolaang is irregular (3 m × 3 m and 3 m × 4 m), while in Poigar it was 6 m x 6 m, in East Dumoga Timur was 5 m × 5 m and 5 m × 6 m. Shade density in Bolaang is denser, thus affecting the microclimate to be cooler and temperatures to be lower, this has an impact on parasitism being high. Microclimate change causes the temperature of maize polyculture to be lower, while without shade the temperature becomes high. Change in microclimate is shown in tea plants, air temperature of tea plants without a shade is 27°C and with the shade of 24°C, humidity is 48% to 74% (Widayat and Rayati, 2011). Geetha and Balakrishnan (2010) stated that cooler microclimates affect *T. chilonis* parasitism and better spread.

C. cramerella egg parasitoids

C. cramerella egg parasitoids are *Trichogrammatoidea* sp, obtained through *C. cramerella* and *C. cephalonica* egg trap. However, there were only two *C. cramerella* eggs we parasitized from 4 times of cacao sampling. The parasitoid morphology of *Trichogrammatoidea* sp. on *C. cramerella* eggs is the same as *C. cephalonica* egg traps. The parasitoid is brownish yellow, the head is brownish yellow and the abdomen has dark patches, the antenna is with a long tassel and the middle coxa is dark brown. Female antennas are in the form of a club, whereas males are not big, but have long hair. The front wing with fringe setae, long tornus section and little trichia on a remigium. The *C. cramerella* egg parasites are *Trichogrammatoidea bactrae fumata, Trichogrammatoidea cojuangcoi* and *Trichogramma chiolonis.* (Alias *et al.*, 2004).

Parasitism shows that the population of *Trichogrammatoidea* sp. in cocoa plantations is relatively low. The effective monitoring of these parasitoids is the *C. cephalonica* egg traps. In Passi and Poigar, the *Trichogrammatoidea* sp. parasitoid through *C. cramerella* eggs have not been found. It was found in Tombariri. *C. cramerella* 's parasitized eggs turn from orange to black (Table 5).

C. cephalonica egg trap is an active trap that uses an agent to attract parasitoids in yellowish-white color. Traps are active with chemical and physical stimuli in the forms of light, color, or chemical compounds making insects are attracted to the trap (Yi *et al.,* 2012). The reported parasitism on the card containing *C. cephalonica* eggs is very low. Low levels of *C. cephalonica* eggs parasitized reported by Kandowangko *et al*. (2015). All parasitized *C. cephalonica* eggs that are bred can become imago parasitoids. However, the dominant population is male as an indication of low parasitism since the parasitoid that parasitizes the egg is female. *Dolichodorus* sp, *Oecophyla* sp., *Iridomyrmex* sp and spiders which are predators of *C. cephalonica* eggs were found in cocoa plant ecosystems. Thus, it consequently affected the low parasitism.

C. cephalonica eggs can be used to monitor *C. cramerella* parasitoids. The cost for *C. cephalonica* egg traps is relatively cheaper than cocoa taking. Besides that, the parasitized eggs of *C. cephalonica* are more easily detected. In Malaysia, to obtain information on *T. bactrae fumata,* yellow and green cylindrical contacts are used. Green is more attractive than yellow (Azhar and Long, 2004). The use of the cylindrical trap method is inappropriate since the trapped parasitoids cannot be bred as they have died.

CONCLUSION

T. armigera parasitoid has spread to maize crop, except for *Trichogramma* sp that is only limited to a few locations and produces female offspring. Increased parasitism of *Trichogramma* sp. and *T. armigera* to *H. armigera* eggs is found in coconut palm shade with the highest in Bolaang (60.91±2.52%). Whereas without the shade of coconut palm, the parasitism was highest in Bolaang (36.46±5.18%). The older female flowers the more decrease the parasitism.

Trichogrammatoidea sp. parasitoid is found in all locations but predominantly found in *C. cephalonica* egg traps compared to *C. cramerella* eggs. The egg trap is the latest breakthrough for parasitoid monitoring of *Trichogrammatoidea* sp. *Trichogrammatoidea* sp. parasitism is still considered very low.

Conflict of interest: None.

REFERENCES

Alba, M.C. (1988). Trichogrammatids in the Philipines. Philipp. Entomol. 7(3): 253-271.

Alias, D.I., Azhar, M. and Schilthenizen (2004). Taxonomic status *Trichogrammatoidea bactrac fumata* Nagaraja, an eggs parasitoid of the cocoa pod borer, *Conopomorpha cramerella* in Malaysia. Malaysian Cocoa Journal. 1: 88-90.

Azhar, I. and Long, G.E. (2004). Dispersal of *Trichogrammatoidea bactare fumata* Nagaraja (Hymenoptera: *Trichogrammatidae* after releases in a Malaysian Cocoa Field. Malaysian Cocoa Journal. 1: 40-45.

Anonim. (2016). Outlook of Agricultural Commodity, Agricultural Subsector, Center of Agricultural Data and Information. Ministry of Agriculture, Jakarta.

Geetha, N. and Balakrishnan R. (2010). Dispersal pattern of *Trichogramma chilonis* Ishii in sugarcane field. Journal of. Biological Control. 24(1): 1-7.

Hawkins, H.H., Cornell, H.V. and Hochberg. (1997). Predator, Parasitoid and Patogen as mortality agens in phytophagous insect population. Ecology. 78(7): 2145-2152.

Hoffmann, M.P., Ode, P.R., Walker, D.L., Gardner, J., Nouhuys, S.V. and Shelton, A.M. (2001). Performance of *Trichogramma ostriniae* reared on factitious hosts, including the target host, *Ostrinia nubilalis*. Biological Control. 10: 1-10.

Huigens, M.E. and Stouthamer, R. (2003). Parthenogenesis associated with wolbachia. In: Insect Symbiosis. [Bourtzis, K., Miller, T.A. (eds)]. Boca Raton: CRC Press. 247-266.

Liu, B., Yang, L., Yang, F., Wang, Q., Yang, Y.Z., Lu, Y.H. and Gardiner, M.M. (2016). Landscape diversity enhances parasitism of cotton bollworm (*Helicoverpa armigera*) eggs by *Trichogramma chilonis* in cotton. Biological Control. 93: 15-23.

Kandowangko, D., Rimbing, J., Assa, B. and Memah V. (2015). Investigation and resaerch on cocoa pod borer in North Sulawesi. Faculty of Agriculture Unsrat Manado.

Karim AI., Iswati R. and Zakaria F. (2013). The level of pod borer attack (*Helicoverpa armigera* Hubner) against maize Bisi-2 variety and local Motorokiki variety. Gorontalo (ID): Universitas Negeri Gorontalo.

Kuzhandhaivel, A.P., Selvaraj, S. and Agnihotri, M. (2016). Seasonal abundance of *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae), A larval parasitoid of *Helicoverpa armigera* (Hubner) hardwick in chickpea. Legume Reasearch. 39(4): 643-647.

Jones, D.B., Barberet, R., Giles, K.L. and Royer, T.A. (2003). Fuctional response of an introduction parasitoid and indigenous parasitoid on greenbug at four temperature. Enviromental Entomolology. 32: 425-432.

Menalled, F.D., Marino, P.C., Gage, S.H. and Landis, D.A. (1999). Does agricultural landscape structure affect parasitism and parasitoid diversity. Journal Ecological Society America. 9 (2): 634-641.

Mills, N.J. and Getz, W.M. (1996). Modelling the biological control of insect pests: A review of host-parasitoid models. Ecological Modelling. 92: 121-143.

Montoya, P., Liedo, P., Benrey, B. and Barrera, J.F. (2000). Functional response and superparasitsm by *Diachasmimorpha longicaudata* (Hymenoptera: Brachonidae) a parasitoid of fruit flies (Diptera: Tephritidae). Annals of the Entomological Society of America. 93: 47-54.

Reddy, G.V.P. and Manjunatha M. (2000). Influence of host plants on parasitism of *Helicoverpa armigera* (Lepidoptera: Noctuidae) by two egg parasitoids, *Trichogramma chilonis* and *Trichogramma achaea*. International Pest Control. 41(6): 223-225.

Romeis, J., Shanower, T.G. and Zebitz, C.P.W. (1999). *Trichogramma* egg parasitism of *Helicoverpa armigera* on pigeonpea and sorghum in southern India. Entomologia Experimentalis et Applicata. 90(1): 69-81.

Russell, James, E., Nunney, L., Saum, M. and Stouthamer, R. (2018). Host and symbiont genetic contributions to fitness in a *Trichogramma*-Wolbachia symbiosis. Peer J. DOI: 10.7717/peerj.4655. pp 1-20.

Tay, W.T., Soria, M.F., Walsh, T., Thomazoni, D. and Silvie, P. (2013). A brave new world for an old world pest: *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Brazil. PLoS ONE 8(11): e80134. https://doi:10.1371/journal.pone.0080134.

Yi, Z., Jinchao, F., Dayuan, X., Weiguo, S. and Axmacher, J.C. (2012). A comparison of terrestrial arthropod sampling methods. Journal of Resources and Ecology. 3(2): 174-182.

Widayat, W. and Rayati, D.J. (2011). The effect of permanent shade tree at mature tea area on microclimate, the population of pest insects and natural enemies and tea shoot production. Journal Penelitian Teh dan Kina. 14(1): 1-7.