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SHORT COMMUNICATION

Effect of genetic modified maize contained *Cry1Ab* gene on the arthropods abundance and diversity in limited test field

Lina Herlina[1] & Bonjok Istiaji[2]

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ABSTRACT

In Indonesia, transgenic products are still considered innovative, and genetically modified (GM) maize has stayed on the market while its impact on environmental biosafety is now being evaluated. Bt corn has been recognized as one of the solutions to the problem of Asian corn and cob borers to preserve maize production. Therefore, this study aimed to investigate the effect of Bt corn on arthropod richness and diversity in a limited testing field using a randomized block design with four different maize varieties as treatments in six replications. The significant findings showed that Bt corn had no significant influence on the diversity, evenness, and abundance index (Margalef and Meinhinick Index) in the limited testing fields, which were all greater at 85 days after plant (DAP) than 60 DAP. Based on the evenness and abundance index, we conclude that Bt corn does not harm the community of existing arthropods.

Key words: abundance, arthropod, Bt corn, diversity, genetic modified

INTRODUCTION

One of the solutions presented by biotechnology practitioners, involving pest-resistant transgenic plants, is gaining popularity due to the intricate nature of pest issues, particularly in the context of strategic agricultural products (ISAAA, 2017; Kumar et al., 2020). Genetically modified crops (GM crops) offer various advantages over conventional breeding, including the time saved in comparison to conventional breeding, which typically demands an extensive period to achieve the desired progeny (Kamthan et al., 2016). It's widely acknowledged that these crops can enhance people's well-being (Kumar et al., 2020; Turnbull et al., 2021). Consequently, certain countries have initiated their adoption despite ongoing debates concerning their safety (Azadi et al., 2017). Over the past 25 years, the cultivation area of GM crops has expanded over 113 times (ISAAA, 2018). GMO technology is widely regarded as the swiftest-adopted technology in modern agriculture (Kumar et al., 2020).

The direct insertion of a single dominant gene into commercial cultivars leads to higher financial gains compared to multigene breeding involving various traits. This is particularly evident in transgenic plants carrying the *Cry* gene from *Bacillus thuringiensis* (Bt), a crystalline protein pesticide that has been in use for over 50 years. This suggests the established efficacy and safety of the gene. Nonetheless, safety management protocols, including environmental monitoring, must be adhered to during production and application. Even though evaluation might support commercial production and application, these procedures are crucial (Chen et al., 2022).

The current environmental monitoring procedures have raised some agricultural concerns related to commercial cultivation (Nawaz et al., 2019). In Bt crops, the target insect pests may develop resistance to the toxin, which could complicate future management efforts (Tabashnik & Carriere, 2017; Campos et al., 2019). The development of resistance in the intended pests poses a threat to the sustainability of Bt crops (Jiang et al., 2016). Although the Indonesian Biosafety Commission declared Bt corn safe in 1999, its commercialization has been a lengthy process (Estiati & Herman, 2015) and has not progressed significantly. The primary maize pests targeted by the first Bt-transgenic maize tested in Indonesia were the Asian corn borer (*Ostrinia furnacalis*) and *Heliothis* species. These pests significantly reduce Indonesia's maize production. There are critical issues associated with these transgenic plants that must not be overlooked and are prerequisites for the acceptability of engineered products.

Corresponding author:
Lina Herlina (tydars66@gmail.com)

[1]Research Center for Genetic Engineering, National Research and Innovation Agency. Jl. Jakarta-Bogor Km.46, Cibinong-Bogor Indonesia 16911
[2]Department of Plant Protection, Faculty of Agriculture, IPB University. Kampus IPB Darmaga, Bogor Indonesia 16980

Numerous studies have examined the impact of Bt plants, such as rice, cotton, and corn, on non-target organisms, particularly natural enemies. Transgenic crops can lead to the persistence of foreign gene expression products in the soil through crop stubble, root exudates, and pollen transmission (Baumgarte & Tebbe 2005). Moreover, they might alter the composition and content of the soil in the vicinity of the plant rhizosphere, thereby influencing the diversity and abundance of soil fauna. This could ultimately jeopardize the varied functions of soil ecosystems (Baumgarte & Tebbe 2005).

These products are still considered novel, and over the past decade, Bt corn has been introduced and is presently undergoing evaluation for its environmental impact. However, GM maize is recognized as an alternative solution to address the corn-borer pest issue that poses a threat to maize production. The study of these products' safety in Indonesia is crucial, particularly concerning their effects on the environment, including non-target organisms like natural enemies and other unintended pests.

The study aimed to investigate the abundance and diversity of arthropods in Bt-maize plots compared to non-transgenic maize plots within a confined testing area. The objective was to assess the impact of Bt corn on the organisms inhabiting the environment from an ecological standpoint. These findings hold significant value, especially as foundational insights for determining the biosafety of transgenic corn plants, including their development, growth, and eventual commercialization in Indonesia.

MATERIALS AND METHODS

Research Site. The experiment was conducted in a restricted test field at the Indonesian Center for Agricultural Biotechnology and Genetic Resources Research and Development (ICABIOGRAD), located in Bogor, West Java (Lat S-6° Long E 106°47'), in the year 2010. The Limited Test Facility serves as a dedicated testing area under the management of ICABIOGRAD, specifically designed for conducting tests related to transgenic products.

Research Design. GM-maize containing *Cry1Ab* gene (Bt-corn) and three popular hybrid maize varieties—Popular Hybrid-1, Popular Hybrid-2, and Popular Hybrid-3—were utilized in the study. Popular Hybrid-1 served as the parent variety for assembling the Bt-corn. The research was conducted using a randomized block design, with four different varieties employed as treatments across six replications.

With a total plot area of approximately 1800 m^2, the corn was cultivated in plots measuring 7.5 × 10 m^2, with a spacing of one meter. Planting utilized a spacing of 75 × 20 cm, involving the placement of two seeds per hole, subsequently thinned at 14 days after planting (DAP). Plant maintenance, including fertilization, weeding, and watering, adhered to Indonesian maize cultivation practices. These practices encompassed treating seeds with the fungicide dimethomorph 50% to counteract downy mildew attacks—a common issue in the field. Additionally, the insecticide carbofuran 3% was applied by injecting it into planting holes at a rate of four grains per hole, aiming to deter seed flies.

Traps positioned within each experimental plot were utilized to assess and examine insect abundance. The yellow-sticky trap or "Kupu" fly insect trap (Infarm, Indonesia), measuring 20 × 16.5 cm, is a potent adhesive trap engineered to capture insects within the plant canopy. The trap's yellow color attracts small insects (parasitoids), and to evaluate populations across different growth stages, the traps were deployed twice—at 60 and 85 days after planting (DAP).

The traps are affixed with wooden stakes that are secured to the canopy plants. The yellow sticky trap is attached to a stake positioned one inch above the plant canopy. As the plant height increases, the yellow sticky trap adjusts its position to match the plant crown's height. In each plot, four traps were positioned based on the cardinal directions: north, south, east, and west. After being in place for two days, the traps were retrieved, and the captured insects were identified within a laboratory setting. The quantity of insects and arthropods, categorized by their functional groups, was assessed.

Insect Identification. Arthropod identification up to the family taxonomic level was carried out using various keys (Borror et al., 1996; Gibson et al., 1997, Noyes, 2019). In addition, identification was also conducted by matching the specimen collected with insect reference specimens in ICABIOGRAD.

Data Analysis. After tallying, observations on the captured insect numbers were analyzed by categorizing them according to their roles as phytophages, predators, parasitoids, pollinators, and saprovores. The analysis of insect diversity indices, richness, and evenness in each plot was calculated and assessed using the Margalef species richness (Richness Index-RI), Meinhinick, Shannon (Diversity Index), and Pielou evenness index (Evenness Index) (Magurran, 1988), which are commonly used in studies involving species abundance and diversity. Arthropod abundance, diversity, and evenness indices between the Bt-corn and other plots (Non-Bt corn plots) were compared using a t-test (with α set at 0.05) to determine any significant differences, employing Minitab 19. The formulas for each index are as follows:

$$R1 = \frac{S - 1}{(\ln N)} \qquad R2 = \frac{SN}{\sqrt{N}}$$

R1 = Margalef's diversity index;
R2 = Meinhinick diversity index;
N = The total number of individuals in the sample;
S = The number of species recorded-here represent by number of family recorded.

The formulas of the Shannon index (H'):

$$H' = \sum_{i=1}^{S} (pi)(\text{In } pi)$$

H' = Diversity index Shannon-Wiener;
pi = The proportion of paricular individuals species found (ni) divided by the total number of individuals found (N).

The formulas of the Pielou/Evennes Index (E):

$$E = \frac{H'}{\ln(S)}$$

E = Diversity index Pielou/Evennes;
S = The number of species.
If all species are represented in equal numbers in the sample, then E = 1.

RESULTS AND DISCUSSION

The Abundance of Arthropods Based on their Ecological Function. Arthropod abundance refers to the number of individual arthropods present at the research site. To facilitate the assessment of the impact of transgenic maize plants on the existing arthropod abundance, the analysis of observed outcomes is categorized according to the ecological roles of these insects within the ecosystem. Specifically, they are classified as phytophages (plant feeders or herbivores that act as insect pests when their populations are high), predators (natural enemies of pests), parasitoids (natural enemies of pests), pollinators, and saprovores (detritivores).

Phytophagous Insects (Potential Pests). The highest abundance of phytophagous insects in the test plot was found in the plots planted with Bt-corn, as depicted in Table 1, where the number was greater in plants aged 85 DAP than 60 DAP. This indicated an increase in the population of phytophagous insects with the increase in the age of corn plants. The phytophagous species that dominated and were observed evenly in all test plots were Agromyzidae (Diptera), Psyllidae (Homoptera), Aphididae (Homoptera), Cecidomyiidae, Drosophilidae (Diptera), Alydidae (Hemiptera), and Thripidae (Thysanoptera). Further observation showed that Delphacidae occupied the largest family trapped in the Bt corn plot and expanded at 85 DAP.

Predatory Insects. There were five types of predators caught, one of which was from the spider group, and the other four were insects, including Dolichopodidae, Gryllidae, Staphylinidae, Formicidae, Sphecidae, and Araneidae, as shown in Table 2. The main predators in all plots were Staphylinidae (Coleoptera) and Formicidae (Hymenoptera). In contrast, the family Aranaea (spider-non-insect group) was only found in the Bt Maize and Popular Hybrid-3 plots.

Parasitoid Insects. Based on the results, a parasitoid from the family Tachinidae (Order Diptera) was found in all test plots, with the highest number in the Popular Hybrid-2 maize plot. Meanwhile, parasitoids from the Order Hymenoptera were Aphelinidae, Scelionidae, Signiphoridae, Encyrtidae, Trichogrammatidae, Perilampidae, and Platygasteridae. In ecological studies, the status of parasitoid abundance becomes one of the parameters used to measure or determine the safety of introducing certain genetically engineered products in certain locations. Phytophages or herbivores, saprovores (detritivors), predators, and parasitoids in a healthy ecosystem are sensitive to environmental changes due to the introduction of genetically engineered products. Based on the ecological role of arthropods, the parasitoid group ranked highest in catches in all plots compared to other groups, reaching 399 individuals in the Popular Hybrid-2 plot, as indicated in Table 3.

Pollinator Insect. The Popular Hybrid-2 maize plot had the most pollinating insects, with a total of seven from four Hymenoptera families, as summarized in Table 4. Pompilidae were the pollinators found in almost all tested plots, including in Bt-corn plots. There were Apidae and Pompilidae captured in the Bt maize plot. The families of Apidae and Pompilidae were crucial pollinators in the environment. Although there were not many captures, their presence in the Bt maize plots was essential, as it indicated that Bt maize did not have a negative impact on pollinator insects. However, it is also important to investigate why Apidae pollinators showed interest in visiting only the Bt maize plots.

Saprovore. Saprovores are organisms that contribute to maintaining the balance of biomass on Earth, making their presence in an agricultural ecosystem extremely important. Therefore, their presence in an ecosystem serves as a measure of how well the ecological system is functioning. Generally, only a few predators were caught, with the Diptera family consisting of seven families accounting for the largest catch (Chironomidae), Gryllidae (Orthoptera), and

Table 1. Accumulative amount of phytophagous insects captured in Bt-corn and non-Bt-corn plots

Order-Family	Amount of phytophages insects in plots							
	60 DAP				85 DAP			
	Bt-corn	Popular-Hybrid-1	Popular-Hybrid-2	Popular-Hybrid-3	Bt-corn	Popular-Hybrid-1	Popular-Hybrid-2	Popular Hybrid-3
Diptera								
Agromyzidae	19	15	27	31	10	11	20	24
Cecydomiidae	17	7	12	11	9	4	8	8
Drosophilidae	19	1	6	6	15	1	4	5
Opomyzidae	-	1	-	-	-	1	-	-
Tephritidae	-	2	-	2	-	-	2	-
∑ Diptera	55	24	47	48	34	17	34	37
Orthoptera								
Acridididae	1	-	-	2	1	-	-	2
Locustidae	-	2	-	2	-	2	-	2
Eumastacidae	-	4	-	-	-	3	-	-
∑ Orthoptera	1	6	-	4	1	5	-	4
Homoptera								
Psyllidae	12	25	40	11	9	13	22	7
Aphididae	17	17	4	14	5	12	4	9
Delphacidae	1	1	-	-	63	1	-	-
Cicadellidae	-	-	-	-	-	46	-	-
∑ Homoptera	30	43	44	25	77	72	26	16
Hemiptera								
Alydidae	1	1	3	1	1	1	2	1
Coleoptera								
Coccinellidae	3	4	-	3	2	4	-	3
Nitidulidae	-	-	-	3	-	-	-	2
Ptinidae	-	-	1	-	-	-	1	-
Rhysodidae	-	1	-	1	-	1	-	2
∑ Coleoptera	3	5	1	7	2	5	1	7
Thysanoptera								
Thripidae	10	6	8	2	5	4	6	2
Acarina								
Acarina (Tetranychidae)	-	3	1	-	-	3	1	2
∑ Phytophagous	100	88	104	87	120	107	70	69

(-) = Not found (0); DAP = Days after planting.

Table 2. Accumulative amount of predatory insects captured in Bt-corn and non-Bt-corn plots

Order-Family	Amount of predatory insects in plots							
	60 DAP				85 DAP			
	Bt-corn	Popular Hybrid-1	Popular Hybrid-2	Popular Hybrid-3	Bt-corn	Popular Hybrid-1	Popular Hybrid-2	Popular Hybrid-3
Diptera								
Dolichopodidae	-	2	7	5	-	2	5	4
Coleoptera								
Staphylinidae	4	1	2	1	3	2	2	1
Araneae								
Araneidae	1	-	-	1	1	-	-	1
Hymenoptera								
Formicidae	6	2	2	3	4	1	2	3
Sphecidae	2	-	-	-	1	-	-	-

(-)= Not found (0); DAP = Days after planting.

Table 3. Accumulative amount of parasitoid insects captured in Bt-corn and non-Bt-corn test plots

Order-Family	Amount of parasitoid insects in plots							
	60 DAP				85 DAP			
	Bt-corn	Popular Hybrid-1	Popular Hybrid-2	Popular Hybrid-3	Bt-corn	Popular Hybrid-1	Popular Hybrid-2	Popular Hybrid-3
Diptera								
Tachinidae	17	9	36	21	15	5	27	16
Hymenoptera								
Aphelinidae	1	2	2	1	2	2	2	1
Scelionidae	19	36	41	37	13	16	17	26
Signiphoridae	69	54	118	37	23	35	65	32
Mutillidae	1	1	11	-	1	1	7	-
Encyrtidae	45	26	12	47	14	14	8	26
Trichogramatidae	2	2	1	2	1	2	1	2
Mymarommatidae	2	-	-	1	2	-	-	1
Eulophidae	1	2	-	-	2	2	-	-
Proctotrupidae	1	-	-	-	1	-	-	-
Ichneumonidae	5	-	3	3	5	-	2	3
Perilampidae	3	2	6	1	3	2	6	1
Bethylidae	1	3	1	-	2	2	1	-
Braconidae	-	1	-	-	1	1	-	-
Platygasteridae	5	17	14	15	4	15	8	10
Agaonidae	1	-	-	-	1	-	-	-
Cynipidae	1	-	-	-	1	-	-	-
Chalcididae	1	1	-	-	2	1	1	1
Ceraphronidae	-	-	1	1	-	-	1	1
Mymaridae	-	3	3	4	-	2	2	4
Eucoilidae	-	1	-	4	4	1	-	2
Diapriidae	-	-	-	1	-	-	-	1

Table 3. Continued

Order-Family	Amount of parasitoid insects in plots							
	60 DAP				85 DAP			
	Bt-corn	Popular Hybrid-1	Popular Hybrid-2	Popular Hybrid-3	Bt-corn	Popular Hybrid-1	Popular Hybrid-2	Popular Hybrid-3
Pteromalidae	-	-	-	1	-	-	-	1
Figitidae (wasp)	-	-	1	-	-	-	1	-
Eumenidae	2	-	-	1	2	-	-	1
∑ Hymenoptera	160	151	214	156	84	96	112	113
Total Number	177	160	250	177	99	101	149	129

(-) = Not found (0); DAP = Days after planting.

Table 4. Accumulative amount of pollinator insects captured in Bt-corn and non-Bt-corn test plots

Order-Family	Amount of pollinator insects in plots							
	60 DAP				85 DAP			
	Bt-corn	Popular Hybrid-1	Popular Hybrid-2	Popular Hybrid-3	Bt-corn	Popular Hybrid-1	Popular Hybrid-2	Popular Hybrid-3
Hymenoptera								
Apidae	1	-	-	-	1	-	-	-
Pompilidae	1	2	-	-	1	2	2	-
Megaspilidae	-	-	1	-	-	-	1	-
Vespidae	-	-	2	3	-	-	2	2
Tiphiidae	-	-	-	3	-	-	-	2
Crabronidae	-	-	3	-	-	-	2	-
∑ Hymenop	2	2	6	6	2	2	7	4
∑ Pollinator	2	2	6	6	2	2	7	4

(-) = Not found (0); DAP = Days after planting.

Table 5. Accumulative amount of saprovor insects captured in Bt-corn and non-Bt-corn test plots

Order-Family	Amount of saprovor insects in plots							
	60 DAP				85 DAP			
	Bt-corn	Popular Hybrid-1	Popular Hybrid-2	Popular Hybrid-3	Bt-corn	Popular Hybrid-1	Popular Hybrid-2	Popular Hybrid-3
Diptera								
Sepsidae	1	4	1	2	1	3	1	2
Phoridae	-	-	-	2	-	-	-	2
Sarcophagidae	-	-	1	-	-	-	1	-
Sciaridae	-	-	1	-	-	-	1	-
Chironomidae	29	30	18	21	31	7	7	13
Culicidae	10	2	20	6	7	2	12	6
Tipulidae	-	1	1	2	-	1	1	3
Collembola								
Isotomidae	1	-	-	1	2	-	-	1
Orthoptera								
Gryllidae	8	9	12	14	5	4	9	7
∑ Saprovor	49	46	54	48	46	17	32	34

Note : - = not found (0); DAP = Days after planting.

just one family from Isotomidae: *Collembola* sp. (Entomobryomorpha), as specifically described in Table 5.

Arthropod Abundance, Diversity and Evenness Index. The number of species present in an ecosystem is referred to as arthropod species richness, and sample size and time can impact the total number within a community. The Margalef Index was used to analyze the species richness of arthropods due to its simplicity in calculation. In all test plots, the order Hymenoptera had the highest rate of insect captures, as shown in Table 6, followed by the order Diptera and Homoptera.

The values of the diversity index (Shannon's H), evenness index (Evenness), abundance index-1 (Margalef-R1), and abundance index-2 (Meinhinick-R2) were all higher at 85 DAP than at 60 DAP, as presented in Table 7a. The Popular Hybrid-3 plot exhibited the highest R1, R2, H', and E values, while the Hybrid-2 plot had the lowest values. The Bt

Table 6. Accumulative amount of arthropods collected based on taxonomic order

Order	Amount of arthropods in plots							
	Bt-corn		Popular Hybrid-1		Popular Hybrid-2		Popular Hybrid-3	
	60 DAP	85 DAP	60 DAP	85 DAP	60 DAP	85 DAP	60 DAP	85 DAP
Diptera	115	90	76	40	134	91	108	84
Orthoptera	9	6	15	9	12	9	18	11
Hymenoptera	170	91	155	99	222	131	165	120
Homoptera	30	77	43	72	44	26	25	16
Hemiptera	1	1	1	1	3	2	1	1
Coleoptera	7	5	6	7	3	3	8	8
Thysanoptera	10	5	6	4	8	6	2	2
Araneae	1	1	-	-	-	-	1	1
Collembola	1	2	-	-	-	-	1	1
Acarina	-	-	3	3	1	1	-	2
Lepidoptera	-	-	-	-	1	1	-	-
Total	344	278	305	235	428	270	329	246

Note : - = Not found (0); DAP = Days after planting.

Table 7a. Indices of arthropod abundance, diversity, and evenness

Plot	∑ Order	∑ Family	∑ Individu (n)	Margalef Index (R1)	Meinhinick Index (R2)	Shannon Index (H')	Evennes (E)
60 DAP							
Bt-corn	9	40	344	6.68	2.16	2.89	0.78
Popular Hybrid-1	8	39	305	6.64	2.23	2.91	0.79
Popular Hybrid-2	8	39	428	6.27	1.89	2.73	0.75
Popular Hybrid-3	9	42	329	7.07	2.32	3.00	0.80
85 DAP							
Bt-corn	9	42	278	7.29	2.52	2.98	0.80
Popular Hybrid-1	8	40	235	7.14	2.61	2.97	0.81
Popular Hybrid-2	9	41	270	7.14	2.50	2.94	0.79
Popular Hybrid-3	10	44	246	7.81	2.81	3.14	0.83

Table 7b. T test for indices between 60 DAP vs 85 DAP

Value tested (indices)	t stat	t tabel	P	NS/S*
∑Order	1.0000	1.9432	0.1780	NS
∑Family	1.5785	1.9432	0.0828	NS
∑ Individu (n)	3.2995	2.4469	0.0082	S
Margalef Index (R1)	2.9815	2.4469	0.0246	NS
Meinhinick Index (R2)	3.9445	2.4469	0.0038	S
Shannon Index (H')	1.7369	1.9432	0.1331	NS
Evennes (E)	1.9973	2.4469	0.0928	NS

* significant at 0.01 probability level.

corn plot ranked second highest, with R1 = 6.68 and H' = 2.89 at 60 DAP, increasing to 7.29 and 2.98 at 85 DAP.

The distribution of individual abundance in each family is even, preventing any species from becoming predominant, as indicated by the Evenness index ranging from 0.75 to 0.83, as shown in Table 7a. This observation is further supported by comparing the values of all arthropod richness indices between the Bt and Non-Bt Corn plots (Table 8), where no significant difference is observed. The abundance of arthropods suggests that Bt maize does not have a negative impact on the community of existing arthropods.

Most of the insects from the families caught were insignificant pests on maize, such as aphids (Aphididae), psyllids (Psyllidae), and thrips (Thripidae). Some other groups might utilize this crop as an "alternative host," as seen with the presence of Leptocorisa sp. (Alydidae) and Delphacidae. However, due to the higher numbers compared to other plots, particular attention should be given to the Delphacidae in Bt maize at 85 DAP. Although Peregrinus maidis has been considered an unimportant maize pest and has not received special attention, this Delphacidae planthopper seems to show greater interest in the tested transgenic maize, indicating its potential to become an important pest. This planthopper belongs to the Delphacidae family (Huang & Qin, 2017), and recent years have seen an increase in studies focusing on hemipteran insect vectors (Pacheco et al., 2022). However, the continued use of Bt crops can prompt target pests to develop resistance actively, including in P. maidis (Xiao & Wu, 2019).

Staphylinid beetles are commonly found in various terrestrial habitats, including under leaf litter or decaying parts of trees in forests, in grass, fruit and wood decay, animal waste, under rocks, or near water sources. Some species associated with flowers prey on a wide range of insects and invertebrates (Frank & Thomas, 2011; Ritanti & Haryadi, 2021). The most prominent species identified was the tomcat beetle (Paederus fuscipes), recognized for its distinct brilliant elytra color, elongated body shape, small size, and short elytra. This insect possesses a poisonous substance called paederin (Ritanti & Haryadi, 2021), and based on the highest number captured, its population was found in the Bt corn area. Additionally, a family of predatory creatures called Formicidae is also frequently encountered. The elevated population of aphids captured in the Bt maize plot was closely linked to the high Formicidae population. Aphids and ants have a symbiotic relationship that is mutually beneficial and results in the production of honeydew for the ants, along with protection from predators. The presence of the Delphacidae planthopper, whose population is relatively high in the plot at 85 DAP and typically produces honeydew, becomes significant under these conditions.

The Signiphoridae had the highest count in each plot, implying that the parasitoid population is uniformly distributed across all test plots. As a minor family within Chalcidoidea, Signiphoridae comprises 88 acknowledged species distributed across four genera. This family is globally distributed, with the majority of its species residing in tropical regions. Some signiphorids function as obligate primary parasitoids on sternorrhynchan hosts, and they are recognized as hyperparasitoids of scale insects, mealybugs, and whiteflies (Hemiptera, Sternorrhyncha) (Noyes, 2019, Schmidt et al., 2019). Signiphoridae is larger in size compared to Encyrtidae. This study revealed that after Signiphoridae, Encyrtidae were the most frequently encountered parasites. Due to the extensive production and research on numerous parasitoids, Encyrtidae has been noted for its significant role in biocontrol (Japoshvili & Hansen, 2017). Alongside the

Table 8. The t- test result for all indices* between the Bt-Corn plot versus Popular Hybrid-1, Popular Hybrid-2 and Popular Hybrid-3 plots

Population	t stat	P	t table	d	NS/S
Bt Corn vs Pop Hyb-1	0.15677	0.43824	1.69726	30	NS
Bt Corn vs Pop Hyb-2	0.11268	0.45552	1.69726	30	NS
Bt Corn vs Pop Hyb-3	0.07057	0.47211	1.69726	30	NS
Bt Corn vs Non-Bt	0.03907	0.48448	1.69726	62	NS

NS: not significant at the 0.05 probability level

* : derived from ∑ Order, ∑ Family, ∑ Individu (n), Margalef Index (R1), Meinhinick Index (R2), Shannon Index (H'), and Evennes (E) of each plots which analyzed simultaneously; Cummulative indices became the variable tested in population (Bt corn vs Non Bt). The t -test results of each population are collected and presented in one table.

Aphelinidae family, they are effective against various pest species, particularly scale insects. Many terrestrial ecosystems heavily rely on parasitoids like encyrtids, which may constitute approximately 20% of all insect species (Japoshvili & Hansen, 2017).

The disparity in maize plant phenology is advantageous for the development of both the host and the parasitoid imago. This variation might be the reason for the decline in captured parasitoids at 85 days after planting (DAP) across all plots compared to the capture at 60 DAP. The mechanisms through which transgenic plants can influence natural enemies are intricate and depend on various factors. These factors encompass feeding on the flowers, sap, nectar, pollen, and nectar of transgenic plants, modifying the emission of volatile compounds by the plant, or altering host behavior to impact parasitoids (Frizzas et al., 2017).

As mentioned earlier, Apidae and Pompilidae were the pollinators captured in the Bt maize plot. It is also essential to ascertain why only Apidae species of pollinators visited the Bt maize plots. The potential reasons behind pollinator reduction have been extensively studied, and predictions about the future impact of climate change on biodiversity are alarming. The primary factors contributing to pollinator loss include decreased floral diversity and abundance, exposure to agrochemicals, parasitic activity, climate changes, alterations in land use, and interactions with environmental stressors (Arpaia et al., 2021).

The potential exposure of pollinators to newly expressed proteins or molecules in pollen or nectar is a significant concern when incorporating insecticidal proteins or molecules. Foragers may collect maize pollen containing the Cry1Ab toxin for the hive. Both adult and juvenile honeybees can consume it, establishing the primary exposure route for bees through direct consumption. A study involving honeybee colonies in flight cages exposed to pollen from GM maize expressing three different insecticidal Cry proteins (*Cry1A.105*, *Cry2Ab2*, and *Cry3Bb1* in the GM maize hybrid MON 89034 MON 88017) showed that nurse bees foraging on conventional and Bt maize had no variations in their survival, body weights, or pollen consumption rates (Li et al., 2021; Arpaia et al., 2021).

Regarding the role of honeybees in dispersing pollen from Bt corn, it is certainly plausible. However, numerous factors influence the quantity of pollen transferred by bees. The flying capacity of honeybees and their complex foraging behavior are the most significant factors (Kleinjans et al., 2012).

Due to their prevalence in agricultural fields and sensitivity to environmental changes, collembolans (Isotomidae) have recently been utilized as indicators to assess the environmental safety of transgenic crop cultivation. Several investigations have explored the potential impact of transgenic crops on environmental safety by examining the population structure of collembolans and how Bt maize has influenced their life history. Some studies have found that the number of collembolans may be influenced by transgenic crops (Szabo et al., 2017).

Based on these findings, the presence of collembolans in the Bt maize plot suggests that the ecological conditions are still considered safe. This is because the normal biological processes continue even in the presence of one of these indicators. The low number of captures may be attributed to the yellow traps used. Instead of yellow traps, pitfall traps, which are designed to capture soil-dwelling insects, would better suit the collection of Collembola.

One of the widely distributed insect decomposers, Chironomidae (Diptera), thrives in wet environments. Although several species within this group serve as decomposers, there have been indications in recent years that they might be transitioning into the status of "urban pests," causing significant disturbance and frequently being associated with disease issues (Sutikno et al., 2021).

Ground crickets, also known as Gryllidae, are surface-dwelling insects categorized as saprovores, indicating their preference for consuming organic matter, decomposing plants, and fungi. These crickets are active mainly at night but tend to hide in dense foliage and leaf litter during the day. They are also commonly found beneath rocks and typically exhibit dull yet distinct colors (Sultana et al., 2021). In environments where food sources are scarce, Gryllidae can exhibit predatory behavior, targeting vulnerable species, especially young ground crickets in their nymphal stage. Despite Collembola being the dominant group of predatory creatures in the ecosystem, the relatively modest catch is understandable due to the influence of the trap design.

The transgenic maize (Bt-corn) plots exhibited higher levels of phytophages, predators, parasitoids, and saprovores compared to the non-transgenic Popular Hybrid-1 plots (Tables 1, 2, 3, and 5). Therefore, the Bt attribute introduced into the hybrid Popular-1 (used as a parent/raw material for Bt-corn) might contribute to the increased population of arthropods. Furthermore, considering the values of diversity index (Shannon - H), evenness index (Evenness), abundance index-1 (Margalef-R1), and abundance index-2

(Menhinick-R2), the arthropod abundance suggests that Bt maize does not negatively impact the existing arthropod community (Table 7a).

According to the t-test results for several abundance indices analyzed by comparing 60 DAP with 85 DAP, nearly all indices displayed non-significant differences at a probability level of 0.01, except for the Menhinick index (Table 7b). This discrepancy is believed to be significantly influenced by the developmental stage of maize plants, which is rapidly advancing and subsequently providing more abundant food resources for arthropods. Consequently, the arthropod abundance also increases.

The increasing population of arthropods, with a crucial ecological role in ecosystem sustainability, will have a positive impact on the corn plantation ecosystem. In order to gather more comprehensive information, an analysis was also conducted by comparing Bt and Non-Bt plots. We performed a t-test analysis on all index values within each plot (referred to as "population" for clarity, see Table 8 for details) as compared to all index values from the Bt corn plot. This resulted in four types of population comparisons: Bt corn vs Popular Hybrid-1, Bt corn vs Popular Hybrid-2, Bt corn vs Popular Hybrid-3, and Bt corn vs Non-Bt. We then summarized each t-value obtained and presented them in the final form in Table 8.

Regarding species abundance, diversity, composition, structure, relative stability of the arthropod community, diversity index, and evenness index, the results indicated no significant differences between transgenic insect-resistant and non-GM maize (Table 8). Despite this analysis being conducted in 2010, the results were corroborated by studies examining the effect of Bt maize on arthropod abundance (Wang & Guan, 2020), Yin et al. (2022), and Chen et al. (2022). These studies suggested that some GM crops have no adverse effects on arthropods, which is consistent with our research.

CONCLUSION

According to the study, Bt-corn does not have a negative impact on the existing arthropod community, as indicated by the diversity index, evenness index, and abundance index. The arthropod community in the limited testing fields showed greater numbers at 85 DAP compared to 60 DAP. Agromyzidae, Psyllidae, Aphididae, Cecydomiidae, Drosophilidae, Alydidae, and Thripidae were the major phytophages in all tested plots. Parasitoids from the Orders Hymenoptera and Diptera were the most commonly caught in all plots, while Pompilidae dominated the pollinator niche. The study concludes that Bt corn is proven to be safe for arthropod abundance in limited-scale testing. However, further testing on a broader scope and larger area is still necessary for validation and confirmation of the level of safety when releasing transgenic products.

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AUTHORS' CONTRIBUTIONS

LH designed the study, carried out the laboratory work, analyzed data and wrote the manuscript. BI carried out the laboratory work and analyzed the data. All authors read and approved the final version of the manuscript.

COMPETING INTEREST

We declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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