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Integrated pest management of cotton aphid using transgenic cotton, intercropping, and botanical pesticide

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Abstract
Cotton is one of the most important natural fibers and plays a significant role in the national economy of Myanmar. Cotton production in Myanmar is quite low, and the major factor for low cotton production is largely due to sucking pest damage. The cotton aphid *Aphis gossypii* is a particularly harmful pest; it causes a number of problems such as yield loss and reduction of fiber quality. However, there are limitations of relying on chemical pesticides alone. In the present study, we examined the effectiveness of IPM combining transgenic cotton, intercropping with green gram and botanical neem pesticides on cotton aphid control. In three consecutive planting seasons, the density of cotton aphids was significantly much lower in IPM cotton fields than BT cotton fields with chemical pesticide application. Moreover, the cotton aphid density with IPM practice was kept below the action threshold level for pesticide application (< 10 aphids per leaf or 10% damaged or infested plants) throughout the experimental seasons. The densities of natural enemies of cotton aphids, *i.e.*, coccinellid beetles, lacewings and spiders, were significantly much higher in IPM fields, suggesting that the low aphid density was at least partly thanks to natural enemies. The use of botanical neem pesticides, particularly early in the cotton season, appeared to reduce cotton pests without the use of chemical insecticides and help conserve numerous natural enemies. Cotton yields were not different between IPM and conventional fields. Thus, combination of the IPM components applied here was effective enough to manage cotton aphids, conserve natural enemies, and reduce chemical pesticide use, while keeping cotton production.

Keywords: Bt cotton, neem, conservation biological control, insect predator, natural enemies

Introduction
Cotton has been a major commercial crop in Myanmar since the introduction of upland cotton (*Gossypium hirsutum* L.) in early 1960 (MCSE, 2006) [6] and now plays a key role in the national economy as it is a main export crop for foreign exchange earnings (Pye Tin, 2003) [12]. In Myanmar, cotton production is confined mainly to the central dry zone between 19°N and 23°N latitudes (Pye Tin, 2006) [13] such as Sagaing, Mandalay, Magwe, Bago, Shan, Chin, and Nay Pyi Taw, with 101 cotton-growing Townships (MOAI, 2014) [7]. Myanmar cotton is grown in three largely overlapped cropping seasons, that is, the pre-monsoon season (February-March to June-July), the monsoon season (May-June to October-November), and the late or post-monsoon season (July-August to December-January). The total sown area was 168,000 hectares with an average yield of 1.75 tons per hectare, and the total production was 294,000 tons in the 2019-2020 fiscal year (MOALI, 2020) [9].
Productivity of cotton seems rather low in the country, and yield stagnation has been a major issue. Causes of the low yield include climate change, soil salinity, inappropriate agronomic practice, but insect pests are the major factor affecting the cotton yield in Myanmar. Cotton production is heavily dependent on some of major insect pests, which often outbreak and threaten the crop annually. Moreover, the insect pests can cause not only quantitative decreases in the whole production but also decreases fiber quality (Nu, 2008) [11]. Crop protection from insect pests is thus important in Myanmar cotton production.
In Myanmar, cotton bollworm and sucking pest damage are the major restriction factors for cotton production. Cotton jassid (*Empoasca* spp.), cotton aphid (*Aphis gossypii*), white fly (*Bemisia tabaci*), thrips (*Thrips tabaci*), cotton stainer bug (*Dysdercus cingulatus* (Fabricius)), mealybug (*Phenacoccus solenopsis*), and spidermite (*Tetranychus* spp.) are widespread and

main pests in cotton-growing areas of Myanmar (Morris and Waterhouse, 2001) [10]. Among them, the cotton aphid *Aphis gossypii* Glover (Homoptera: Aphididae) is one of the most important pests of cotton in Myanmar because of its severity of damage and difficulty of control.

Since decades ago, the cotton aphid *Aphis gossypii* has become the biggest menace to the cotton crop, and, in Myanmar, this pest can result in the complete yield loss of the crop (Lungyaw Cotton Research Farm, 2018) [4]. The degree of damage depends on the period of the attack and the size of the aphid populations (Matthews and Tunstall, 1994) [5]. Also, the aphid is an important vector of cotton diseases, and can cause serious losses of yield (Razmjou *et al*., 2006 [14]; Kumar *et al*., 2022) [2]. Thus, management of the cotton aphid is crucial to stable production of cotton. Cotton aphid management has become more difficult in recent years, however. Chemical control is often ineffective, possibly due to the aphid's resistance to many common insecticides. The development of integrated pest management (IPM) on cotton was then emphasized, encouraging incorporation of natural pest control mechanisms (Nu, 2008) [11].

In 2017, the cotton aphid became a serious pest in cotton production not only at the Lungyaw Cotton Research Farm but also at cotton farmers' fields in Myanmar. Cotton yield dropped below 101.4 kilograms per hectare in this year (Lungyaw Cotton Research Farm, 2018) [4]. Although chemical control using insecticides was applied to suppress outbreaks of sucking pests, this control was found not effective. Also, heavy reliance and misuse of insecticides would result in the development of insect resistance, environmental contamination, adverse effects on non-target organisms and development of secondary pests. IPM program for cotton aphid control is thus an urgent need.

Accordingly, field and laboratory studies were undertaken to assess the feasibility of practices for IPM in cotton. Our previous studies were found that use of a Bt cotton variety or a botanical pesticide neem helped suppress cotton aphids but aphid control was not satisfactory enough with the practices (Lungyaw Cotton Research Farm, 2015) [3]. In the present paper, we examined the usefulness of IPM practices combining Bt cotton and botanical pesticide with an intercropping system for the conservation of the natural enemies. For this purpose, we used green gram or mung bean (*Vigna radiata* L.) and Bt cotton (*G. hirsutum* L.) in the intercropping practice. We chose green gram because this is a major pulse crop in Myanmar (MOALI, 2019) [8], which means this intercropping practice should be rather acceptable for Myanmar farmers, and because previous studies have demonstrated the usefulness in intercropping for pest management purpose. Based on the present results, we discuss the feasibility of IPM practices in cotton production.

Materials and Methods

Experiments were carried out at the Cotton Research and Technology Development Farm in Lungyaw, Kyaukse Township, Mandalay Region, Department of Agriculture, Ministry of Agriculture, Livestock, and Irrigation, Myanmar. Lungyaw Farm is located at latitude 20˚ N, longitude 96˚ E, 367 meters above sea level. Experiments were conducted in the three consecutive seasons, *i.e.*, the post-monsoon seasons (mid-July to end November) of 2019, the pre-monsoon season (mid-February to end of June) of 2020, and the post-monsoon seasons (mid-July to end November) of 2020. Bt cotton from Shwe Daung-10 was used in the present research, and this transgenic cotton is bollworm-tolerant and is also moderately resistant to sucking pests due to its high hair density. Shwe Daung-10 was mutant from Ngwe Chi-6 and distributed or released in 2018 to Myanmar farmers. Ngwe Chi-6 was produced using an Indian Bt variety backcrossed with a local long staple variety at the Lungyaw Cotton Research Farm. The cotton seeds were acid-denatured before sowing.

In this study, we prepared two experimental cotton fields. The size of the experimental fields was 0.25 acre (40 m x 30 m) each. In one field, IPM practices were applied and cotton plants were intercropped with green gram (3 rows of cotton to 1 row of green gram), and a botanical pesticide, *i.e.*, neem pesticide (0.75% SC), which is commercialized and widely used in Myanmar, was applied, when necessary, with weekly interval. Another field was used as control, and conventional practices including chemical control were applied. Chemical pesticides used in the control field were Acetamiprid, Abamectin, Cartap Hydrochloride 50% SP, Acephate, Neonicotinoid; they were applied depending on the economic threshold level of the cotton aphid. For other cultivation practices, such as inter-cultivation, weeding, hoeing, irrigation, and manure and fertilizer application, recommended farming practices were carried out for both fields from sowing to harvesting.

Field survey was made to examine the density of cotton aphid and its potential natural enemies. Cotton aphid populations were examined in both experimental fields starting 25 days after sowing with a weekly interval. Ten sample plants were taken across diagonally and selected randomly in each treatment plot (10 cotton plants in each survey, 10 plants * 10 times * 2 fields with weekly interval). Aphid population was counted per plant, *i.e.*, three leaves per plant, each from top, middle and bottom of the selected plant and examined by using hand lens magnifier (10x). The observations recorded on cotton aphids were later averaged to per leaf basis.

Predatory arthropods were monitored using a sweep net. The terminals of cotton plants were swept while walking along the row of cotton plants, and 20 sweeps per plot were done with a weekly interval. All arthropods collected were brought back to the laboratory for identification and count. The major species of predatory arthropods collected in this study area were ladybirds, lacewings, and spiders, and, hence, we monitored these natural enemies as the index of conservation biological control. All relevant data were subjected to statistical analyses (one way analysis of variance ANOVA) using the Statistix 8 program, and JMP version 14. Mean comparisons were computed using the Least Significant Difference (LSD) test ($\alpha = 0.05$).

Results and Discussion

During the three experimental seasons, cotton aphid was detected in most of cotton plants for both experimental fields. It was found that the mean numbers (±SE) of cotton aphids per leaf in the intercropped IPM field were 5.15±0.42, 5.77±0.26, and 5.28±0.30, in 2019 post-monsoon, 2020 pre-monsoon, and 2020 post-monsoon seasons, respectively, whereas those in the conventional field were 13.74±0.42, 15.65±0.26 and 14.51±0.30, respectively (Fig. 1 and Table 1). In all three seasons, the aphid densities were significantly lower in the former field than the latter (Table 1).

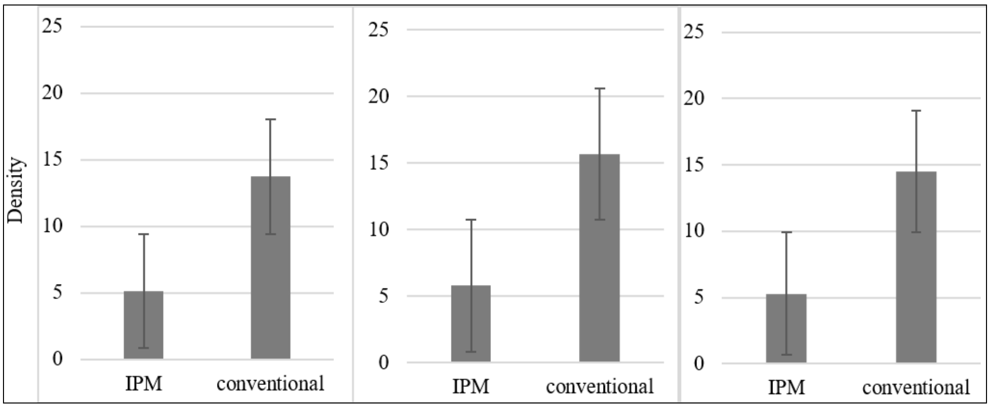

Fig 1: Mean number of aphid densities in IPM and conventional cotton field for 2019 post-monsoon, 2020 pre- monsoon, and 2020 post-monsoon seasons (From left to right)

Table 1: Mean number of cotton aphid per leaf in three experimental seasons

Treatment	Mean number of cotton aphid/leaf		
	2019 post	2020 pre	2020 post
IPM field	5.15 [b]	5.77 [b]	5.28 [b]
Conventional field	13.74 [a]	15.65 [a]	14.51[a]
LSD $_{0.05}$	1.25	0.77	0.88
SE (mean)	0.42	0.26	0.30
Prob	<.0001	<.0001	<.0001
CV %	14.10	7.70	9.46

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

Natural enemies were commonly found in the IPM field, and main predator groups of *A. gossypii* were identified as Coccinellidae (ladybird beetles), Chrysopidae (lacewings), Linyphiidae (spiders) and Thomisidae (spiders). The mean numbers (±SE) of ladybirds in the IPM field were 2.38±0.10, 2.16±0.12 and 2.24±0.13 in 2019 post-monsoon, 2020 pre-monsoon, and 2020 post-monsoon seasons, respectively, whereas those in the control field were 0.3±0.10, 0.26±0.12 and 0.21±0.13, respectively (Fig. 2 above). Ladybird densities were significantly higher in the IPM field (Table 2). Similarly, lacewings were significantly more abundant in the IPM than control fields (Table 2). The mean numbers of lacewing in the IPM field were 1.2±0.04, 1.17±0.05 and 1.12±0.06 (Fig. 2 middle) in 2019 post- monsoon, 2020 pre-monsoon, and 2020 post-monsoon seasons, respectively, whereas those in the control field were 0.03±0.04, 0.07±0.05 and 0.02±0.06, respectively. Also, the densities of spiders (Linyphiidae and Thomisidae) were significantly higher in the IPM than control fields 1.12±0.04, 1.05±0.03 and 1.11±0.04 (Fig. 2 below; Table 2) in 2019 post-monsoon, 2020 pre-monsoon, and 2020 post-monsoon seasons, respectively, whereas those in the control field were 0.04±0.04, 0.04±0.03 and 0.03±0.04, respectively (adult and larval numbers were pooled for ladybirds and lacewings). Thus, abundance of natural enemies of cotton aphids was much higher in the IPM fields than those in conventional Bt cotton fields.

There were 3000 cotton plants in the IPM and 4000 cotton plants in the control fields with the same area of 0.25 ac (40 m x 30 m). Average seed cotton yields were 687 viss/acre (478 kg/ha) in the intercropped IPM field and 703 viss/acre (487 kg/ha) in the conventional control field for the three seasons. Although seed cotton yield in the IPM field was somewhat lower than the control field, there were no significant difference between the fields (Table 2).

This finding was the same as Kadam *et al*. (2014) [1], who reported that the lowest incidence of aphid populations of 3.28 aphids per 3 leaves in cotton and green gram intercropping and the maximum incidence of 16.57 aphids per 3 leaves in sole cotton were recorded and concluded that the population dynamics of sucking insect pests of Bt cotton in different intercropping systems were the same.

During the three experimental seasons, pesticides were applied six times or more in the IPM fields and eight times or more in the control field. The cost of neem pesticides used in the IPM field is lower than that of chemical pesticides used in the control field. In IPM practices, it is important to monitor the occurrence or density of target pests; whether the density of a target pest is kept below the economic injury level is a key point for effective IPM. To do so, we use the threshold value (=density); when the density of a pest reaches the threshold, management action, such as pesticide spray, is to be applied. In Myanmar, we set the action threshold for the cotton aphid as 10 aphids per leaf and/or 10% of damaged or infested cotton plants. In the present study, we observed in the intercropped IPM field the mean cotton aphid densities had been below the action threshold level during the three consecutive experimental seasons (Fig. 1). The present results have therefore demonstrated that our IPM practices in cotton field is feasible in terms of cotton aphid management. In contrast, aphid densities in the conventional field were frequently above the threshold level despite frequent chemical pesticide use (Fig. 1). Chemical pesticides used in the present study appeared still effective, though not enough, and the outbreak of cotton aphid did not occur. Given that cotton aphid can develop resistance to such pesticides, their effectiveness would not be guaranteed into the future.

In the present study, we also have shown that predators of the cotton aphid are more abundant in the IPM fields than the conventional fields throughout the three consecutive seasons between 2019 and 2020 (Fig. 2), whereas the target pest aphid remains low in density (Fig. 1). Main natural enemy predators detected in our experimental fields were Coccinellidae (ladybird beetles), Chrysopidae (lacewings), Linyphiidae and Thomisidae (spiders) (Fig. 2). It is considered that the higher densities of predatory natural enemies are partly due to use of botanical pesticides, which are less harmful to natural enemies than synthesized chemical pesticides. Also, the presence of green gram adjacent to cotton plants may increase the densities of natural enemies. We suggest that there should be a combined positive influence of botanical pesticides and intercropping to natural enemies, promoting the establishment and increase of natural enemies including ladybirds, lacewings and spiders. The presence of these natural enemies should then help suppress cotton aphid populations. Although we did not observe other potential natural enemies of the cotton aphid, such as parasitoid wasps, such natural enemies would also be conserved in our IPM practice.

Lastly, we found crop yield was not different between the two types of fields (Table 2). The results suggest that the occurrence of pests and diseases of cotton does not differ in the fields. Although the density of the cotton aphid was higher in our conventional fields, it was not so high enough to cause a serious damage to cotton yield, possibly due to use of Bt variety and frequent use of chemical pesticides. However, given cost of the chemical pesticides applied was much higher, our IPM practice would be better in terms of the total cost. Total cotton production per each field is lower in IPM fields because the area of cotton plants cultivated is 75% in comparison of conventional fields. A reduction of the yield, however, can be compensated by the production of green gram. Taken together, IPM with a combination of botanical pesticides and green gram intercropping is a feasible effective approach for cotton aphid management.

Table 2: Mean number of lady beetle, lace wing and spider in three experimental seasons and seed cotton yield

Treatment	Mean number of Ladybeetles/plant			Mean number of Lacewings/plant			Mean number of Spiders/plant			Seed cotton yield
	2019 post	2020 pre	2020 post	2019 post	2020 pre	2020 post	2019 post	2020 pre	2020 post	
IPM field	2.38 ^{a}	2.16 ^{a}	2.24 ^{a}	1.20 ^{a}	1.17 ^{a}	1.12 ^{a}	1.12 ^{a}	1.05 ^{a}	1.11 ^{a}	687 ^{a}
Conventional field	0.30 ^{b}	0.26 ^{b}	0.21 ^{b}	0.03 ^{b}	0.07 ^{b}	0.02 ^{b}	0.03 ^{b}	0.03 ^{b}	0.03 ^{b}	703 ^{a}
LSD 0.05	0.30	0.36	0.39	0.12	0.13	0.16	0.11	0.09	0.11	79.85
SE (mean)	0.10	0.12	0.13	0.04	0.04	0.05	0.03	0.03	0.04	13.12
Prob	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.47
CV%	24.22	31.70	34.06	21.95	23.34	30.51	20.66	17.67	22.27	3.27

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

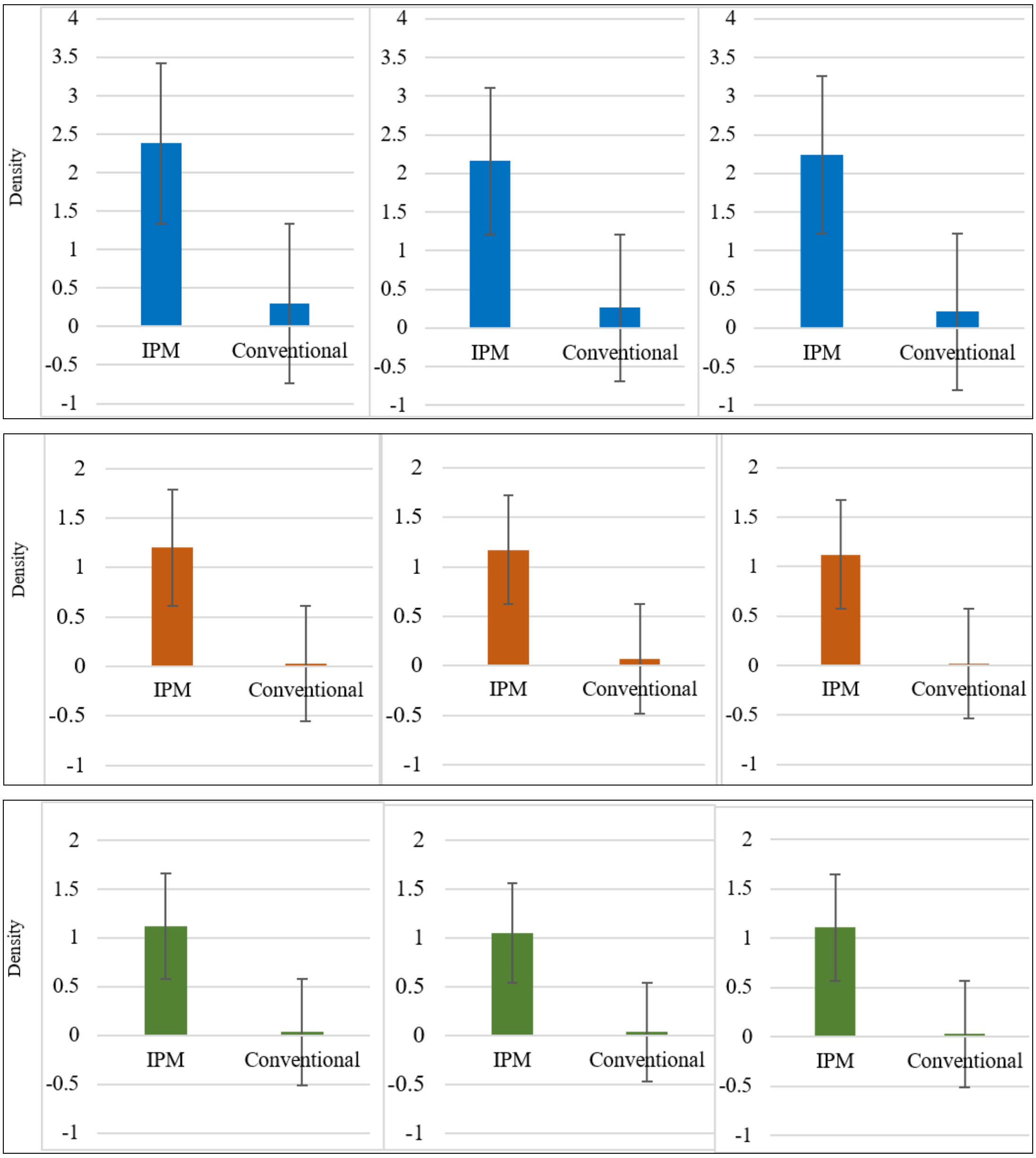

Fig 2: Mean number of predator densities in IPM and conventional cotton field for 2019 post-monsoon, 2020 pre-monsoon, and 2020 post-monsoon seasons (From left to right); ladybeetle (above), lacewing (middle), and spider (below)

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Conclusion
In conclusion, this study highlights the effectiveness of Integrated Pest Management (IPM) combined with green gram intercropping in significantly reducing cotton aphid densities in cotton fields compared to conventional practices. Over three experimental seasons, aphid populations in IPM fields consistently remained below the action threshold, demonstrating effective pest control, whereas conventional fields frequently exceeded this threshold despite more frequent pesticide applications. This indicates that the IPM approach, which utilizes botanical pesticides, is more efficient in maintaining aphid populations at manageable levels.
Natural enemies of cotton aphids, such as ladybird beetles, lacewings, and spiders, were significantly more abundant in IPM fields. The higher predator densities are attributed to the use of botanical pesticides, which are less harmful to beneficial insects, and the presence of green gram, which supports a more favorable habitat for these natural enemies. This abundance of predators in IPM fields contributes to the suppression of aphid populations, showcasing the ecological benefits of this approach.
Although the average seed cotton yield in the IPM fields was slightly lower than in conventional fields, the difference was not statistically significant. The reduced cost of botanical pesticides and the additional production of green gram make the IPM approach more cost-effective overall. These findings are consistent with previous research, reinforcing the conclusion that intercropping and IPM can effectively manage pest populations while maintaining crop yields.
The study concludes that IPM practices, including the use of botanical pesticides and green gram intercropping, offer a sustainable and economically viable solution for managing cotton aphids. This approach promotes the conservation of natural enemies, reduces dependency on chemical pesticides, and supports sustainable agricultural practices. Given the potential for pests to develop resistance to chemical pesticides, IPM practices provide a more durable and ecologically friendly alternative for cotton cultivation.

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