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Effectiveness of *Menochilus sexmaculatus* (Fabricius) (Coleoptera: Coccinellidae) to control cotton aphid *Aphis gossypii* Glover on cotton plants

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Abstract
The cotton aphid *Aphis gossypii* is a worldwide pest insect on a variety of agricultural crops. This pest is also a major pest of cotton in Asia, causing not only a decrease in the yield but also decreases in the fiber quality. Cotton aphid management with chemical control has become more difficult due to high resistance to many common insecticides. The aim of the present study was to determine the usefulness of the predatory ladybird beetle *Menochilus sexmaculatus (*Fabricius) for conservation biological control of cotton aphid. For this purpose, the effective predator/prey ratio was examined using larval and adult *M. sexmaculatus* in greenhouse conditions. The ladybirds (1-4) and 50 cotton aphids were released on each cotton plant, and the number of aphids were counted until they were eaten out. When the ratio was 1/50 with adult ladybirds, the time required until aphid populations had reached zero was 16 days. As the ratio increased, aphid populations decreased at a greater rate. Although the days taken were longer when test was made with 1st instar of *M. sexmaculatus*, the density of cotton aphids reached zero until 20 days of initiation of the test, regardless of the ratios. In contrast, aphid density became double or nearly three times larger when no predator was present until 20 days. Predator/aphid ratio had a significant effect on aphid density, and higher ratio resulted in faster decrease of aphids. The present result suggests that *M. sexmaculatus* is an effective natural enemy of the cotton aphid since it can suppress the aphid population even when predator/aphid ratio is 1/50 ratio. Augmentative release of a number of *M. sexmaculatus* would provide satisfactory control for the pest. Conservation of this predator via IPM practices could also provide a satisfactory control of the cotton aphid.

Keywords: Natural enemies, insect predator, biocontrol, cotton production

Introduction

Cotton *Gossypium hirsutum* L. is one of the major crops in the world and is mainly used for fiber production. It is also cultivated widely in Myanmar and is an important commercial crop used for exports in the country (Pye Tin, 2003) [26]. In Myanmar, it is grown in three main seasons: the pre-monsoon (February–March to June–July), monsoon (May–June to October–November), and late or post-monsoon seasons (July–August to December–January). The production in the country is not high, and the yield stagnation has been a frequently raised issue. Apart from some important causes, such as climate change and soil salinity, insect pests are a main factor for low yield of cotton in Myanmar. In addition to their negative impact in yield, cotton pests can even cause a reduction of fiber quality. Establishing effective pest management program is therefore on strong demand.

Globally aphids are very severe pest of agriculture (Minks and Harrewijn, 1987) [20]. The cotton aphid *Aphis gossypii* Glover is an important agricultural pest (Andrews and Kitten, 1989; Cartwright, 1992) [6, 7]. It is a cosmopolitan, polyphagous species widely distributed in tropical, subtropical and temperate regions of the world (Parajulee, 2007) [25]. This aphid pest has been known as a principal pest of cotton in the world (Ahmad *et al*., 2003) [4] and is also a major pest of Myanmar's cotton (Morris and Waterhouse, 2001) [21]. The cotton aphid is particularly a harmful pest causing not only a quantitative decrease in the whole production but also a decrease of product quality due to direct damage inducing plant deformation by feeding and indirect damage due to honeydew (Razmjou *et al*., 2006) [27]. Furthermore, this pest can be a plant disease vector to transmit plant viruses Ebert and Cartwright, 1997 [11]; Razmjou *et al*., 2006 [27].

Insecticidal control is not only expensive but also pollutes the environment and also leads to many health problems. Indiscriminate use of pesticides causes phytotoxicity and destruction of beneficial organisms such as predators, parasitoids, microorganisms and pollinators (Luckman and Metcalf, 1978) [17]. Subsequently, Integrated Pest Management (IPM) has been opted in which biological control as one of the strong components (Debach and Rosen, 1991) [8]. Thus, IPM incorporating several practices should be essential to controlling the cotton aphid.

Biological control of agricultural pests uses natural enemies such as predators and parasitoids to suppress target pest populations (Hoy, 1994) [13]. In developing a biological control strategy for cotton aphid, potential natural enemies need to be selected. Besides parasitoids and pathogens, predators play an important role in regulating the aphids. Coccinellidae, Hemerobiidae, Chrysopidae, Syrphidae and Anthororidae are the dominant predators of aphids (Wagiman, 1996) [30].

Ladybird beetles (Coccinellidae) are one of the major predators of the cotton aphid. Ladybird beetles have been used on greenhouse crops (e.g., tomatoes, sweet peppers, and cucumbers) to control several pests, including aphids, thrips, whiteflies, mites, and lepidopteron eggs (Omkar, 2004) [23]. They are capable of regulating the aphid population due to their voracity, search efficiency, predation capacity, and reproduction rate (Dixon *et al*., 1997; Dixon, 2000; Magro *et al*., 2002; Kindlmann *et al*., 2015) [10, 9, 18, 16]. Thus, ladybird beetles can be a good candidate agent for biological control and IPM in cotton.

The Coccinellid *Menochilus sexmaculatus* Fabricius has been reported as a good candidate for biological control of aphids (Wagiman, 1996) [30]. This ladybird beetle is known widely from many Asian countries and seems be one of the commonest species of Coccinellidae in agricultural areas (Khan and Khan, 2002) [15]. *M. sexmaculatus* was reported to be a common predator of the cotton aphid (Jalali and Singh, 1994) [14]. Our preliminary field observations also suggest that this predatory beetle is very common and abundant in crop fields, including cotton plants, in Myanmar. We therefore have started to assess the usefulness of *M. sexmaculatus* in cotton aphid IPM program in Myanmar.

The aim of this study was to determine the efficacy of the ladybird as a predator of the cotton aphid. The effective released number of *M. sexmaculatus* for suppressing the cotton aphid population was determined in the present study. Although several methods have been used to measure the effectiveness of a predator on aphid populations (Hodek *et al.*, 1972) [12], the present study performed a greenhouse experiment. Based on the results, we demonstrate that *M. sexmaculatus* is potentially an effective predator and discuss the usefulness of this predatory ladybird beetle.

Materials and Methods

The experiments were carried out at the Cotton Research and Technology Development Farm in Lungyaw, Department of Agriculture, Ministry of Agriculture, Livestock, and Irrigation, Myanmar, which is located in Kyaukse Township, Mandalay Region in the country. The experiments were conducted in enclosed green houses with a 4 months period between February 2022 and June 2022.

Aphids and ladybird beetles

Cotton aphids and ladybird beetles were collected from cotton fields at Lungyaw Cotton Farm. After the collected aphids and the predatory coccinellids were identified to species, rearing of the two insects was initiated under a laboratory condition of 27 °C and 70% RH. Cotton plants were grown in plastic bags (30 cm x 30 cm x 45 cm). Cotton aphids were introduced onto the leaves of cotton plants when the plants reached the squaring stage (25 days after sowing), and the plants were then placed in a rearing cage (150 cm x 100 cm x 100 cm). Around 30 cotton plants with aphids have been kept *in* order to obtain sufficient numbers of aphids to maintain aphid and ladybird beetle populations in the laboratory.

Ladybird beetles were reared on cotton aphids. A pair of male and female *Menochilus sexmaculatus was transferred in* a plastic petri dish (9 cm x 2 cm). Fresh cotton leaves (1-2 leaves) with appropriate number of cotton aphids were then placed in the petri dish. Cotton leaves with aphids were replaced every day to provide the beetles with fresh prey. The paired beetles were checked every day. When freshly laid egg masses were found, the egg masses were collected from the dish, separated and incubated in petri dishes. When hatching was observed, 1st instars were gently collected and were reared individually in a petri dish containing cotton leaves with aphids.

Greenhouses

A greenhouse (15 m x 6 m x 6 m) covered with mesh nylon net (Diameter: 0.24 mm) was used in the present study. It was maintained as a biological control greenhouse by keeping it away from insecticide contamination. Also, the nylon net was enough to avoid entry of other insect pests. The greenhouse was divided into five sectors (3m x 3m). The sectors were separated each other with the mesh net attached to the frames of the greenhouse; each sector was completely isolated to avoid movement of insects from one sector to another. Cotton plants for experiments (see below) were placed in each sector upon initiating the experiments.

Cotton plants for experiments

Cotton plants were prepared as follows. A total of 40 plastic square pots (30 cm x 30 cm x 45 cm in length, width and height) were prepared for the experiment. Two-3 cotton seeds (Ngwe chi-11 variety) were sown in each pot. Thinning was made 18 days after sowing to adjust the number of cotton plants per bag, and each bag with a single plant of 13-15 cm height was prepared. Then, cotton plants were transferred from the plastic pot through hoeing to each sector in an experimental greenhouse. Each cotton plant was separated at least (150) cm in order to avoid a migration of aphids (and ladybirds) between plants as possible. The cotton plants were used for testing after one week. Thus, 40 cotton plants in all were prepared for the following experiments, and one plant was used as one replication.

Experimental Design

To assess the efficacy of *M. sexmaculatus* as a predator, growth of aphid populations was monitored when the numbers of the ladybirds introduced were changed. The numbers of ladybirds were 1 or 2 or 3 or 4 per plant, and, as control, we monitored predator-free aphid populations during the experimental period. Ladybird beetles used in the experiments were newly hatched 1st instars or unsexed adult beetles; the effect of ladybirds on aphid populations was compared between 1st instars versus adults, in order to assess how the developmental stage of predator could interact with the effect. Fifty adult aphids were transferred with a fine camel brush on each cotton plant in the experimental greenhouse (See above). Because the exact number of aphids could decrease due to dropping off or moving from the plant, a pre-count of aphid was carried out just before releasing ladybird beetles. When necessary, additional aphids were placed so that the initial density of the aphids was exactly 50. Then, newly hatched first-instars or newly emerged adults of *M. sexmaculatus* were released on each cotton plant. The larvae and adults were taken from mass-rearing petri dishes, and a single ladybird larva or adult was gently placed on each plant with 50 aphids. Releases of *M. sexmaculatus* were done on the 25th day after sowing of cotton plants.

Data collections: The number of *A.* g*ossypii* was counted at 5-day intervals until their population dropped near zero. The presence or absent of the beetle on plant was also checked while the number of aphids were counted. Cotton plants were inspected directly to count aphids with the help of a hand lens (10x magnification). Daily temperature and rainfall were noticed during the cotton growing season for the experiment.

Data Analysis

All relevant data were subjected to statistical analysis using Statistix 8. Mean comparisons were computed using the Least Significant Difference (LSD) test ($\alpha = 0.05$). Percentage data were statistically analyzed after arcsine transformation.

Results and Discussions

Our experiments have shown that the ladybird beetle *M. sexmaculatus* can suppress the cotton aphid population on cotton plants to a sufficient level though the efficacy of suppression depends on the number of predators released.

The aphid population became 1.75 after 20 days from the release of one *M. sexmaculatus* 1st instar (Table 1). Also, aphid density reached 0, thus completely suppressed, after 20 days, 15 days and 10 days after release of 2, 3 and 4 larval predators, respectively (Table 1). Although only one 1st instar of the lady beetle could finally eat up all cotton aphids, the days required to make the aphid density zero was a little bit long, and, with the larval ladybird number increased, the days were shortened more and, hence, decrease of aphid populations was much faster when more predators had been released (Fig. 1).

Table 1: Average number of *A. gossypii* populations per plant after releasing *M. sexmaculatus* larvae at different treatments under field cage conditions on cotton plants

Treatment	Initial aphid population	5 days after released predators	10 days after released predators	15 days after released predators	20 days after released predators
T_1	50	31 ^{b}	22 ^{b}	13 ^{b}	1.75 ^{b}
T_2	50	18 ^{c}	10 ^{c}	2.5 ^{c}	0 ^{b}
T_3	50	14 ^{d}	3 ^{d}	0 ^{c}	0 ^{b}
T_4	50	1.75 ^{e}	0 ^{e}	0 ^{c}	0 ^{b}
T_5	50	69 ^{a}	92 ^{a}	113 ^{a}	130 ^{a}
$LSD_{0.05}$	-	2.01	2.17	7.28	3.35
SE	-	0.65	0.70	2.36	1.08
Prob	-	<.0001	<.0001	<.0001	<.0001
CV %	-	4.83	5.56	18.30	8.27

Means followed by different letters in same column are significantly different from each other at 5% level of significance

Similarly, release of adult *M. sexmaculatus* resulted in a complete suppression of cotton aphids. When predator/aphid ratios were 1/50, 2/50, 3/50 and 4/50 with adult ladybirds, days required until aphid populations had reached zero were around 16, 15, 10, and 5, respectively (Table 2). Decrease rate of aphids was faster as the ratio or the number of ladybirds released increased (Fig. 2). Adult ladybird beetles reduced aphid populations more than 1st instars did (Fig. 1 vs 2) though the reduction difference diminished when 4 predators had been released.

Table 2: Average number of *A. gossypii* populations per plant after releasing *M. sexmaculatus* adults at different treatments under field cage conditions on cotton plants

Treatment	Initial aphid population	5 days after released predator	10 days after released predator	15 days after released predator
T_1	50	20.5^b	7^b	2.25^b
T_2	50	6.25^c	1^c	0^b
T_3	50	1.75^d	0^c	0^b
T_4	50	1.25^d	0^c	0^b
T_5	50	70^a	94^a	115.5^a
LSD 0.05	-	2.13	3.44	6.25
SE	-	0.69	1.11	2.03
Prob	-	<.0001	<.0001	<.0001
CV%	-	6.99	10.96	17.25

Means followed by different letters in same column are significantly different from each other at 5% level of significance.

In the control cages, where no predator had been released, the number of cotton aphids increased steadily, and aphid density became nearly double or three times larger (Tables 1 and 2; Fig. 1 and 2). The sharp increase of cotton aphids caused a heavy damage on the experimental cotton plants while such damage was not detected in predator-released cotton. These results confirmed again that the predator *M. sexmaculatus* suppressed cotton aphids and, as the result, protected cotton plants.

Al-Eryan *et al*. (2001) [5] demonstrated that releasing *Coccinella undecimpunctata* (adult female) against *A. gossypii* at predator/prey ratios of 1:100, 1:50, and 1:25 resulted in a reduction of aphid populations by 99.6, 99.4, and 99.4%, respectively, within 28 days. Adashkevich (1975) [2] also reported that the best control of aphids was gained in 10 days when 1st instars of *Coccinella septempunctata* were released against *A. gossypii* at the ratio of 1:50 or 1:100. The present results, together with the above mentioned previous studies, indicate that ladybird beetles are superior predators of the cotton aphid, and *M. sexmaculatus* can be useful in controlling this serious pest.

In addition, we confirmed that aphid populations remained to be zero for a period of days after they reached zero once. Similar findings were made by Abdel-Salam *et al*. (2005) [1], and it was mentioned that the number of aphids remained zero for a period of 13 days after the release of *C. undecimpunctata* adults in predator-prey ratios of 1:60 and 1:75. Previous literatures indicate that *M. sexmaculatus* attacks a wide variety of aphid species (Agarwala *et al*., 2001) [3] and some coccids pests, etc. (Sugiura and Takada, 1988) [29]. Since ladybird beetles including *M. sexmaculatus* are not an exactly specialist predator and can exploit other prey items, including other aphid species, whiteflies, mealybugs, etc., they may keep staying on cotton plants even when the cotton aphid disappeared, and help prevent establishment of a new cotton aphid colony. In our study, we did not check whether other potential prey items occurred in our experimental greenhouse. The effect of the occurrence of other alternative prey items on maintaining *M. sexmaculatus* populations in cotton fields would be a future subject.

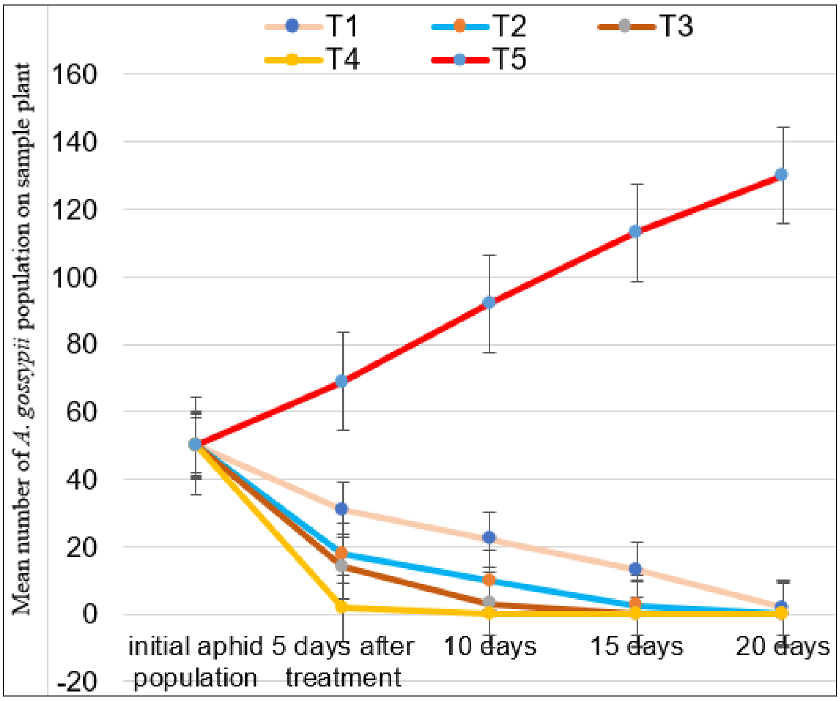

Fig 1: Change of A. gossypii densities in relation to the number of M. sexmaculatus larvae released (1, 2, 3, or 4) and the day after release (T_5 = control). See the text for details

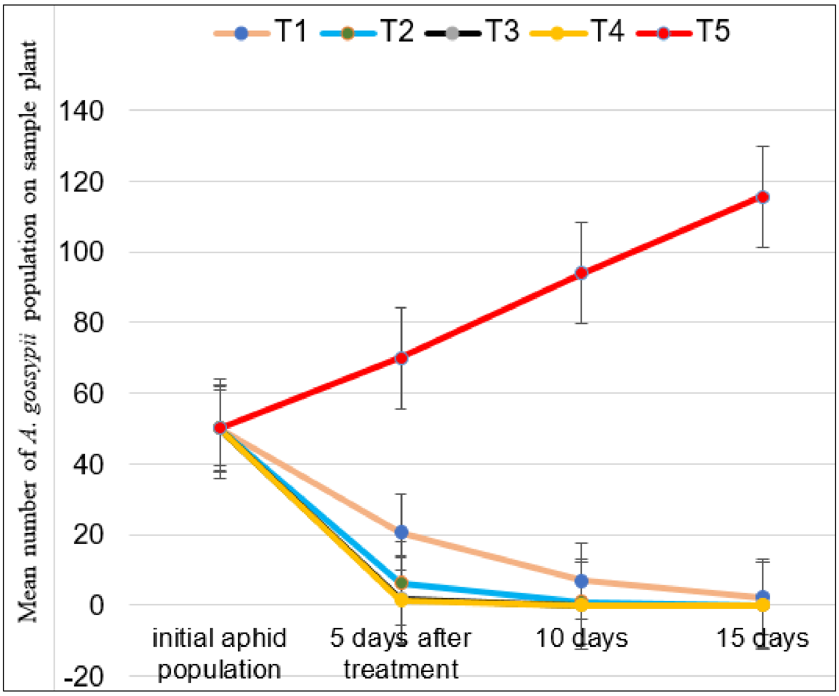

Fig 2: Change of *A. gossypii* densities in relation to the number of *M. sexmaculatus* adults released (1, 2, 3, or 4) and the day after release (T_5 = control)

The present study thus demonstrated that *M. sexmaculatus* could be employed as the biological control agent against *A. gossypii*. This study also suggested that one *M. sexmaculatus* per plant was satisfactory enough to suppress the cotton aphid when the initial density was 50. Cotton aphid populations with the density 50 per plant can be reduced to nearly 0 within 2 weeks when multiple adult ladybirds occur on each cotton plant (Fig. 1 and 2). Even when only one adult predator occurs, cotton aphids can disappear within 20 days, attaining a complete suppression of the pest (Fig. 2). Also, when 4 1st instars of the predator are present on a cotton plant, aphid populations are almost completely suppressed in 5 days of ladybird egg-hatching (Fig. 1). Female *M. sexmaculatus* normally lay an egg-mass with 10-15 eggs (Nu, 2008) [22]. Therefore, the presence of the egg-mass on a cotton plant should lead to a quick reduction of aphid populations after hatching.

In our study, *M. sexmaculatus*/aphid ratio was demonstrated to affect readiness of aphid suppression, implying the importance of predator/prey ratio in aphid control. Similarly, Omkar and James (2003) [24] mentioned that a predator-prey ratio of 1:50 may be considered optimal for the augmentative release of *Coccinella transversalis* for biological control of *A. gossypii*. Zaki *et al*. (1999) [31] reported that a single release of *C. undecimpunctata* (1:50 predator/aphid ratio) resulted in a 99.97% reduction of *A. gossypii*. Semyanov (1997) [28] found that the release of the first instar of *Harmonia dimidiate* was highly effective against *Macrosiphum euphorbiae* on cucumbers at a predator-prey ratio varying from 1:50 to 1:100. Likewise, in *M. sexmaculatus, 5 larvae could* reduce the aphid population by 80.67 and 90.63% in 72 hours of release against 150 and 160 aphids on caged potato plants, respectively (Mannans *et al*., 2001) [19].

Conclusions

Our study demonstrated that one *M. sexmaculatus* per plant, regardless of the developmental stage (first instar larva or adult), was enough to suppress *A. gossypii* populations on the cotton plant, suggesting that *M. sexmaculatus* has promising potential for biological control of *A. gossypii*. A predator-prey ratio of 1:50 was considered an effective ratio suitable for control of the aphid under field conditions. It was also indicated that the searching efficiency and rate of consumption were at their maximum when one predator was searching and at a prey density of 50 aphids.

It may be suitable for the release of predators in the infected field and monitoring the prey populations under field conditions. It can help make the critical decision on whether it is time for pesticides to be sprayed or delayed in the field, leading to economic savings and eco-friendly management if spraying is unnecessary. Therefore, the release of *M. sexaculatus* into a large number of cotton aphids could provide satisfactory control for the pest. Further investigations on the performance of the predator against the pest in the field would provide more information.

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