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The Release of *Trichogrammatoidea armigera* parasitoid for the Parasitization of *Helicoverpa armigera* Eggs in Maize

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Abstract

Background: *Helicoverpa armigera* (Hübner) is a common polyphagous pest on corn cob. *H. armigera* that is controllable through *Trichogrammatoidea armigera* (Nagaraja) parasitoid release. Successful inundated release of parasitoids depends on parasitization and parasitoid dispersal.

Methods: The experiment consisted of 2 parasitoid release plots and 1 no release plot. *T. armigera* parasitoid was released twice, at 45 DAP and 52 DAP (Day after planting) in the middle of the experimental plot. The dose of parasitoid released during the corn generative phase was 1000 individuals per 254 m^2 (~40000/ha). Evaluation was carried out by collecting the eggs of *H. armigera* 3 days after release at radius 1, 3, 6 and 9 m in all cardinal directions from the release point.

Result: The release of parasitoids increased the eggs parasitization of *H. armigera* in 45 DAP tot he tune of 51.91% and 52 DAP was 38.57%. The wind direction did not affect the parasitoid movement which is active and almost uniform in all cardinal directions. Parasitoid movement was slightly similar in all distances and cardinal directions. The distribution of parasitoids reached 9 m from 40 release points ha^{-1} in colonies. *T. armigera* parasitoids were grouped hence, the monitoring in corn was carried out systematically.

Key words: Corn plants, Dispersal, Egg parasitoid, *Helicoverpa armigera*, *Trichogrammatoidea armigera* parasititazion.

Introduction

The main problem in corn cultivation is controlling *Helicoverpa armigera* pest, a polyphagous that mainly attacks soybean, tomatoes and cotton. *H. armigera* larvae damages corn cobs and feeds internally thus makes it difficult to control using insecticides. However the moths of *H. armigera* lay eggs on cobs silk and amenable for egg parasitisation. Egg parasitoid *Trichogrammatoidea armigera* has been found effective in controlling this pest. The parasitoid is broadly found in corn plantations in North Sulawesi, naturally 13.1 to 18.6% parasitisation but it has not been used as a controlling agent (Rimbing *et al.*, 2013).

The systematic study in this region is not made by inundative release of this parasitoid to establish how much it will control the pest. How parasitoid *T. armigera* controls *H. armigera* should be examined to assess the inundation release of the parasitoid in controlling *H. armigera*. Parasitoids can control specific pests and suppress pest populations. Successful parasitoids release requires data on parasitization and parasitoid behavior.

In North Sulawesi, Major egg parasitoid on the eggs of *H. armigera* is *Trichogrammatoidea armigera* in maize (Rimbing *et al.*, 2013). Hassan (1993) stated that local parasitoid species are better because these species have adapted to the local environment. Selecting a parasitoid strain is an important step for successful biological control (Hassan 1993; Sigsgaard *et al.*, 2017). Each parasitoid strain showed different levels of parasitization in the same host species.

Released parasitoid dispersal determines the success that the distance dispersal radius should be measured to determine the number of parasitoid release spots, distribution pattern of the parasitoid and the dose of the parasitoid can be determined for more effective results (Knipling 1992). Inundated release of parasitoids performance if governed by parasitoid behavior, its ability of the parasitoid to find a host, the pattern of distribution of the parasitoid, the time of release and the dose of the released parasitoid (Hassan 1993; Jalali and Singh, 2006; Marwoto 2010). Dispersal pattern holds essential practical and theoretical importance (Southwood and Henderson, 2000). To date, proper strategy for optimal release of parasitoid *T. armigera* remains unknown. This study examined the parasitoid ability of *T. armigera* to *H. armigera* eggs after release, distribution of parasitoids and distribution patterns of parasitoids.

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Materials and Methods

Setting

Parasitoids were released to corn plants in Minahasa, North Sulawesi, Indonesia at an altitude of 727 meters above sea level. The point of release was at 01°18′17.2″N and 12°48′

54.04″E. The temperature in the Entomology Laboratory of the Faculty of Agriculture at 07.00 AM was 26.71±0.611°C and 28.05±0.86°C at 02.00 PM. The experiment was carried out from May to October 2016.

T. armigera parasitoid propagation

Green beans granules were ground as *Corcyra cephalonica* (Stainton) feed (Rimbing *et al*., 2013). *H. armigera* eggs collected from the field were incubated in the laboratory. The egg parasitoid hatch out from incubated eggs were utilized to parasitize *C. cephalonica* eggs from the first day and continuously cultured on *C. Cephalonica* eggs until the 7th generation. *T. armigera* took 1-2 days to parasitize *C. cephalonica* eggs. Black *C. cephalonica* eggs that hatched were put into glass tubes with 5 × 20 cm in diameter and 10% honey was smeared as food for the parasitoids. ± 100 UV irradiated (for 45 minutes) eggs of *C. Cephalonica* were glued on 2 × 8 cm white cardboard and then kept in a glass tube containing parasitoids.

Parasitoid release on corn plants

Two parasitoid release plots and one no parasitoid release plot were mentained. The distance between parasitoid release plots with no release was ± 100 m, while the distance between release plots was 50 m. in a hectare corn crop, then demarked into a circle area of 254 m^2 as an experimental plot. The release was made at the center of the experimental plot. *Helicoverpa. armigera* eggs were collected at 4 radial distance of 1, 3, 6 and 9 m from the parasitoid released point with four replications at the east, west, south and north areas of both parasitoid released and unreleased plot (Fig 1).

The release of 1000 individual parasitoids per 254 m^2 was carried out twice during the generative phase shown by corn silk on the cob. The first release was on 45 DAP and the second was on 52 DAP at 06.30 AM. Cardboard of 2 × 8 cm in size containing parasitized *C. cephalonica* eggs was inserted in a pipe which end was covered with perforated gauze for the parasitoid to come out, while the other end was covered with cloth without the parasitoid imago coming out. Pipes of 5 cm in diameters and a length of 40 cm were

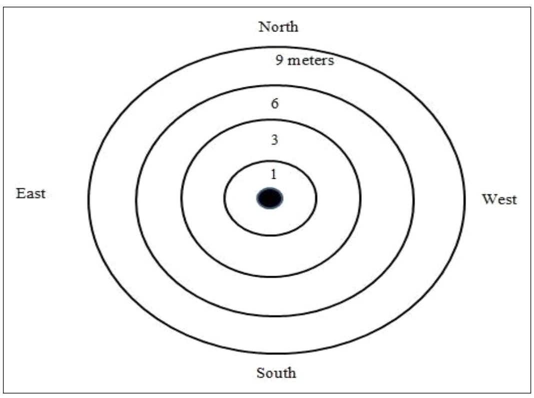

Fig 1: *T. armigera* parasitoid distribution map.
●=Parasitoid release point.

placed in the center of the experimental plot using bamboo as support poles of 1.30 m height where pipes were put. After the release of the parasitoids, within 3-day interval, *H. armigera* eggs were collected from each radius distance. Six plants with corn silk were selected at each radius distance to obtain *H. armigera* eggs, while at a radius of 1 m, only 2 plants were taken due to insufficient population. *H. armigera* eggs from collected cobs were brushed from corn silk in a pattriplate and observed for parasitoid emergence. in the laboratory. The parasitization rate of *H. armigera* eggs is described as follows:

$$P = \frac{n}{N} \times 100\%$$

Where:
P = Parasitization.
n = Number of parasitized eggs.
N = Total eggs of *H. armigera*.

Data analysis

Varian analysis followed with Duncan's multiple range test (DMRT) at p<0.05 were employed to examine the parasitization of *T. armigera* on its eggs using SPSS program version 23, while distribution pattern was examined using by Morsita, Krebs's formula (1978):

$$Id = n\frac{\Sigma xi^2 - \Sigma xi}{(\Sigma xi)^2 - \Sigma xi}$$

RESULTS AND DISCUSSION

The population of _H. armigera_ eggs

Each *H. armgera* eeg was put on the corn silk during the generative phase shown by yellowish white that turned to black after being parasitized. *H. armigera* egg population was found in all plant ages as treatment, with the highest population density in 45 DAP (Table 1). *H. armigera* eggs were laid on the corn silk, with a dominant population of 2 eggs per each. Analysis of variance (ANOVA) on the number of eggs of *H. armigera* in maize plots showed a significant difference p<0.017.

Paratisization of _T. armigera_ parasitoid

The release of parasitoid *T. armigera* cultured on eggs of *C. cephalonica* increased the per cent d parasitization on *H. armgera* eggs. Analysis of variance showed a significant effect on parasitization of *H. armigera* eggs p<0.005. Parasitization of the released parasitoid plot was higher than that of the no-release plot due to increased *T. armigera* parasitoids population. Parasitoid release of 45 DAP and 52 DAP parasitoids increased to 54.84 and 64.89% parasitization, respectively. However, at 45 DAP, a decrease by 25.69% was confirmed. Parasitoids parasitized the eggs *H. armigera* 45 DAP at higher rate due to varied plant ages and corn silk still undergoing peak development from pink to dark red 95%, where the female insect *H. armigera* prefers to lay eggs on such corn silk. At 52 DAP release, only 60% pink corn silk was found and the rest was blackish brown to black which was less preferred by *H. armigera* to lay eggs.

Higher host egg density relates to more parasitic egg. High host density allows parasitoids to easily find hosts and increased the parasitization (Vinson 1977). Besides phenological factors, some microclimate factors, parasitoid survival and release time intervals also have contributions.

During the study up to 21 DAP until the harvest, dry season occurred, except 2 days before the release of the parasitoid at 45 DAP since heavy rain for 2 consecutive days occurred and the ground was covered with rickshaws. The microclimate on 45 DAP was cooler because of heavy rain received for 2 consecutive days before release and was more suitable for parasitoids, resulting in higher parasitization than on 52 DAP. Parasitoids are poikilo thermal organisms which body temperature depends on the temperature of the surrounding environment. Changes in air temperature affects metabolic processes of the insect's body which affects the parasitization. Geetha and Balakrishnan (2011) found cooler microclimate affecting parasitoid *Trichogramma chilonis* Ishii parasitization, resulting in better dispersion. It is likely that on 52 DAP, the air temperature increased and caused weaker parasitoid activity to host eggs. The parasitization of *Trichogrammatoidea lutea* Girault increased from 21 to 27°C and decreased at 30°C. Optimum parasitization of *T. lutea* against *H. armigera* eggs was highest at 27°C (58 %) (Mawela *et al*., 2013). The longer the age of imago of the parasitoid *T. armigera*, the higher the total parasitization, while the shorter the lifespan of the parasitoid results in lower total parasitoid of the eggs of *C. cephalonica* (Rimbing *et al*., 2013). Insects' activities including parasitoids are faster and more efficient at high temperatures, but the life of the parasitoids is shorter (Mavi and Tupper, 2004). How temperature and humidity affected parasitoid *T. armigera* was not specifically analyzed in this study. According to Hunt *et al*. (2001), plant environments at high humidity are more likely to be visited by insects than those with low humidity.

The release of parasitoids on 52 DAP occurred while the corn silk of the surrounding corn crops was still blooming between 40 to 45 DAP. It is hypothesized that the parasitoids released on 52 DAP migrated to corn plants which silk was still blooming. Corn silk still blooms pink and the egg population is high. The impact of the migration of parasitoids caused the parasitoid on 52 DAP might be resulted in low parasitization. On the other hand, the low parasitization released on 52 DAP could predict that most *H. armigera* eggs were 3 days old. Rimbing *et al*. (2013) explained that egg aged 3 day showed drastic decrease in parasitization. Older *T. armigera* parasitoids decrease the parasitization. The release of parasitoids should be at intervals of 3 days to increase parasitization since *H. armigera* lays eggs gradually.

The parasitoid dose of 1000 individuals per 254 m^2 equivalent to 40,000 individuals ha^{-1} was able to increase the parasitization of *H. armigera* eggs. Release of parasitoid *Trichogrammatoidea bactrae-bactrae* Nagaraja 500,000 individuals ha^{-1} resulted in parasitized eggs of *Etiella* sp. of 18.77 and 44.04% (Marwoto and Supriyantin, 1999). On the contrary, the release of *T. armigera* on maize plants with low parasitoid doses resulted in the maximum parasitization of *H. armigera* eggs of between 38.57 and 51.91% (Table 2). Prior to the release of parasitoids, the quality of the parasitoid needs to be examined, including its parasitization characteristics and the lifespan of parasitoid. The life span of imago *T. armigera* was 4 days on eggs of *C. Cephalonica* cultured on mung bean bran, whereas those cultured with corn bran only had one day parasitoid life. Parasitization of parasitoids in eggs of *C. Cephalonica* cultured on mung bean bran was higher than in corn bran (Rimbing *et al*., 2013).

The dispersal of *T. armigera* parasitoids

After 3 days of release, the parasitoids reached *H. armigera* eggs to be parasitized. The parasitoid release plot was higher without the release of parasitoids. Based on the parasitization of eggs of *H. armigera* and its tilapia confirmed by the Morisita Id index>1, the spatial pattern of the parasitoid *T. armigera* followed the clustering pattern. The analysis of variance on the dispersal range of the parasitoids showed insignificant gap; release 45 DAP $p>0.575$, 52 DAP $p>0.448$; without parasitoid release 45 DAP $p>0.920$, 52 DAP $p>0.853$.

T. armigera was able to disburse up to 9 m from the release point even reaching>9 m (Fig 2). Geetha and Balakrishnan (2011) mentioned that the distribution of the parasitoid *T. chilonis* could reach 30 m, but even distribution occurs at a radius of 10 m. The distribution of the parasitoid *T. armigera* is rather active. Passive parasitization accumulates in cardinal directions due to the wind. During the study, weak wind blew from the southeast. Chen and Chiang (1993) wrote that parasitoids *Trichogramma* spp. tend to fly against the wind. Parasitoids dispersed in all directions to the north, south, east and west, meaning that the parasitoids did not gather in one of the cardinal directions. The highest parasitization trend was found in 45

Table 1: Number of *H. armigera* eggs in corn fields.

Plant age	Number of *H. armigera* eggs/......
Plot of second release without parasitoids at 52 DAP	2.90±0.44 a
Plot of second release at 52 DAP	3.50±1.52 ab
Plot of first release without parasitoids at 45 DAP	3.78±1.12 ab
Plot of first release at 45 DAP	4.00±1.25 b

Table 2: Parasitization of *T. armigera* on the parasitoid release and without release.

Plant age	Percentage of parasitism (%)
Without release plot on 52 DAP	13.54±3.61 a
Without release plot on 45 DAP	23.44±5.92 ab
Release plot on 52 DAP	38.57±2.51 bc
Release plot on 45 DAP	51.91±5.23 c

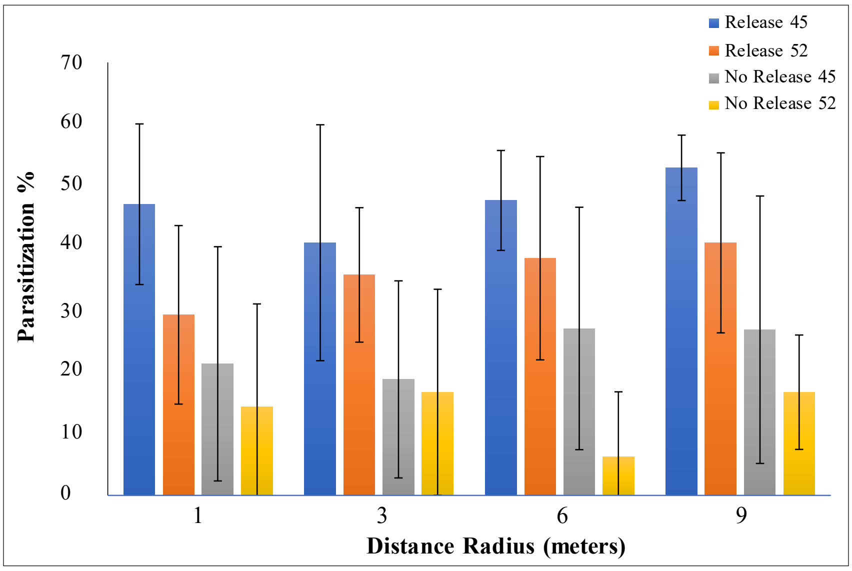

Fig 2: The dispersal of *T. armigera* on corn.

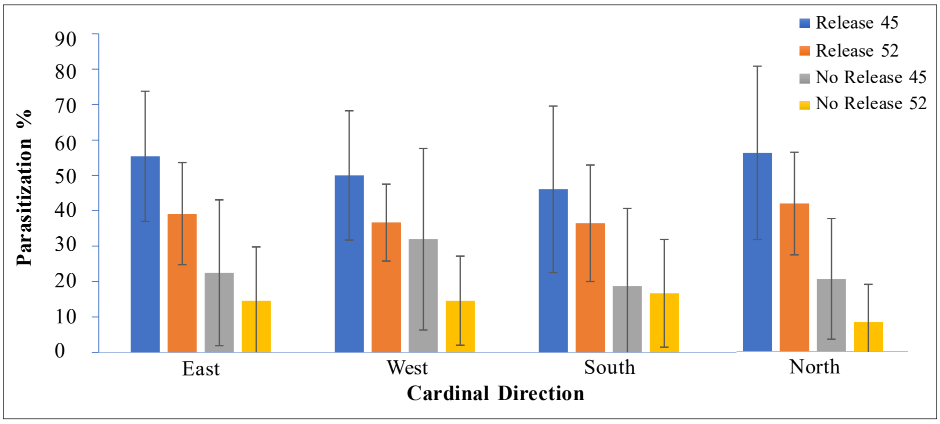

Fig 3: Parasitization of *T. armigera* against *H. armigera* eggs in the cardinal directions.

DAP and 52 DAP in all cardinal directions (Fig 3). Analysis of variance results was not significantly different; plot of release 45 DAP p>0.92, 52 DAP p>0.97; without release 45 HST p>0.97; 52 DAP p>0.94.

The parasitoid *T. armigera* tends to cluster in release and no release plots. The plot of the release of parasitoids with clusters leads to regular dispersal. Smaller Morisita index indicates lower clustering population (Rosenberg and Anderson, 2011). The clustering pattern was homogeneous corn ecosystem, with minimum individual competition. The distribution of *T. armigera* parasitoids on maize is in clusters, requiring the use of regular or systematic sampling method.

The distribution range of the parasitoid *T. armigera* and parasitization showed that the number of parasitoid release points in maize is 40 ha^{-1}. The release of *T. chilonis* at 100 stations ha^{-1} can suppress *Chilo sacchariphagus* Boj infestations, increasing the sugarcane production by 23% (Marquier *et al*., 2008). Parasitoid release did not determine the number of stations. In 8-time releases of *Trichogramma sp*. with a dose of 100,000 individuals ha^{-1}, no significant difference in the mortality of shoot borer eggs and sugar cane stem borer was found (Nurindah *et al*., 2016). This poor result might be due to in appropriate number of release points and low-quality parasitoids.

CONCLUSION

The release of 1000 *T. armigera* parasitoids per 254 m^2 increased the parasitization of *H. armigera* eggs on corn plant. The increase in parasitization on 45 DAP reached 51.91% and 38.57% on 52 DAP. The released parasitoids were evenly dispersed to all radius, even up to a radius of 9 m. The parasitoid *T. armigera* moved actively and dispersed evenly to north, east, south and west directions. The distribution of *T. armigera* was clustered for more systematic parasitoid monitoring. Based on the dispersal radius of the parasitoid, 40 release points ha^{-1} were determined as the release points of *T. armigera* in corn crops for effective management of cob borer.

Conflict of interest: None.

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