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Arthropod Diversity in Soybean (*Glycine max* (L.) MERRILL) with *Tagetes erecta*

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Abstract

Habitat manipulation aims to conserve insect population on agricultural landscapes by augmenting and conserving the population of natural enemies of pests or biological control agents. The study aimed to evaluate the effect of habitat manipulation on the yield of soybean by assessing the population of insects and other associated arthropods. Companion planting of *Tagetes* with soybean using inter-row and border row planting designs was followed. A total of 4, 388 individuals representing 11 insect Orders, and 128 species were collected through net sweeping and yellow sticky board traps. These two methods of insect collection were further compared using the two-planting designs. Border row planting design revealed a low population of insect pests, and thus better than the inter-row design. Also, higher number of biological control agents such as coccinellids was found in these plots than in soybean inter-row with *Tagetes erecta*, and soybean alone.

Keywords: soybean agroecosystem, companion planting, arthropod diversity

Introduction

Soybean, scientifically known as *Glycine max*, is an erect, bushy annual that varies in height from 1 foot to 8 feet depending upon the cultivar, day length, temperature, moisture, and nutrition requirement (Smith,1995) and also known as one of the "world's wonder crops" (Agcopra and Piadozo, 2018). Soybean is an important global crop, providing oil and protein. The bulk of the crop is solvent extracted for vegetable oil and the defatted soy meal is used for animal feed. A small proportion of the crop is consumed directly by humans. Soybean products appear in large varieties of processed food (Wilson and Clifford, 1975). Vegetable soybean is a rich and cheap source of vitamins, minerals, protein, energy, and fiber.

Soybean can be grown in the tropics and subtropics throughout the year. However, numerous factors threaten soybean production by directly reducing the yields and quality. Abiotic factors include temperature, relative humidity, and rainfall. Biotic factors include insects, pathogen, nematodes and weeds

(Heinrichs and Muniappan, 2018). The common insect pests in soybean plant are the bean fly or stem fly, soybean aphids, green stink bug, common cutworm, soybean leaf folder, green looper, soybean pod borer, and corn earworm that can be controlled using biological control such as weekly application of *Trichogramma chilonis* and spraying of vermi tea or naturally fermented solutions (NFS). For chemical control, spraying of appropriate insecticide following the recommended rate is suggested (Sicat and BuÒo, 2014). However, pesticides have been gaining infamy by leaving behind consequences that are equally important than controlling pests alone such as health concerns brought about by pesticide residues and environmental contamination.

Habitat manipulation is one way that can promote lessened pest population by focusing on the enhancement of natural enemies. It is another form of conservation biological control. "Habitat manipulation is the manipulation of agricultural area and surrounding environment with the aim of conserving or augmenting population of natural enemies". It is one of the approaches promoting bottom-up control. Manipulation within the crop, such as green mulches and cover crops (first trophic level) will act on pests directly (Lalbabu et al., 2013).

The planting of companion plants such as marigold with the soybean is an example of bottom-up control. Marigolds possess rich nectar and can help support local populations of bees and other pollinating insects (Comba et al., 1999) and successful control of the nematodes in vegetable crops. Marigold plants are more recognized for their nematicidal properties and are mostly grown as ornamental plants in home gardens. Inter-row and border planting designs using a companion plant (Marigold) in soybean are examples of planting designs that may increase the density of natural enemies. It is assumed that the inter-row planting design of marigolds may cause confusion in insects. In border planting design, marigold is intended to serve as a barrier from pests reaching the soybean plants.

Materials and Methods

Site Selection

The experiment was conducted at Ramon Magsaysay Center for Agricultural Resources and Environmental Studies (RM CARES). The site is well-drained, and the type of soil is clay loam suitable for soybean plants. The area practices minimal or zero pesticide application.

Layout and Design

The field experiment was conducted in a 16 x 13 m^2 area with a total of 208 m^2. The number of plots in the area was twelve, where the sizes of the plots were 2 x 3 m^2. The distance between plots was 1m and 2m between blocks. There were four replications and three treatments.

The treatments were as follows:
T_1 – control or untreated
T_2 – inter-row companion planting design
T_3 – border row companion planting design

Test Plant. Soybean variety (main crop)

The CLSOY – 1 (SJ – 2) variety of soybean was used in this experiment. CLSOY – 1 (SJ - 2) is a non-photoperiod variety with a maturity of 90 to 95 days. The variety has a uniform maturity, non-lodging, moderate resistance to shattering and bacterial pustule, and strain of rust. The variety has a yield potential of 1.5 to 2.5 tons per hectare (Francisco & Arimado, 2015).

Marigold (Companion plant).

The companion plant of soybean used in this field experiment was *Tagetes* (Marigold or Amarillo). These were planted ahead in pots so that their flowering will coincide with the growth of soybean.

Sampling Method

A sampling of insects in the experimental field was conducted at weekly intervals. Using the sweep net, each plot was swept 10 times beginning at 6:00 AM for each sampling schedule. One yellow sticky board trap (YSBT) measuring 5 x 8" was installed in every plot between the two rows of soybean after the net sweeping. The sticky boards were retrieved after 24 hours, and the trapped insects were collected and sorted. Some insects that stuck were rinsed off using 70% ethyl alcohol. Visual observation on the soybean plants was conducted from 3:00 – 5:00 PM. The plants were directly examined for the presence of insects. Data were recorded in appropriate data sheets.

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Results and Discussion

A total of 4388 Arthropods were collected through net sweeping and yellow sticky board traps; the 3549 collected by net sweeping is distributed as follows: 1,458 from control or untreated, 1067 were from inter-row and 1024 were from border row planting designs. For YSBT, a total of 839 individuals were collected where 324 were from control or untreated, 263 from inter-row, and 252 from border row. Ten orders of insects (Class Insecta) were represented which include Diptera, Hemiptera, Hymenoptera, Coleoptera, Lepidoptera, Odonata, Orthoptera, Ephemeroptera, Thysanoptera, and Mantodea. Collections from the Class Arachnida include the Araneae (spiders).

The insects were classified into pests, natural enemies, or miscellaneous. The insect pests associated with soybean that were collected from the experimental field are presented in Table 1. The following are considered major pests namely, beanfly (*Liriomyza*), aphids (*Aphis*), green stink bug (*Nezara)*, legume shield bug (*Piezodorus*), bean bug (*Riptortus*), bean plataspid bug (*Coptosoma* and *Megacopta*), seed bug (*Nysius*), leafhopper (*Amrasca*). Other leafhoppers that were not known pests of soybean but occurred frequently and in substantial numbers, were *Nephotettix* and *Recilia*. There were pests that were not found during the study such as the common cutworm, corn earworm, and soybean podborer. The larval stage of the soybean leaffolder and green looper were noted during the visual observation.

Table 1. List of insect pests collected from soybean and soybean combined with marigold in two planting designs inter-row and border row from December, 2017 to April, 2018 at RM CARES experimental field

PEST		NUMBERS					
		Sweep Net			YSBT		
		T1	T2	T3	T1	T2	T3
Liriomyza	Diptera:Agromyzidae	58	47	44	13	19	10
Aphis	Hemiptera:Aphididae	2	2	0	44	31	25
Nezara	Hemiptera:Pentatomidae	4	0	3	0	0	0
Piezodorus	Hemiptera:Pentatomidae	3	0	0	0	0	0
Riptortus	Hemiptera:Alydidae	1	1	2	0	1	0
Coptosoma	Hemiptera:Plataspidae	41	8	10	54	47	41
Megacopta	Hemiptera:Platasidae	1	0	0	0	0	0
Nysius	Hemiptera:Lygaeidae	0	2	3	0	0	0
Amrasca	Hemiptera:Cicadellidae	485	342	314	1	2	2
Nephottetix	Hemiptera:Cicadellidae	23	11	10	0	0	2
Recilia	Hemiptera:Cicadellidae	36	17	25	3	4	1
Sogatella	Hemiptera:Delphacidae	4	5	6	0	0	0

Leafminer (*Liriomyza*)

This was the most frequent fly caught by net sweep method of sampling. *Liriomyza* of the family Agromyzidae are small flies whose larvae feed on the internal tissue of plants, often as leafminers and stem miners. Larvae feed mostly in the upper part of the leaf, mining through the green palisade tissue. Mines are usually off-white, with trails of frass appearing as broken black lines along the length of the leaf. The resulting leaf discoloration of the mine, with dampened black and dried brown areas appearing, usually as the result of plant-induced reactions to the leafminer (EPPO, 2005). These may be found in different locations of

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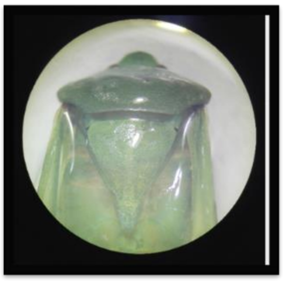
the plant and surrounds depending on the life stages, from the egg stage is inserted just below the leaf surface, larval stage is inside mines on leaves, pupal stage found in crop debris, in the soil or sometimes on the leaf surface and from adult stage the free flying or on leaf surfaces while producing feeding and oviposition punctures (IPPC, 2016). The kind of damage done is the reason this fly is considered a major pest of soybean.

Soybean Aphid (*Aphis*)

Soybean aphids have sucking mouthparts and damage is done by extracting sap or phloem. Aphids spread throughout the plant and attack flowers, pods, stems and leaves. They feed on the undersides of newly emerged or emerging leaves. Yellowing and warped leaves are the symptoms of plant damaged of soybean aphids, although other pests and stressors can cause similar damage, necessitating proper scouting (Grettenber and Tooker, 2011). Some dried-up leaves were found as evidence of their damage.

Green Stink Bug and Legume Shield Bug (*Nezara and Piezodorus*)

Both the nymphal and adult stages of green stink bug and legume shield bug attack primarily the seeds and pods of soybean plants. They also feed on soybean plant stems, foliage, and blooms that cause small brown or block spots. The direct feeding damage can lead to a reduction in seed quality and quantity. Young seeds can be deformed, undersized or even aborted (Boyd and Bailey, 2000).



Bean Bug or Bean Pod Sucking Bug (*Riptortus*)

The eggs of bean bugs or bean pod sucking bug are laid on bean leaves. Bean bug is the major pest of beans. Both adult and nymphs sting the bean pods and extract the juices that the beans fail to mature. The pods turn brown, shrivel and die (Tsatsia and Jackson, 2017).

Bean Plataspid Bug (*Coptosoma* and *Megacopta*)

Bean plataspid bugs prefers to feed on new plant growth, where it extracts photosynthate from the phloem (Zhang et al. 2012). It can commonly found feeding along plant stems, petioles, leaves, pods, and possibly flowers (Zhang et al. 2012, Seiter et al. 2013) of various plants. Damage resulting purple spots that later coalesce to form large black necrotic regions (Thippeswamy and Rajagopal 454 2005), and extensive feeding may result in defoliation (Chaterjee 1934). As it is a phloem feeder, they produce copious amounts of honeydew that results in secondary plant issues such as black sooty mold leading to reductions in photosynthetic ability of the plant (Zhang et al., 2012).

Seed Bug (*Nysius*)

These bugs feed on seeds, it is common to observe damage of the vascular tissues (Ashlock, 1977) and it is a suctorial insect that draws water and nutrients from plants. The symptoms of damage are distortion, chlorosis and wilting of cotyledons (Molinari and Gamundi, 2010). The damage in the field was not evident which was not surprising since the numbers of the seed bug was low.

Leafhopper (*Amrasca*)

Both adult and nymphs feed on soybean, but the most serious damage is caused by the nymphs. Leafhoppers uses its piercing-sucking mouthparts to remove plant juices, it injects a toxin into the plant which causes a decrease in the ability to produce photosynthesis. Yellowish patches on the leaves with crinkling and cupping is the symptoms damage by leafhoppers. Other hoppers observed were *Recilia* and *Nephotettix* whose numbers were substantially high. However, since the study did not deal with yield loss, the observation was included for documentation purposes.

Planthopper (*Sogatella*)

Planthoppers can directly damage the crop by sucking the phloem and indirectly by transmitting viral diseases (Heong and Hardy, 2009). Plants turn yellow and dry up rapidly. Heavy infestation creates brown patches of dried plants known as hopperburn. In the same way as leahoppers, damage from this cannot be substantiated. In contrast, the numbers of the planthoppers were minimal during the conduct of the study.

Low/Non-Occurrence of Lepidopteran Pests

Lepidopteran larvae are the common pests that are normally found in soybean. But in this research there was no larvae of Lepidopteran insects observed or caught in net-sweeping and yellow sticky board traps. It was seen at times during visual observation alighting on the flowers of *Tagetes* but not that frequent. According to Srinivasan et al. (1994), *Tagetes*, can be used as a trap crop because the female moths are highly attracted to the floral parts of the plants for both ovipositing and nectar feeding (Burguiere, Marion-Poll and Cork. 2001). Female moths tend to lay eggs on *Tagetes*.

Arthropod Diversity in Soybean with *Tagetes* as Companion Plant

The comparative counts of arthropods classified by insect orders, collected through net sweeping as affected by the planting designs, inter row and border row using marigold are shown in Figure 2. It is notable that two insect orders, Hemiptera and Diptera are apparently the most represented in terms of numbers from all the plots respective of the treatments. Lagging behind were the other arthropod groups, but amongst these, Hymenoptera and Araneae revealed slightly more numbers. Lepidopterans which were expected to have more numbers were evidently kept at low numbers. Mayflies or Ephemeropterans which are not known to occur in traditional agroecosystems were found and observed within plots. Generalist predators such as Odonatans and mantises were also present. The rest, like Orthopterans and Coleopterans known to be common in fields were also found

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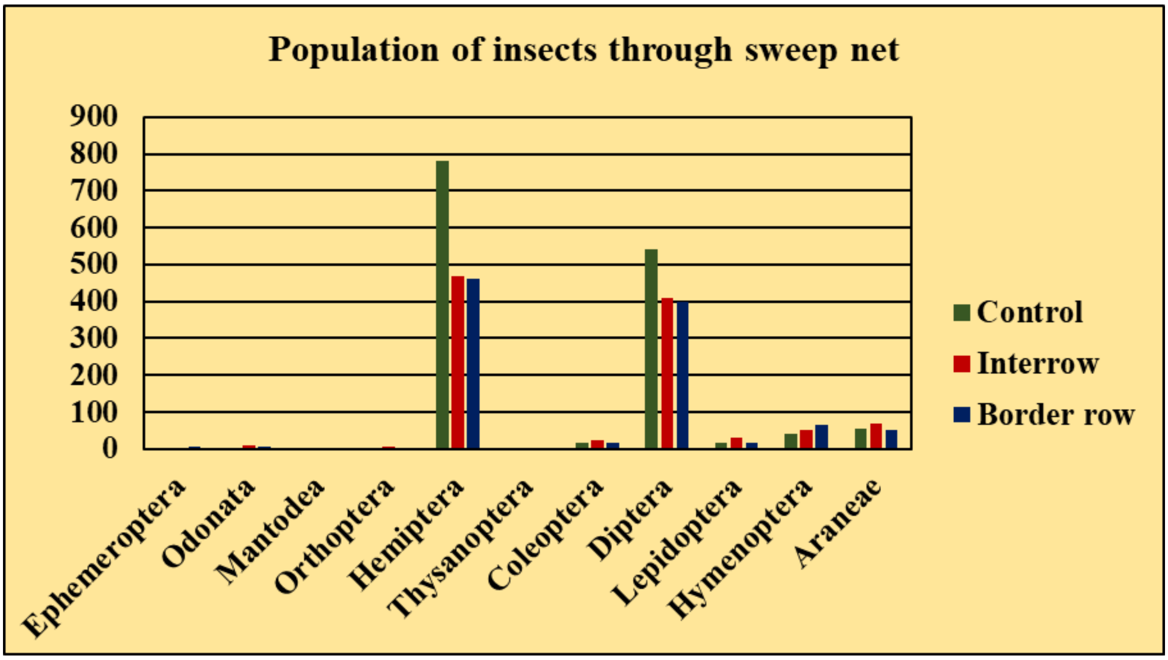

Figure 2. Comparative counts of arthropods (insects and spiders) between border, inter row and border row planting designs of marigold in soybean plants

In control or untreated, Hemiptera had the highest population with a total number of 780, followed by Diptera with a total of 542, Araneae (56), Hymenoptera (41), Lepidoptera (18), Coleoptera (15), Orthoptera (4), Odonata (1), Thysanoptera (1). (Appendix Table 1). The highest population of insects recorded in inter row planting design of marigold in soybean plants belongs to Hemiptera with a total population of 468, followed by Diptera with a total count of 410, Araneae (69), Hymenoptera (52), Lepidoptera (29), Coleoptera (22), Odonata (8), Orthoptera (5), Ephemeroptera (2), Thysanoptera (2). Data is detailed in Appendix Table 1.

In border row planting designs of marigold in soybean plants, Hemiptera had the highest population with the total of number of 463, followed by Diptera with a total population of 399, Hymenoptera (64), Araneae (51), Coleoptera (17), Lepidoptera (17), Odonata (5), Ephemeroptera (5), Orthoptera (2) and Mantodea (1).

The Hemiptera and Diptera had the highest insect populations which is presented in figure 2. Comparison of the three treatments showed a similar trend as the over-all tally of populations from both yellow sticky board and net-sweep. The control or soybean alone had the highest population of Hemipterans, and the most abundant insects represented were the hoppers (*Amrasca*) and bean plataspid bug (*Coptosoma*). Similar to the over-all counts, Diptera were the next represented comprising of the beanfly (*Liriomyza*) and midge (*Chironomus*). The Hymenopteras (most are known natural enemies) have low counts coming from the soybean alone, i.e. when compared to the two treatments with soybean and Tagetes in the inter-row and the border row designs. The Tagetes from the mixed planting probably attracted them because most are known pollinators and flowers are their main source of food (Bhardwaj et al., 2012). Although some Dipterans are pests, predators and parasitoids represented here are the dipterans that parasitize hoppers (*Amrasca*) such as the longlegged fly (*Condylostylus*) and big-headed fly (*Pipunculus*). The known hovering agents like the hover fly (*Eristalis*) were also collected. The larvae of which are known to feed on aphids (Aphis). The tachinid fly (*Chetoptilia*) is the known parasitoid of caterpillars and also other insects (Shepard, Barrion and Litsinger, 1987). These were also found occurring in the experimental plots. Visual observations showed some insects not collected by yellow sticky board trap and netsweeping. These included fast fliers like dragonflies and damselflies. There were some mayflies (*Caenidae*) seen also. The comparative counts of arthropods collected through YSBT between control, inter row and border row planting designs of marigold into soybean plants are presented in Figure 3.

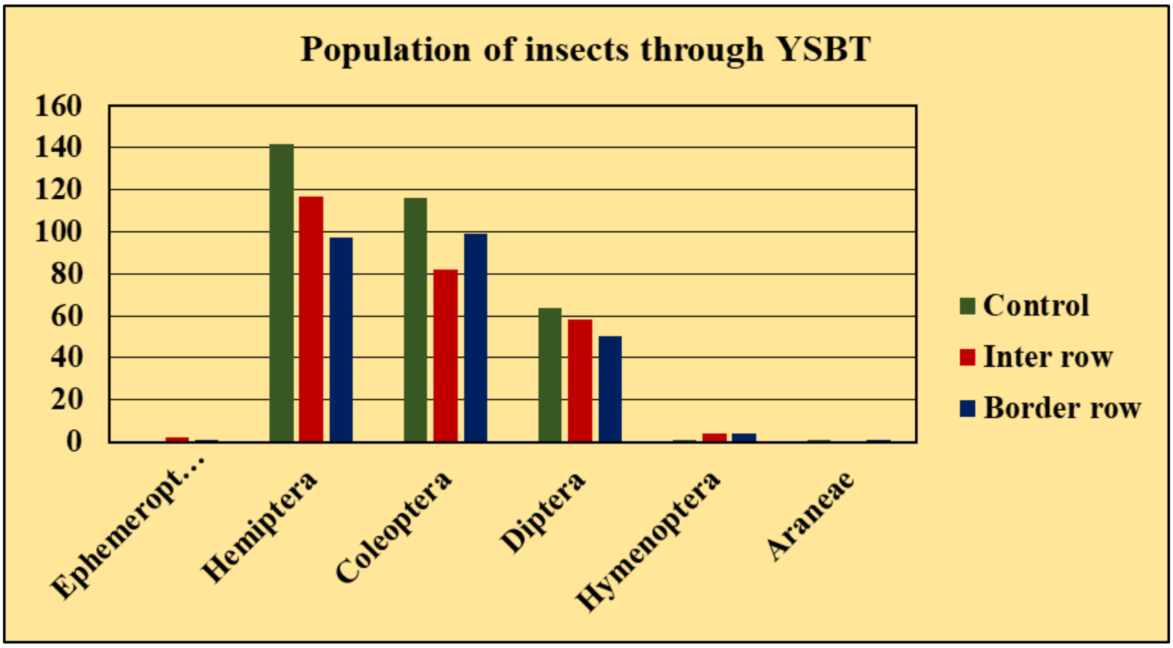

Figure 3. Comparative counts of arthropods (insects and spiders) between border, inter row and border row planting designs of marigold in soybean plants

In control or soybean alone, Hemiptera had the highest population with a total number of 142, followed by Coleoptera with a total population of 116, Diptera (64), Hymenoptera (1), Araneae (1). Numbers are recorded further in Appendix Table 2. It is notable that Coleoptera was represented more through yellow sticky board trapping and was even higher than the Diptera. Hemipterans were remained the highest using the aforementioned technique. In the same vein, in inter-row planting design with marigold and soybean plants, the Hemiptera had a total population of 117, followed by Coleoptera with a total count of 82, Diptera (58), Hymenoptera (4), Ephemeroptera (2).

In border row planting design of marigold and soybean plants, Coleoptera had the highest population with the total number of 99, followed by Hemiptera with a total population of 97, Diptera (73), Hymenoptera (4), Ephemeroptera (1), Araneae (1).

The results for border row was different from the first two, with Coleoptera having the highest population recorded. The coccinellid beetles are known predators especially of aphids. It is likely that the Coccinellids were attracted to the yellow color and were cornered or contained within the borders of border row planting design.

Yellow sticky board trap is a commonly used method for population monitoring of many pests (Lu et al., 2012), and were also used to lessen populations within plots as in the leafminer control in the country. The control or soybean alone had the highest population counts of Hemiptera, Coleoptera and Diptera. The most abundant insect trapped by yellow sticky board traps were bean plataspid bug (*Coptosoma*), lady beetle (*Coccinellidae*) and aphid (*Aphis*). According to Stephen and Losey (2004), it was found that yellow sticky board traps collected more lady beetles than net sweep. According to Weinzierl et al. (2005), the winged aphids (*Aphis*) and whitefly (*Bemisia*) are attracted to yellow objects.

Weekly Population Trends Between Pests and Natural Enemies

The population trends of natural enemies plotted against selected pests that were found during the sampling period are shown in Figure 4. The trend of pests versus natural enemies in the three treatments were similar. Although still at a low, the pests increased commencing from the growth of crops and dwindled at weeks 3 to 4. From the reproductive stage of soybean (weeks 5 to 11).

During the first four weeks, the most abundant insect pests were bean fly (*Liriomyza*), leafhoppers (*Amrasca, Nephottetix* and *Recilia*) and plant hoppers (*Sogatella*). It is noted though, that *Nephottetix* and *Recilia*, are not known pests of soybean but of rice. However, rice was not in the vicinity during the field trial. This observation infers that soybean can be a refugia. It remains to be tested whether there, build-up was evident. The low population of pests recorded from week 1 to week 4, coincided with the vegetative stage of soybean and the blooming stage of marigold flowers. Further increase in the population of the pests were observed during these can be pests of soybean or just attracted to the *Tagetes* or marigold. Week 7 was the sampling schedule that revealed peak populations of pests in all treatments. The natural enemies also reached their highest populations two weeks from the peak pest populations. The two treatments with mix planting designs increased from week 6 to 8 and were higher earlier than that of the control. At week 9, the natural enemies in all treatments increased and the pests decreased.

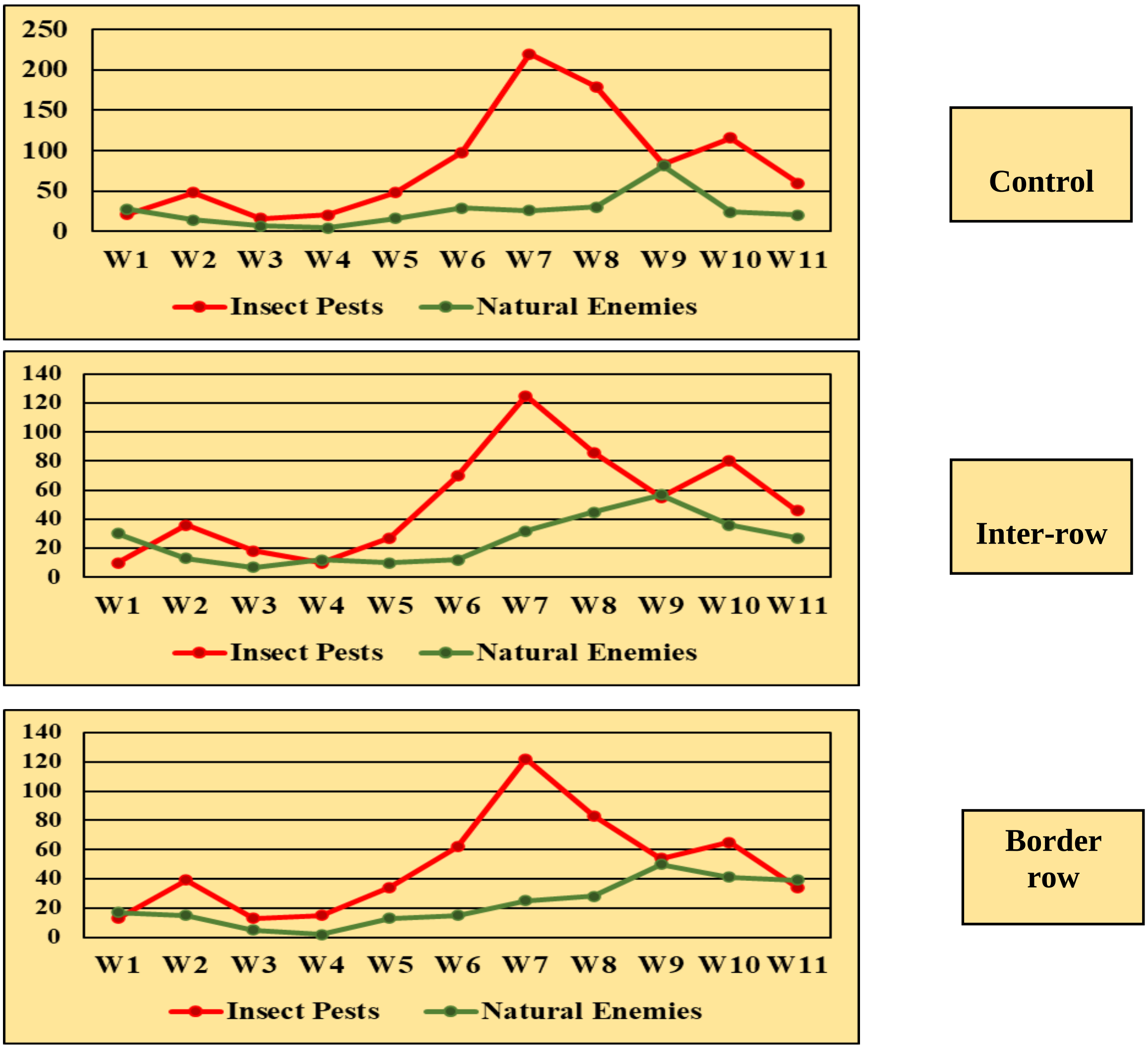

Figure 4. Population of insect pests and natural enemies in control or soybean alone, inter-row and border row planting design of marigold in soybean

This observation implied that planting marigold enhanced the presence of natural enemies. The subsequent increase in the numbers of natural enemies from the control plots could be an over-all impact on the entire experimental site. The size of the field was quite small as compared to plot sizes required of ecological studies. It would be interesting to find out the impact of mix planting (inter-row and border row) on a bigger experimental field so that diversity indices could be determined. It will also be interesting to conduct studies on a longer time frame (annual) to correlate other factors affecting the presence of natural enemies.

Another research that could be pursued will be to determine the effect of miscellaneous insects on sustaining the presence of natural enemies.

Soybean Yield

Data on yield is presented in Table 3 and photographs of bagged yield is shown in Figure 5. It was observed that yields from control plots were numerically higher than the inter-row and border row design - treatments. However, the differences among means were statistically insignificant. The benefits of the mixed planting design could have increased the natural enemies in the entire experimental site, hence pest pressure expected in the control plots were also lessened as in the case of the soybean plants with marigold inter-row and soybean plants bordered with the same.

Table 3. Yield of soybean as affected by the combination of *Tagetes* planted as inter-row or border row in the experimental field at RM CARES on December 2017 to April 2018

TREATMENTS	Yield ($6m^2$)	Projected Yield (ha)
T1 – Control	4.5 kg	7,500 t/ha
T2 - Inter-row	4.4 kg	7,333 t/ha
T3 - Border row	4.35 kg	7,250 t/ha

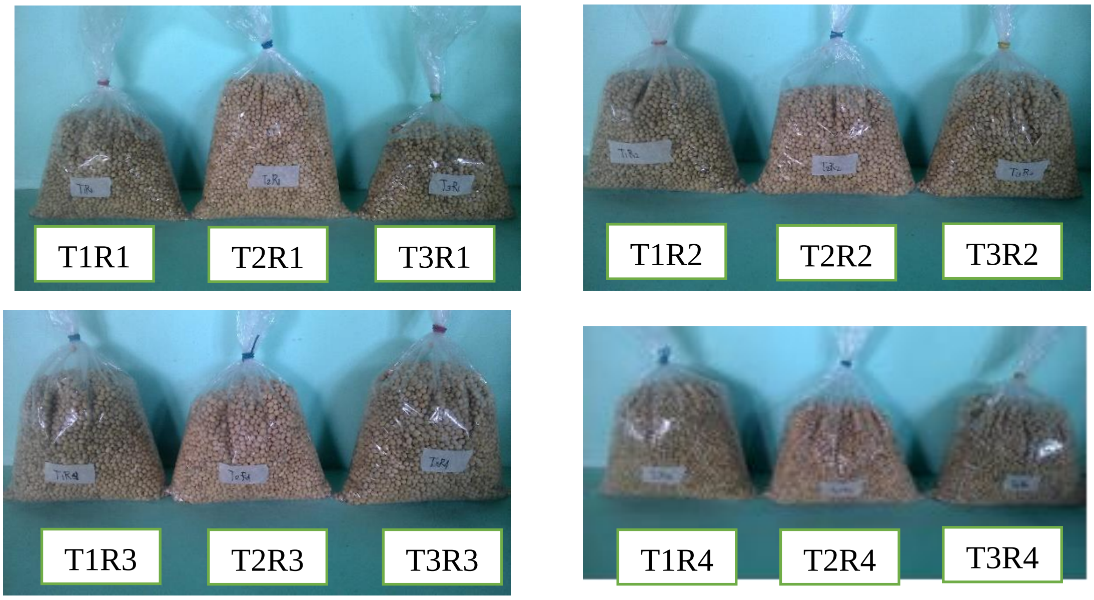

Figure 5. Yield of the three treatments with four replications from the experimental plots planted with soybean from December 2017 to April 2018

Based on the results of this research, the benefits of incorporating flowering plants whether as inter-row or as border row proved efficient in increasing natural enemies and enhancing diversity of arthropods within soybean fields.

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