



The Entomopathogen Consortium's Application to Save Corn Production against *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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ABSTRACT

Background: *Helicoverpa armigera*, a major cob borer pest in corn production, is commonly managed using chemical insecticides. However, intensive use of these chemicals poses serious long-term environmental and health risks. Therefore, there is an urgent need for sustainable and eco-friendly pest control alternatives. This study aimed to evaluate the effectiveness of entomopathogenic fungal consortia as an environmentally safe biocontrol strategy for managing *H. armigera* infestations in corn.

Methods: Twelve entomopathogenic fungal isolates were obtained from corn rhizosphere soil and tested *in vitro* against third-instar larvae of *H. armigera*. The evaluation was based on larval mortality rate, infection speed and behavioral changes. Based on these parameters, the isolates were grouped into three microbial consortia (A, B and C) and further assessed for synergistic compatibility. Field experiments were then conducted using a randomized block design (RBD). Each consortium was applied at a standardized spore density and compared with both a chemical control (Carbofuran) and an untreated control.

Result: All fungal isolates achieved 100% larval mortality. Consortia A and B demonstrated significantly higher pathogenicity and synergistic interaction, effectively reducing pest attack intensity and severity. Field applications of Consortia A and B produced cob damage levels comparable to Carbofuran treatment and significantly improved cob length and weight. These findings confirm that entomopathogenic fungal consortia, particularly Consortia A and B, represent a promising and sustainable biological control approach to safeguard corn production against *H. armigera* infestations.

Key words: Consortium, Corn, Entomopathogenic, *Helicoverpa armigera*, Microbe.

INTRODUCTION

Corn is one of the main staple foods in Indonesia and it has the second-highest carbohydrate content after rice. As the demand for corn increases every year, the challenge of maintaining optimal corn production becomes increasingly important. Based on data from the Indonesian Ministry of Agriculture in 2019 shows that the need for corn has continued to increase since 2016, from 23.6 tons to 28.9 million tons in 2017 and then increased again in 2018 by 30 million tons. The increase in demand shows the importance of corn plants and so many efforts are being made to maintain and increase sustainable corn production. One of the problems in corn production is the attack of plant pests, especially the cob borer (*Helicoverpa armigera*), which can cause a significant decrease in yields. The reduction in yields due to *H. armigera* attacks in Sulawesi reached 51.9-53.4% and the average damage to corn cobs in East Java reached 21.5%. The period 2005-2015, with corn land productivity reaching 4.34 tons per hectare with a growth rate of 3.81% per year (Asriani *et al.*, 2023).

Control of *H. armigera* attacks is generally carried out using chemical insecticides intensively. Although effective, excessive use of insecticides can have long-term negative impacts, such as environmental pollution, increased chemical residues in agricultural products and the death of natural enemies of insects (Tiwari, 2024). This triggers the need for alternative control methods that are more

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sustainable and environmentally friendly. The use of entomopathogenic consortia, a combination of microorganisms that are pathogenic to insects, is one promising approach to controlling *H. armigera* pests (Das *et al.*, 2024). Previous studies have shown that entomopathogens such as *Lecanicillium* sp. can inhibit egg hatching and cause significant mortality of *H. armigera* larvae (Swathy and Vivekanandhan, 2024). However, the use of a single entomopathogen is often less effective in the long term because it only focuses on one stage of pest development.

The approach using an entomopathogen consortium, which combines several types of pathogenic microbes, has proven more effective in controlling pests than single isolates. This consortium works synergistically by complementing the functions of various enzymes each microorganism produces to provide better results in suppressing pest populations (Karthi *et al.*, 2024a). According to (Shukla *et al.*, 2023), a combination of entomopathogenic fungi such as *Metarhizium anisopliae*, *Beauveria bassiana* and *Lecanicillium lecanii* can reduce armyworm attacks on plants by up to 34.74%. Seeing the great potential of the entomopathogen consortium in controlling pests, this study aims to test the effectiveness of the consortium application in saving corn production threatened by *H. armigera* attacks. It is expected that the use of this consortium will not only increase corn production but also produce better-quality corn without chemical insecticide residues (Zhao *et al.*, 2024). This approach is important in developing a more sustainable and environmentally friendly pest control strategy for corn farmers. Several recent studies have confirmed the virulence and efficiency of these fungi against various insect species, including Lepidopteran and Hemipteran pests (Beemrote *et al.*, 2023; Qubbaj and Samara, 2022; Thanuja *et al.*, 2025). These findings highlight the broad potential of entomopathogenic fungi as sustainable biocontrol agents.

MATERIALS AND METHODS

Media preparation

For 1 liter of Potato Dextrose Agar (PDA), 200 g of potatoes, 20 grams of Dextrose E-Merck, 20g of agar E-Merck and two capsules of 500 mg of chloramphenicol were used. Chloramphenicol inhibits bacterial growth, making the medium safer from bacterial contamination.

Bioassay test

The bioassay test was carried out using a vial; all tests had three replications, each containing one caterpillar. They were feeding in the form of sweet corn, with as many as five seeds in each vial. Spraying pests with fungi was carried out twice daily, in the morning and evening. Observations were made every day for one week, with observation variables including mortality (%), speed of infection (hours), when there was a behavior change (hours) and symptoms of fungal infection in caterpillars (Dash *et al.*, 2020; Septia *et al.*, 2024).

Synergy test

After the hypovirulent test and bioassay stage, the test was continued by testing the synergism between isolates. The trick is to grow the three best isolates in the middle of a petri dish. Then, the microbes that can develop are in contact and whether there is an inhibition zone with the best microbes will be used for further tests (Aguín *et al.*, 2020).

Field test

Field tests were carried out when corn begins to appear in cobs and this is because *H. armigera* pests start a lot during that phase. Application of enteropathogen fungi consortium was carried out in the morning and afternoon (Costa and Mortoni, 2023). Randomized block design (RBD) was used in the field test. The treatment in field test consists of 3 types of Formula A (strong), B (medium) and C (weak) with a dose of application of 1 L microbes/200 m² of land area with a spore density of 10⁶ conidia/ml. The field test parameters consist of:

1. Attack rate *H. armigera*, Calculating the attack intensity of attack rate *H. armigera* calculated using formula:

$$I = x \times 100\% \frac{a}{b}$$

I = Attack intensity (%).

a = Number of corn cobs affected.

b = number of plants observed.

2. The severity of the attack $\frac{\text{Width of pest attack (\%)}}{\text{Plant}}$

Data analysis

Data were analyzed based on *in vitro* and *in vivo* experiments. Qualitative descriptive and quantitative analysis was used *in vitro*, while field data were analyzed using variant analysis (ANOVA). The interpretation was carried out based on descriptive analysis compared with related literature. In addition, the interpretation of the results for the *in vivo* experiment was carried out with a follow-up test of Duncan with a level of 5%.

RESULTS AND DISCUSSION

Bioassay testing of *H. armigera* larvae

Twelve fungi obtained the results of the hypovirulence test isolates that did not affect the growth of corn plants. They then tested the effectiveness of entomopathogenic fungi using the bioassay method *in vitro* with *H. armigera* larvae that have been instar 3, with characteristics of the size of needles and can already eat fruit meat, with a larval length of 8-13 mm. Then, spray every 12 hours and the data is presented in Table 1.

Fungi J5S1U2 has the lowest inhibition of 38% with a mortality score of 11 and J3S2U1 has the highest food inhibition of 63% with a mortality score of 14. The death score is calculated every 12 hours until the larvae die. All fungi tested by bioassay can kill larvae with 100% mortality. J3S2U3 can infect *H. armigera* pests at a time of 24 hours faster than other types of fungi. Fungi J3S1U2, J1S1U1 and J3S2U1 experienced the same behavioral changes 36 hours more quickly than other types. Symptoms of infection are predominantly white in bioassay tests.

Microorganisms in the soil that are good describe abundant food sources coupled with suitable temperatures and other environmental conditions that support the growth of microorganisms in the soil, such as fungi and bacteria

(Cao *et al.*, 2024; Chauhan *et al.*, 2023). The slow growth of fungi depends on how the isolate can absorb the nutrients in the media to the maximum (Septia and Sukorini, 2024). The component affects the growth and development of fungi, such as dextrose, which acts as the growth and development of fungi. Factors that affect the growth and development of fungi include nutrients such as sugars, polysaccharides, nitrates, ammonia, amino acids, polypeptides and proteins (Cao *et al.*, 2024). In addition, environmental impurities, including temperature, pH and light, can also affect fungi's growth, especially in mycelium growth (Wao and Agnihotri, 2022; Singh *et al.*, 2024).

The slow death of *H. armigera* larvae was also affected by the inhibition of feeding activity, which can affect larval development. This was evidenced by the decreased feeding activity of the larvae when given isolating treatment of various entomopathogenic fungi (Russo *et al.*, 2024a; Sui *et al.*, 2024). Entomopathogenic fungi are reasonable pest control, environmentally friendly and harmless to beneficial organisms (Costa Mortoni, 2023; Sari *et al.*, 2023). Consortiums A and B were the best for controlling *H. armigera*. This is because those consortiums are dominated by types of microbes that can control plant pests. The power of conidia of fungi in consortiums A and B has to quickly increase the resulting sporulation to increase its ability as a plant pest controller. It is known that the combinations of entomopathogenic fungi are very effective as endophytes in attacking the main pests of corn crops, especially in attacking survival in the larvae and pupa of corn main pests (Hassuba *et al.*, 2024; Sui *et al.*, 2024).

H. armigera pest death depends heavily on several factors such as temperature, media, pH and spore moisture to grow. The process of attacking fungi in pests through contact and mouth, where pests will eat a number of infective units in plants that have been applied with fungi, then in good conditions, spores will germinate and penetrate deeply into the body of the host insect (Pawar *et al.*, 2024; de Souza *et al.*, 2022). Then hyphae secrete

the enzymes chitinase, lipase and protease that help decompose insect cuticles. Then mycelia develops until it reaches hemolymph, so it becomes thick and pale, slowing down until it finally stops. After that, the insect weakens and eventually dies (de Souza *et al.*, 2020). Entomopathogenic fungal endophytes may be important bodyguards having a negative effect on polyphagous and sucking insect pests. Entomopathogenic fungi asymptotically colonize plant tissues and can even promote growth and protect the plant against biotic stresses, pests and diseases, or abiotic ones such as water deficit, nutritional deficiencies, etc (Hassuba *et al.*, 2024; Nelly *et al.*, 2020). The degree of EF colonization of the different tissues and organs of the plant and fungal persistence over time varies according to the plant species and fungal strain, from local to systemic colonization of the plant tissues, with even vertical transmission detected (Thakur *et al.*, 2021).

The boxplot in Fig 1 shows that food blockers (%) have the least variability, with most values clustered between 50-60%, indicating a consistent blocking efficiency across samples. In contrast, both Infection Speed (hours) and Changes in behavior (hours) display wider ranges, suggesting greater variability in how quickly infections progress and how behaviors change, with median values around 70 and 65 hours, respectively. This highlights that while food blocking is relatively uniform, the infection and behavioral responses vary significantly among the samples.

The boxplot illustrates the distribution of three key metrics entomopathogen fungi consist of Food blockers (%), Infection Speed (hours) and Changes in Behavior (hours). Food blockers (%) shows a relatively small range with a consistent distribution, suggesting that the effectiveness of food blocking remains stable across the samples, despite a minor outlier (Monica *et al.*, 2024; Slowik *et al.*, 2024). Infection Speed (hours), on the other hand, displays significant variability, with the interquartile range spanning from approximately 40 to 80 hours, indicating

Table 1: *H. Armigera* larva bioassay test.

Isolate code	Food blockers (%)	Death score	Mortality (%)	Infection speed (hours)	Changes in behavior (hours)	Symptoms of infection (discoloration)
J3S1U2	45	11	100	84	36	Black-whitish
J3S2U3	48	14	100	24	132	Greenish
J3S2U1	63	14	100	108	36	Black-whitish
J2S2U1	49	11	100	72	48	white
J4S2U2	57	14	100	60	96	White
T5U1O	42	12	100	72	60	Black-greenish
J2S2U5	43	12	100	84	48	Whitish yellow
J2SU4	45	12	100	48	84	White
J1S1U1	52	14	100	120	36	Whitish red
J2S1U4	45	13	100	36	108	White
J4S1U1	41	11	100	60	60	Black-whitish
J5S1U2	38	11	100	36	84	Yellow

Where, J= Fungi, S= Soil, U= Replication.

differences in how rapidly infections spread among the samples. Changes in Behavior (hours) also exhibits notable variation, similar to Infection Speed, which suggests that behavioral responses are linked to infection progression but are not uniform across all samples (Leonard *et al.*, 2023). The wide ranges for both Infection Speed and Changes in Behavior highlight the complexity and variability in the infection process and its influence on behavior, whereas Food blockers (%) remains more predictable and consistent in its effects (Patel, 2023; Zhang *et al.*, 2024).

The correlation heatmap reveals several key relationships between the metrics illustrated in Fig 2. Food blockers (%) have a strong positive correlation with Death Score (0.74) and Infection Speed (hours) (0.49), indicating that higher food-blocking effectiveness is associated with increased death scores and faster infection speed. Changes in Behavior (hours) is negatively correlated with Infection Speed (hours) (-0.88), suggesting that as infection

speed increases, changes in behavior occur more slowly. Death Score has a positive but moderate correlation with Changes in Behavior (0.30), implying that higher death scores are somewhat linked to more significant changes in behavior over time. Overall, the heatmap shows that infection dynamics and behavioral changes are closely interlinked, with food blocking playing a significant role in both outcomes. Similar patterns of mortality caused by *Beauveria bassiana* and *Metarhizium anisopliae* were reported (Qubbaj and Samara, 2022; Thanuja *et al.*, 2025), indicating a consistent pathogenic potential of these fungi across different insect species.

The correlation heatmap highlights important relationships among key metrics entomopathogen fungi. A strong positive correlation between Food blockers (%) and Death Score (0.74) suggests that as the efficiency of food blocking increases, so does the death rate, indicating a significant impact of food deprivation on mortality(Kryukov

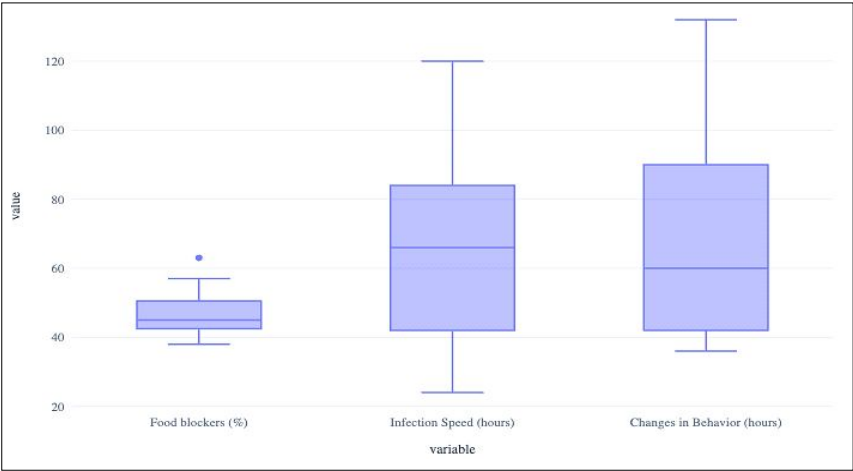


Fig 1: Distribution of key metrics bioassay test.

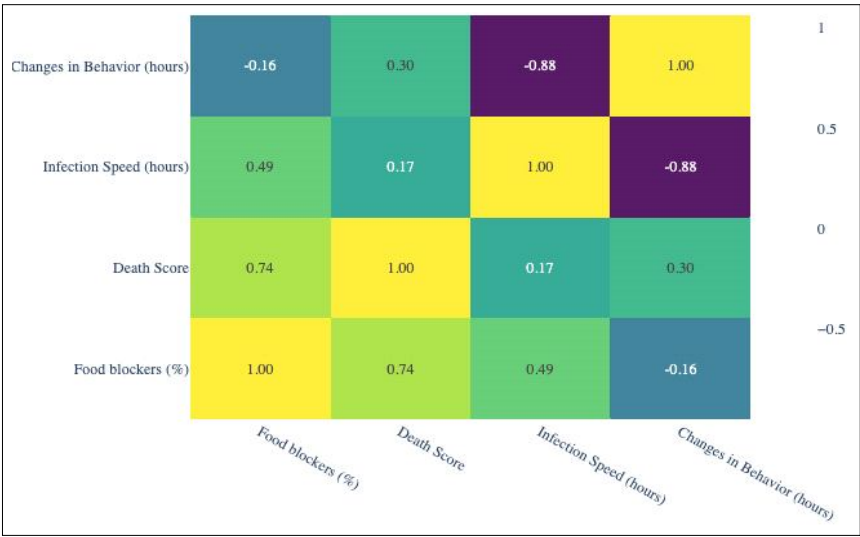


Fig 2: Correlation heatmap of key metrics bioassay test.

et al., 2020; Zembruski et al., 2023). Similarly, Food blockers (%) shows a moderate correlation with Infection Speed (hours) (0.49), implying that food deprivation may accelerate the infection process. However, there is a strong negative correlation between Infection Speed (hours) and Changes in Behavior (hours) (-0.88), meaning that faster infection times result in quicker changes in behavior (Csata et al., 2024). This inverse relationship suggests a critical link between the speed of infection and behavioral responses, which may help in understanding how infection progression influences host activity (Kryukov et al., 2020). The overall pattern underscores the importance of food blocking and infection dynamics in shaping both mortality and behavioral changes.

Symptoms of infection due to the attack of fungi on caterpillars *H. armigera* resulted in a change in the color of the caterpillar's body and the appearance of fungi on some

parts of the caterpillar. Over an extended period, the caterpillar will be dry and destroyed due to entomopathogen fungi from the corn soil. Symptoms due to an attack of entomopathogen fungi are presented in the form (Fig 3).

Based on Fig 3, Symptoms that arise in caterpillars due to the attack of fungi begin with lazy caterpillars moving, appetite reduced and hyphae from fungi all over the body. Hyphae will appear on the skin, feet (legs), mouth (thoracic) and abdomen. In addition, the state of the dead caterpillar's body will harden, wrinkle and dry. The findings are consistent with previous reports (Thanuja et al., 2025). Such responses suggest that fungal infection triggers host defense mechanisms that can influence mortality outcomes.

Fungi consortium synergy test

The synergy test was carried out by combining various fungi obtained from the soil of the corn rhizosphere. First,

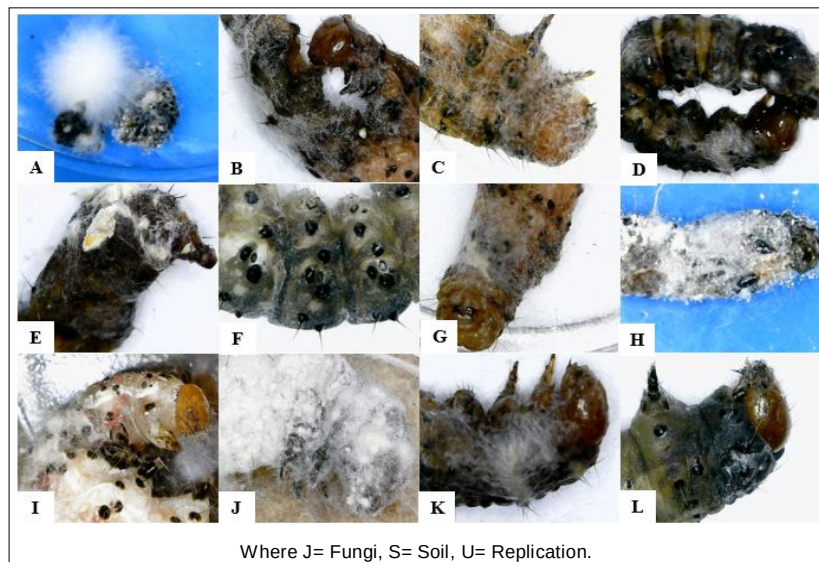


Fig 3: *H. armigera* larva bioassay test dies after fungi application (A) J4S2U2, (B) J4S1U1, (C) J5S1U2, (D) J3S2U1, (E) J3S1U2, (F) J3S2U3, (G) J2S2U5, (H) J2S2U1, (I) J2S1U4, (J) J2SU4, (K) J1S1U1, (L) T5U1O.

Table 2: Entomopathogenic fungi synergy test results.

The nature of synergy	Genus	Front view	Rear view
Strong (Combination A)	a. J5S1U2		
	b. J4S1U1		
	c. T5U1O		
	d. J2S2U5		
Medium (Combination B)	a. J2S1U4		
	b. J3S1U2		
	c. J2SU4		
	d. J3S2U3		
Weak (Microbe C)	a. J2S2U1		
	b. J1S1U1		
	c. J4S2U2		
	d. J3S2U1		

Where, J= Fungi, S= Soil, U= Replication.

Table 3: *H. armigera* field test on corn cobs.

Treatment	Average attack intensity (%)	Average attack severity (%)
Control + (Carbofuran)	20a	1a
Control - (water)	100c	15.5c
Combination A	25a	1.5a
Combination B	35a	3.5a
Combination C	75b	7.5b

Description: Numbers followed by the same letter in each column show no significant effect according to Duncan's 5% test.

Table 4: Corn cob length and fresh weigh.

Treatment	Average of corn cob (cm)	Average of fresh weight (g)
Control + (Carbofuran)	12.57a	210.6bc
Control - (water)	14.35b	187.15a
Combination A	13.88ab	227.25c
Combination B	14.54b	217.15c
Combination C	13.17ab	189.8ab

Notes: the numbers followed by the same letter in each column show no significant effect according to Duncan's test, 5%

synergy testing using fungi from the bioassay tests as many as 12 isolates. Then, the fungi of the 12 isolates are divided into three large parts: Strong, Medium and Weak. The grouping category was taken from the percentage data inhibiting. Finally, the test results are presented in Table 2.

Table 2 indicates a strong consortium of fungi (J5S1U2, J4S1U1, T5U1O and J2S2U5), medium consortia (J2S1U4, J3S1U2, J2SU4 and J3S2U3) and weak consortia (J2S2U1, J1S1U1, J4S2U2 and J3S2U1) can synergize and merge between fungi one and the other fungi are indicated by not forming or slave zones. fungi images are marked with a circle, thus allowing all types of fungi to be combined. The growth that does not exist in the inhibitory zone is usually characterized by the growth of colonies that do not inhibit each other and the absence of clear zones around fungi. This synergy is a compatible interaction in the inhibitory zone characterized by the presence of clear boundary lines, normal growth, not inhibiting each other, no *over-growth* and the unification of hyphae (20).

Field test

The analysis of the average intensity of corn cob attacks showed an authentic influence of the fungi consortium on the average attack of *H. armigera*. Further test results with Duncan's 5% level against the average intensity of caterpillar attacks were presented in Table 3.

Table 3 combination A and Combination B had an effect that was not significantly different from the positive control (Carbofuran) and has a significant impact compared to the negative control and combination C; this was seen from the average percentage of attack intensity and severity. The lowest average attack intensity occurred in the positive control (Carbofuran) 20% Combination A 25% and Microbial

Combination B 35%, then on the severity of attacks positive control (carbofuran) 1% and Combination A 1.5% and Combination B 3.5%. The smallest percentage was negative control and Combination C.

Jointly inoculating two different strains of *B. bassiana* (Bov 3 and Bov 2) into *D. fovealis* caterpillars, insect mortality was considerably higher than when either strain was used separately (Russo *et al.*, 2024b). Furthermore, cultivating these fungi together increased the production of several important enzymes involved in biological control processes, like chitinase and cellulase (Karthi *et al.*, 2024b). The present study, therefore, aims to evaluate the metabolic alterations caused by a consortium of the Bov3 and Bov2 strains to ascertain the reasons for increased mortality in *D. fovealis*. One of the main defence mechanisms of insects against entomopathogenic fungi like *B. bassiana* is the formation of oxidative molecules such as reactive oxygen and nitrogen species (ROS and NOS). These oxidative molecules can damage important cellular components, including proteins, lipids, carbohydrates and nucleic acids. In this regard, the production of antioxidant molecules by fungi is extremely important to circumvent the host's immune system and settle the infection (Melkumyan *et al.* 2024; Oktariana *et al.* 2024).

Corn yield

The analysis of variance on the average Length of the corn cobs showed a very significant effect of the fungus consortium on the average Length and fresh weight of the cobs. Duncan's further test results, with a level of 5% on the average severity of caterpillar attacks, were presented in Table 4.

It shows the longest corncob length was in the negative control treatment of 14.35 cm and was not significantly different from Microbes B, 14.54 cm, Microbes A, 13.88 cm and Microbes C, 13.17 cm. The use of Microbes A, Microbes B and positive control (carbofuran) had the highest wet weight of cobs.

CONCLUSION

Based on research on the effectiveness of the application of the entomopathogenic fungi consortium against the attack of cob borer larvae (*H. armigera*) as an effort to save corn production, it can be concluded as follows: Various consortia of entomopathogenic fungi on corn soil can be used-proven by the discovery of 12 types of entomopathogenic fungi. Microbe A and the B Microbial Consortium effectively suppress pest attacks at attack intensity, severity and wet weight of corn cobs.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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