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Evaluation of the efficiency of entomopathogenic fungi in controlling mustard aphids and promoting the growth of green cos lettuce

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Abstract Entomopathogenic fungi (EPF) have significant potential as biological control agents for insect pests and as integral components of integrated pest management systems. This study assessed the efficacy of nine entomopathogenic fungal isolates in controlling mustard aphids under laboratory conditions. Among the isolates, NM*Met*_KTLS7/3 exhibited the highest mortality (88.89%) and infection rates (85.57%), which were significantly different from those of the other isolates ($p \leq 0.05$). Additionally, NM*Met*_KBR5/1 demonstrated the highest phosphate solubilization index (PSI) of 1.54, comparable to NM*Met*_KTLS7/3 (1.49) and NM*Met*_LTMC3/3 (1.44). Green cos lettuce plants treated with soil drench applications of entomopathogenic fungi exhibited significantly enhanced growth parameters, including plant height, leaf number, leaf area, fresh weight, and dry matter content ($p \leq 0.05$). The total fresh weight (shoot+root), shoot weight, and root weight of lettuce treated with NM*Met*_KBR5/1 demonstrated significantly higher values compared to other EPF isolates, with values of 105.79 g, 87.40 g, and 14.47 g, respectively. These values were not statistically different ($p > 0.05$) from those treated with NPK fertilizer (116.05 g, 90.65 g, and 20.60 g, respectively). In conclusion, all nine EPF isolates controlled aphids, solubilized phosphate, and promoted lettuce growth. NM*Met*_KTLS7/3 and NM*Met*_KBR5/1 are recommended for further field evaluation.

Keywords: Entomopathogenic fungi, Biological control, Phosphate solubilization

Introduction

Aphids, as plant pests with sucking mouthparts, cause damage by feeding on plant sap from tender leaves, shoots, and flower clusters, ultimately hindering plant growth and causing economic losses in various agricultural crops. The damage they inflict can be categorized into two types: direct damage resulting

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from sap extraction and plant reactions to aphid feeding, and indirect damage caused by the transmission of viruses (Dedryver *et al.*, 2010; Erol *et al.*, 2020). Furthermore, indirect damage occurs when plants become contaminated with aphid honeydew. Honeydew contributes to economic losses by physically contaminating plants and providing a nutrient source for fungi, which can contaminate produce and reduce photosynthesis rates by blocking sunlight (Ebert and Cartwright, 1997). Although chemical pesticides, such as pyrethroids, neonicotinoids and chlorpyrifos, are commonly used to control aphids, their excessive use poses significant risks to both human health and the environment. Consequently, safer strategies involving entomopathogenic fungi, natural enemies, and plants are being utilized for aphid management (Chaker *et al.*, 2021).

Entomopathogenic fungi (EPF) play a crucial role in controlling insect pests, with an ability to destroy over 700 species of insects (Zimmerman, 2007; Maina *et al.*, 2018). EPF are considered superior to synthetic insecticides because they are safe for humans, environmentally sustainable, and target-specific. Many EPF are pathogenic to economically significant insect pests, enabling effective pest control. They are cost-effective in the long term, exhibit fewer residual effects, and can overcome resistance issues. EPF degrade the insect's cuticle, proliferate in the hemolymph as hyphal bodies, and secrete toxins, leading to the insect's death. Subsequent saprophytic growth results in the production of fungal spores capable of infecting other hosts (Sharma *et al.*, 2023).

Moreover, these EPF also possess endophytic properties, with an ability to reside in the intercellular spaces of plants without causing disease to the host (Vidal and Jaber, 2015). Once inside plant cells, EPF produce various secondary metabolites, such as benzopyranones, phenolic acids, quinones, and steroids, which have antimicrobial properties and act as antioxidants (Strobel, 2003). Additionally, they benefit plants in various ways, including promoting plant growth, enhancing resistance to diseases and insect pests (Hao *et al.*, 2021; Raya-Díaz *et al*., 2017).

Various EPF species have been utilized to enhance soil conditions in agricultural systems, there by promoting plant growth. For example, species of the *Metarhizium* fungus can colonize the roots of various plants, including switchgrass, haricot beans, tomatoes, wheat, soybeans, and hemp (Bidochka *et al*., 2001; Hu *et al*., 2023). When fungi are applied through a soil drench, foliar application, or seed or transplant treatment, they colonize and benefit the plants through one or multiple mechanisms. In return, EPF can survive in, on, or around the plants in the absence of their arthropod hosts, obtaining nutrition from the plant. Several EPF can colonize the roots of both monocot and dicot plants, transferring nitrogen from insect remains directly to the roots and establishing a

mutualistic association (Behie *et al.,* 2017). This endophytic colonization stimulates the plant's defense system and the production of secondary metabolites (Agbessenou *et al.,* 2020; Hu and Bidochka, 2021). EPF can also produce phytohormones such as auxins, improve water transportation, and increase the availability of nutrients (phosphate dissolution, potassium, and siderophore production). Indole Acetic Acid (IAA), a type of auxin, plays a crucial role in these processes, resulting in improved plant growth (Yusniwati *et al*., 2022).

The ability of EPF (Endophytic *B. bassiana* and *M. brunneum*) to enhance nutrient uptake and promote plant growth was demonstrated by improved iron availability, chlorophyll content, root length, and abundance of fine roots in sorghum grown on calcareous substrates (Raya-Díaz *et al*., 2017). Additionally, Dara (2019) reported that EPF can colonize plant roots, act as extensions of the roots, and potentially enhance nutrient and water absorption, aiding plants to withstand stress factors. Russo *et al.* (2018) observed notable enhancements in soybean attributes following the foliar application of *B. bassiana.* These improvements included increased plant height, number of branches, pod weight per branch and plant, number of pods per branch and plant, number of seeds per pods, branch, and plant, as well as seed weight and yield. Similarly, Jaber and Enkerli (2016) found that treating broad bean seeds (*Vicia faba*) with *B. bassiana* and *M. brunneum* led to significant improvements in seedling emergence, plant height, number of leaf pairs, and the weights of fresh shoots and roots. Ahmad *et al.* (2020) reported that inoculating seeds of Austrian winter pea (AWP, *Pisum sativum* L.) with *M. robertsii* significantly increased the height and above-ground biomass of both AWP and cereal rye.

This study aimed to identify entomopathogenic fungal isolates capable of effectively controlling mustard aphids, solubilizing phosphate, and promoting the growth of green cos lettuce.

Materials and methods

Entomopathogenic fungi

Soil samples were collected in Nakhon Ratchasima province area and used to isolate entomopathogenic fungi using the baiting technique. Yellow mealworms (*Tenebrio molitor* L.) at the 3–5 instar stage were used as bait, following the method described by Bidochka *et al.* (1998). After serving as bait, the mealworms were surface-sterilized by immersing them in a 0.5% sodium hypochlorite solution for 3-5 minutes, followed by two rinses with sterile water. Subsequently, the sterilized mealworms were placed in a moist chamber under dark conditions at 28-30°C and 60-80% RH., and left until the fungi produced

spores. The spores were then separated to obtain pure fungal cultures, and their morphological characteristics, such as conidia, conidiophores, and spore structures (conidiomata), were examined to confirm their classification as entomopathogenic fungi based on the criteria outlined by Samson *et al.* (1998); Humber (2012).

Pathogenicity test against mustard aphid under laboratory conditions

Entomopathogenic fungi (EPF): All isolates of EPF were cultured on PDA (Potato Dextrose Agar, Himedia®). The culture was maintained in darkness at a temperature of 28-30°C for 21 days. Subsequently, the spores were harvested using a surfactant (Tween 20® (Loba Chemie PVT. LTD), 0.05%). The fungal culture was diluted in a petri dish, and a sterilized loop was employed to scrape the mycelium and spores. The spore suspension was then quantified using a Hemocytometer under a microscope at 40X magnification. The concentration of the spore suspension was adjusted to a density of 10^8 conidia/mL.

Mustard aphid: Adult aphids were collected from a vegetable field area around Nong Raweing Education Center, Rajamangala University of Technology Isan, Nakhon Ratchasima, Thailand. They were reared in plastic mesh cages sized 1.5x1.5x1 meters, with mustard plants serving as host plants. The aphids were maintained until they reached adulthood, laid eggs, and hatched into first-generation. Subsequently, 30 aphids were then transferred to test cages (plastic cylinders with a diameter of 10 cm and a height of 25 cm) containing mustard plants, one day before the experiment.

Mustard aphid assay: Five milliliters of EPF spore suspensions, each at a concentration of 1×10^8 conidia/mL, were sprayed onto cages containing aphid for each treatment. After spraying, the cages were covered with insect-proof nets and incubated in a growth chamber at a temperature of 28-30 ºC with a 12:12 dark/light cycle. Control treatments included unsprayed cages and spraying with surfactant (Tween 20®, 0.05%). The experimental design was a Completely Randomized Design (CRD) consisting of 3 replicates per treatment, with each replicate consisting of 30 aphids.

Measurement: After 24 hours of inoculation, dead aphids were collected. The insects' skin surfaces were treated with a 0.5% sodium hypochlorite solution for 3-5 minutes, followed by rinsing with sterile water for 3-5 minutes; this process was repeated twice. The aphids were then transferred to a moist chamber and incubated in the dark at a temperature of 28-30°C and 60-80% RH. Daily observations were recorded for mortality and fungal infection.

Determination of phosphate solubilization index on PVK agar

The EPF isolates obtained from pure cultures were preliminarily screened for their potential to solubilize phosphate on Pikovskaya (PVK) agars (Himedia®) (Pikovskaya, 1948). The PVK medium was autoclaved at 121°C for 15 min. Sterilized PVK agar was poured into sterilized petri dishes. Entomopathogenic fungal mycelium plugs of each isolate, grown on PDA at 28-30°C. for 10 days, were cut from the edges of each actively growing colony using a sterile cork borer (Ø5 mm). Fungal mycelium plugs were then placed on the central part of petri dishes containing PVK agar for 7 days at 28-30°C. Plugs of sterile PDA were used as controls. Three replicates, each consisting of 5 petri dishes, were tested for each treatment. The diameter of clear zones surrounding the colony of each isolate was measured after 3, 5, and 7 days of incubation. The phosphate solubilization index was calculated according to the formula below (Shukla and Vyas, 2014).

$$\text{Phosphate Solubilization Index (PSI)} = \frac{\text{colony diameter + clear zone diameter}}{\text{colony diameter}}$$

Plant growth promoting potential of entomopathogenic fungi in green cos lettuce under pot culture conditions

Four- week- old lettuce seedlings were grown in sterile peat moss then transplanted into plastic pots measuring 7. 5 inches in height with an inner diameter of 10. 25 inches. These pots were filled with sterile soil and manure mixture in a 2:1 ratio. One week after planting, a spore suspension of the fungus (1×10^8 conidia/mL) was applied as a 150 mL soil drench to each plant once per week. Applications continued until the lettuce plants were 28 days post-transplant. Each treatment was replicated 10 times using separate pots. Observations were recorded for various parameters, including plant height, total number of leaves, leaf area per plant, leaf size (width x length, fresh weight (shoot, root, and total (shoot+root)), and dry matter content (shoot and root).

Data analysis

Statistical analyses were conducted using the Statistical Analysis System (SAS) software (Version 9.00; SAS Institute Inc., 2006). An analysis of variance (ANOVA) was performed to assess differences among treatments, followed by Duncan's New Multiple Range Test (DMRT) to compare mean differences between each treatment group.

Results

Entomopathogenic fungus

Eight isolates of EPF were obtained from soil samples (Table 1), including seven isolates of *Metarhizium* spp. (NM*Met*_KTLS7/ 3, NM*Met*_NS7/ 2, NM*Met*_SS9/ 2, NM*Met*_LTMC3 / 3, NM*Met*_KBR5/ 1, NM*Met*_BAL7/ 1, and NM*Met*_WNK5/ 1) , one isolate of *B. bassiana* (NM*Bb*_KTLS2/ 3) , and an additional isolate (NM*Met*_DOA) supplied by the Nakhon Ratchasima Agricultural Technology Promotion Center (Plant Protection). When cultured on PDA for 21 days, each EPF isolate exhibited different growth rates. NM*Met*_BAL7/ 1 had the largest colony diameter (80. 50 mm) , while NM*Bb*_KTLS2/3 showed the slowest growth (62.83 mm). Colony characteristics varied among isolates: *Metarhizium* spp. had smooth, greenish-brown mycelium that turned green- brown with age, with cylindrical and uniseptate spores. In contrast, *B. bassiana* mycelium was white and powdery, growing smoothly on the nutrient surface, with spherical, single-celled, green, shiny spores (Figure 1).

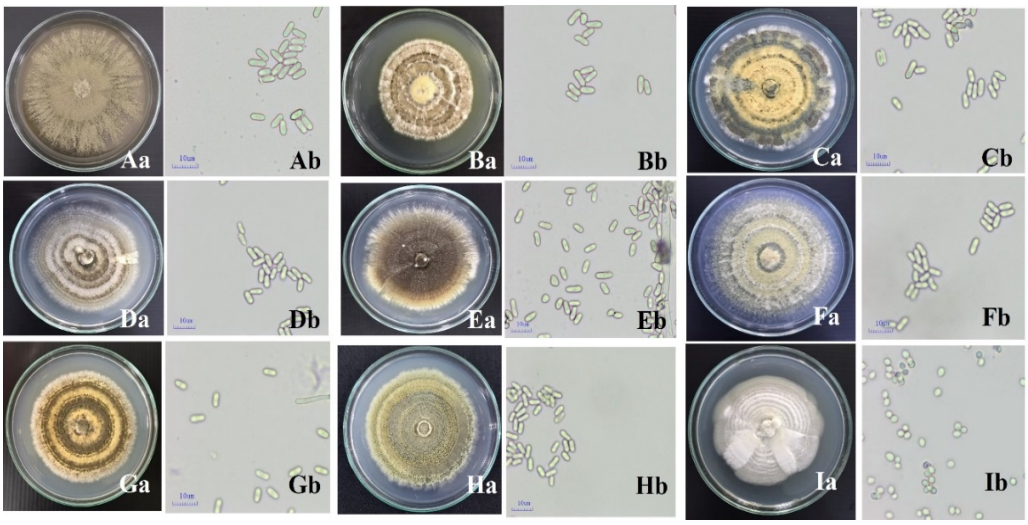

Figure 1. The characteristics of the colony (a) and conidia (b) of the entomopathogenic fungi cultured on PDA for 21 days; A: NM*Met*_KTLS7/3, B: NM*Met* _NS7/2, C: NM*Met*_SS9/2, D: NM*Met*_LTMC3/3, E: NM*Met*_KBR5/1, F: NM*Met*_BAL7/1, G: NM*Met*_WNK5/1, H: NM*Met*-DOA, I: NM*Bb*_KTLS2/3

Table 1. The entomopathogenic fungal isolates, along with their colony diameters, were assessed on Potato Dextrose Agar (PDA) after 21 days of incubation

Isolates	Soil sample data			Colony characteristics	Colony diameter (mm±sd.)
	Entomopathogenic fungi	Source (Habitat)	Location (Village, sub-district, district)		
NM*Met*_KTLS7/3	*Metarhizium* sp.	Rice field	Ban Nong Kok, Nong Suang Kham Thale So	The mycelium is initially white and subsequently produces greenish-brown to brown spores.	65.00±6.54^{bc}
NM*Met*_NS7/2	*Metarhizium* sp.	Forest soil	Ban Donree, Don Chomphu, Non Sung	The mycelium ranges from yellow to brown and produces green to dark green spores. The culture medium gradually develops a light-yellow pigmentation	64.83±2.08^{bc}
NM*Met*_SS9/2	*Metarhizium* sp.	Forest soil	Ban Nong Phaiyai, Soeng Sang ,Soeng Sang	The mycelium is white to yellow in color and produces green to dark green spores.	72.00±4.36^{b}
NM*Met*_LTMC3/3	*Metarhizium* sp.	Rice field	Ban NongPhong, Khui, Lam Thamenchai	The mycelium exhibits a white coloration and produces spores ranging from brown to dark brown.	73.00±2.29^{ab}
NM*Met*_KBR5/1	*Metarhizium* sp.	Eucalyptus field	Ban Oraphim, Oraphim, Khon Buri	The mycelium is white to yellow in color and produces brown to dark brown spores.	72.67±1.76^{ab}
NM*Met*_BAL7/1	*Metarhizium* sp.	Forest soil	Ban Bom, chaoraka, Ban Lueam	The mycelium is white to yellow to greenish in color and produces green to dark green spores.	80.50±5.29^{a}
NM*Met*_WNK5/1	*Metarhizium* sp.	Forest soil	Ban Butako, Wang Nam Khiao, Wang Nam Khiao	The mycelium is white to yellow to brown in color and produces dark green to black spores.	71.33±1.26^{b}
NM*Bb*_KTLS2/3	*Beauveria bassiana*	Forest soil	Phan Dung, Phan Dung ,Kham Thale So	The mycelium was white and powdery, growing smoothly on the medium surface, with spherical, single-celled, green, shiny spores	62.83±7.51^{c}
NM*Met*_DOA	*Metarhizium* sp.	Brown plant hopper	Supported by Nakhon Ratchasima Agricultural Technology Promotion Center (Plant Protection)	The mycelium ranges from white to greenish-dark brown and produces spores that are green to dark green.	68.83±3.55^{bc}
			p-value		**0.0034**

Means ± sd. in the column followed by the same superscript letters were not significantly different (DMRT, p>0.05); sd. = standard deviation.

Pathogenicity test against mustard aphid under laboratory conditions

All nine EPF isolates effectively controlled mustard aphids, with statistically significant differences ($p \leq 0.05$) observed among the treatments. Mortality rates ranged from 15.56% to 88.89%, while infection rates varied between 13.33% and 85.57%. Among the isolates, NM*Met*_KTLS7/3 showed the highest mortality and infection rates, whereas NM*Met*_NS7/2 exhibited the lowest. In contrast, the control treatment and Tween 20® at 0.05% achieved mortality rates of 2.22% and 5.56%, respectively, with no observed infections. (Figure 2).

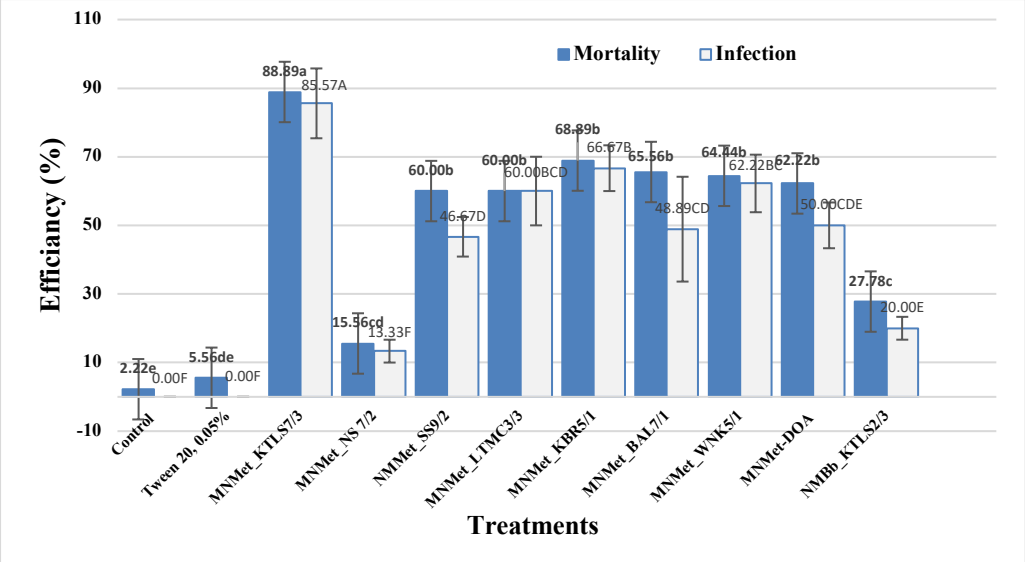

Figure 2. Percentage of mortality and infection in mustard aphids inoculated with entomopathogenic fungi under laboratory conditions. (Means with the same letters are not significantly different ($p>0.05$), as determined by DMRT)

Phosphate solubilizing activities

Phosphate- solubilizing activities were assessed on PVK medium using $Ca_3(PO_4)_2$ as the phosphate source for EPF. All isolates were capable of solubilizing $Ca_3(PO_4)_2$, with Phosphate Solubilizing Index (PSI) values ranging from 1. 16 to 1. 54. NM*Met*_KBR5/ 1 exhibited the highest phosphate solubilization activity, with a PSI value of 1.54, though this was not statistically significantly different ($p>0.05$) from NM*Met*_KTLS7/3 and NM*Met*_LTMC3/3. However, it did show a statistically significant difference ($p \leq 0.05$) compared to the other isolates (Table 2).

Table 2. Phosphate solubilizing index (PSI) of entomopathogenic fungal isolates

Isolates	Phosphate solubilization index (PSI±sd.)		
	3 days	5 days	7 days
NM*Met*_KTLS7/3	1.32±0.02^{ab}	1.36±0.08^{ab}	1.49±0.15^{a}
NM*Met*_NS7/2	1.10±0.17^{c}	1.06±0.12^{e}	1.16±0.01^{b}
NM*Met*_SS9/2	1.17±0.05^{bc}	1.16±0.07^{cde}	1.24±0.08^{b}
NM*Met*_LTMC3/3	1.45±0.15^{a}	1.40±0.11^{a}	1.44±0.04^{a}
NM*Met*_KBR5/1	1.42±0.06^{a}	1.38±0.06^{ab}	1.54±0.05^{a}
NM*Met*_BAL7/1	1.30±0.06^{abc}	1.26±0.08^{abc}	1.27±0.09^{b}
NM*Met*_WNK5/1	1.10±0.17^{c}	1.24±0.02^{bcd}	1.26±0.04^{b}
NM*Met*_DOA	1.19±0.07^{bc}	1.11±0.04^{de}	1.16±0.03^{b}
NM*Bb*_KTLS2/3	1.08±0.14^{c}	1.21±0.04^{cd}	1.23±0.03^{b}
p-value	**0.0028**	**0.0002**	**<0.0001**

Means ± sd. in the column followed by the same superscript letters were not significantly different (DMRT, p>0.05); sd. = standard deviation.

Plant growth-promoting potential of entomopathogenic fungi

Twenty-eight days after planting (DAP) (Table 3), the lettuce treated with NM*Met*_KBR5/1 showed the greatest height (16.96 cm). However, no statistical difference (p > 0.05) was observed compared to the application of NPK fertilizer or other fungal isolates. In the control treatments, green cos lettuce exhibited the lowest height.

Table 3. Effect of entomopathogenic fungi applied as foliar treatments on plant height in green cos lettuce

Treatments	Plant height (cm ± sd.)			
	7 DAP	14 DAP	21 DAP	28 DAP
Control	6.92 ±0.96^{c}	7.76±0.55^{bc}	11.90±1.41^{bc}	14.32±1.26^{c}
NPK Fertilizer	7.40 ±0.68^{b-d}	8.18±0.71^{ab}	13.44±1.21^{a}	16.86±1.126^{a}
NM*Met*_KTLS7/3	7.56±0.69^{a-d}	8.22±0.54^{ab}	12.66±0.65^{ab}	16.76±0.75^{ab}
NM*Met*_NS7/2	6.72±0.41^{d}	7.20 ±0.20^{c}	11.56±0.61^{bc}	15.58±0.61^{b}
NM*Met*_SS9/2	8.02±0.75^{ab}	8.32±0.60^{ab}	12.20±0.93^{a-c}	16.42±0.79^{ab}
NM*Met*_LTMC3/3	7.48±0.92^{a-d}	8.02±0.98^{ab}	11.74±0.66^{bc}	16.22±0.61^{ab}
NM*Met*_KBR5/1	7.66±0.47^{a-d}	8.24±0.63^{ab}	12.12±1.06^{a-c}	16.96±1.07^{a}
NM*Met*_BAL7/1	7.86±0.54^{a-c}	8.16±0.54^{ab}	10.94±1.41^{c}	16.26±0.60^{ab}
NM*Met*_WNK5/1	8.00±0.28^{ab}	8.22±0.50^{ab}	11.68±1.18^{bc}	16.04±0.82^{ab}
NM*Met*_DOA	8.50±1.17^{a}	8.76±0.69^{a}	12.60±0.68^{ab}	16.26±0.64^{ab}
NM*Bb*_KTLS2/3	8.32±0.58^{ab}	8.88±0.35^{a}	12.68±0.19^{ab}	15.98±0.72^{ab}
p-value	**0.0088**	**0.0102**	**0.0217**	**0.0012**

Means ± sd. in the column followed by the same superscript letters were not significantly different (DMRT, p>0.05); sd. = standard deviation; DAP = days after planting.

The number of lettuce leaves (Table 4) at 7, 14, and 21 DAP showed no statistically significant differences (p>0.05). At 28 DAP, the application of NPK fertilizer resulted in the highest number of leaves; however, this was not significantly different (p>0.05) compared to the application of NM*Met*_SS9/2 and NM*Bb*_KTLS2/3. In addition, the application of NPK fertilizer resulted in the largest leaf area per plant, measuring 1,333.5 cm², but this was not significantly different (p>0.05) compared to the application of NM*Met*_SS9/2, NM*Met*_LTMC3/3, NM*Met*_WNK5/1, NM*Met*_KTLS7/3, NM*Met*_NS7/2, NM*Met*_KBR5/1, NM*Met*_DOA, NM*Bb*_KTLS2/3, and NM*Met*_BAL7/1.

Regarding leaf width, NPK fertilizer application resulted in the greatest values; however, these did not differ significantly (p > 0.05) from those of the other treatments. For leaf length, NM*Bb*_KTLS2/3 produced the longest leaves, although no significant differences (p > 0.05) were observed compared with the other EPF treatments.

The fresh weight (total weight, shoot weight, and root weight) (Table 5) of NM*Met*_KTLS7/3, NM*Met*_BAL7/1, and NM*Met*_KBR5/1 treatments showed trends similar to that of NPK fertilizer- treated group, with no statistically significant differences (p> 0.05). In terms of dry weight, the NPK fertilizer treatment resulted in the highest shoot dry weight, although no significant difference (p>0.05) was observed compared to the treatments with NM*Met*_KBR5/1, NM*Bb*_KTLS2/3, and NM*Met*_BAL7/1. Regarding root dry weight, NPK fertilizer showed the highest value, while NM*Met*_DOA had the lowest.

Table 4. Effect of entomopathogenic fungi applied as foliar treatments on the number of leaves, leaf area, and leaf size in green cos lettuce

Treatments	Number of leaves (leaves ± sd.)				Leaves area/plants (cm^2 ± sd.)	Size of leaves (cm ± sd.)	
	7 DAP	14 DAP	21 DAP	28 DAP		Width	length
Control	3.60±0.54	4.20±0.45	7.00±0.71	11.00±2.23^{d}	803.70±212.89^{b}	4.20±0.45	7.00±0.71
NPK Fertilizer	3.60±0.54	4.60±0.89	7.40±0.54	14.60±0.89^{a}	1,333.5±326.71^{a}	4.60±0.89	7.40±0.54
NM*Met*_KTLS7/3	3.60±0.54	4.40±0.54	6.80±0.45	12.80±0.83^{bc}	1,231.7±218.33^{a}	4.40±0.54	6.80±0.45
NM*Met*_NS7/2	3.60±0.54	4.40±0.54	7.00±0.70	12.40±1.14^{bcd}	1,229.3±198.11^{a}	4.40±0.54	7.00±0.70
NM*Met*_SS9/2	3.80±0.44	4.00±0.00	7.20± 0.44	13.20±0.83^{ab}	1,259.3±133.85^{a}	4.00±0.00	7.20±0.44
NM*Met*_LTMC3/3	3.40±0.54	4.00±0.00	6.80±0.44	12.20±1.09^{bcd}	1,167.2±158.69^{a}	4.00±0.00	6.80±0.44
NM*Met*_KBR5/1	3.60±0.54	4.20± 0.44	7.00±0.70	12.20±0.83^{bcd}	1,246.1±153.97^{a}	4.20± 0.44	7.00±0.70
NM*Met*_BAL7/1	3.80±0.44	4.00± 0.70	7.20±0.44	12.40±0.89^{bcd}	1,228.9±131.58^{a}	4.00± 0.70	7.20±0.44
NM*Met*_WNK5/1	3.60±0.89	3.60±0.54	6.80±0.83	11.20±1.48^{cd}	1,088.4±105.11^{a}	3.60±0.54	6.80±0.83
NM*Met*_DOA	3.40±0.54	4.20± 0.44	7.00±0.70	12.60±1.14^{bcd}	1,237.3±163.18^{a}	4.20± 0.44	7.00±0.70
NM*Bb*_KTLS2/3	3.40±0.54	4.20±0.44	7.60±0.54	13.20±1.09^{ab}	1,127.4±188.28^{a}	4.20±0.44	7.60±0.54
p-value	**0.9774**	**0.2639**	**0.5516**	**0.0027**	**0.0035**	**0.2639**	**0.5516**

Means ± sd. in the column followed by the same superscript letters were not significantly different (DMRT, $p>0.05$) ,
sd. = standard deviation; DAP = days after planting.

Table 5. Effect of entomopathogenic fungi applied as foliar treatments on the fresh weight and dry matter in green cos lettuce

Treatments	Fresh weight (g ± sd.)			Dry matter (g ± ad.)	
	Shoot + Root	Shoot	Root	Shoot	Root
Control	59.01±16.48^{c}	43.74±11.85^{c}	10.15±2.89^{c}	2.24±0.46^{c}	0.82±0.09^{b}
NPK Fertilizer	116.05±17.97^{a}	90.65±17.99^{a}	20.60±4.30^{a}	5.20±1.05^{a}	1.95±0.51^{a}
NM*Met*_KTLS7/3	100.06 ±18.85^{ab}	81.84±16.47^{ab}	13.29±3.17^{bc}	3.98±0.68^{b}	0.78±0.11^{b}
NM*Met*_NS7/2	97.04±8.34^{b}	79.40±5.07^{ab}	12.53±1.47^{bc}	4.10±0.22^{b}	0.73±0.16^{b}
NM*Met*_SS9/2	98.06±8.10^{ab}	79.89±7.72^{ab}	11.88±2.52^{bc}	4.20±0.39^{b}	0.70±0.09^{b}
NM*Met*_LTMC3/3	93.54±9.72^{b}	78.11±8.58^{ab}	12.24±1.57^{bc}	4.34±0.37^{b}	0.86±0.20^{b}
NM*Met*_KBR5/1	105.79±13.73^{ab}	87.40±12.33^{ab}	14.47±2.55^{b}	4.55±0.59^{ab}	0.82±0.11^{b}
NM*Met*_BAL7/1	98.82±10.24^{ab}	82.29±9.13^{ab}	13.98±2.38^{bc}	4.48±0.46^{ab}	0.72±0.06^{b}
NM*Met*_WNK5/1	87.69±9.71^{b}	72.03±6.84^{b}	11.12±1.44^{bc}	3.76±0.40^{b}	0.70±0.11^{b}
NM*Met*_DOA	98.38±9.35^{ab}	81.02±5.91^{ab}	12.52±2.55^{bc}	4.19±0.52^{b}	0.68±0.21^{b}
NM*Bb*_KTLS2/3	92.07±10.21^{b}	76.32±9.58^{ab}	11.18±1.95^{bc}	4.49±0.22^{ab}	0.82±0.16^{b}
p-value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**

Means ± sd. in the column followed by the same superscript letters were not significantly different (DMRT, $p>0.05$);
sd. = standard deviation.

Discussion

All the EPF isolates effectively targeted aphids, although their impacts varied. Notably, the NM*Met*_KTLS7/3 fungus exhibited superior efficacy against aphids, aligning with findings by Popoonsak and konkarn (2021). They conducted experiments using *M. anisopliae*, *B. bassiana*, and *Isaria javanica* to control black aphids infesting long beans (*Aphis craccivora* Koch). Their results showed that different EPF fungi exhibited distinct levels of efficacy against black aphids. For instance, DOA-M8 demonstrated the highest aphid mortality rate (95.00%), statistically comparable to DOA-M8, DOA-M1, and DOA-B4, both of which had the lowest mortality rates. Similarly, Gebeyouohans *et al*. (2021) reported variations in effectiveness among different strains of *M. anisopliae* (MEI1 and MEI2) and *B. bassiana* (BEI1 and BEI2) against cabbage aphids (*Brevicoryne brassicae* L.), with BEI1 being the most effective (100%), followed by MEI1 (83.4%), and MEI2 showing the least impact. Furthermore, Qubbaj and Samara (2022) noted differing virulence levels of EPF isolates and strains (*B. bassiana*, *M. anisopliae*, and *L. lecanii*) against the black bean aphid (*Aphis fabae* Scop.), with *V. lecanii* demonstrating the highest virulence compared to the other entomopathogenic isolates.

The ability of EPF to invade insects is dependent on many factors, including the species and virulence of the EPF, the target insect, the duration of interaction between the EPF and the insect, and environmental conditions such as temperature, humidity, sunlight, and rainfall (Bugti *et al.,* 2020; Quesada Moraga *et al.*, 2024). Some species of EPF have specific host ranges. The destruction of insects by EPF fungi may be due to secondary metabolites or toxins produced by the fungi. However, some species of EPF only invade and compete for essential mineral nutrients inside the insect's body to sustain their own life (Bihal *et al*., 2023).

After a 7-day incubation on PVK medium, indigenous isolates of the EPF demonstrated significant phosphate solubilization activity. Notably, strain NM*Met*_KBR5/1 exhibited the highest solubilization index, although this was not statistically different from strains NM*Met*_KTLS7/3 and NM*Met*_NS7/2. The formation of distinct clear zones on PVK media also indicated the capacity of the EPF isolates to secrete extracellular enzymes, specifically the phosphatase enzyme. This enzymatic activity closely mirrors the role of fungi in natural environments, where they act as phosphate solubilizers (Khastini *et al*., 2015).

The effect of the EPF on lettuce growth showed that all 9 isolates promoted growth and yield of green cos lettuce. Compared to the control group, all isolates of the EPF resulted in higher growth indices, including plant height, leaf number, leaf area, fresh weight, and dry matter. Notably, strains

NM*Met*_KBR5/1 and NM*Met*_KTLS7/3 demonstrated higher growth indices in terms of fresh weight (above-ground and total), without statistical differences (p>0.05) compared to the use of NPK Fertilizer (15-15-15). These findings are consistent with Liu *et al*. (2022), who reported that *B. bassiana* and *M. anisopliae* promoted the growth of hydroponically grown maize. These fungi were able to systematically colonize all maize organs within 1 week through maize roots. Similarly, Yusniwati *et al*. (2022) reported that *B. bassiana* isolates possessed the ability to act as phosphate solvents and produce IAA hormones. Subsequently, the capacity of *B. bassiana* fungus isolates to function as growth promoters became evident through the observed increase in plant height and the number of chili leaves. In okra cultivation, the application of *M. anisopliae* MetA1 significantly increased the shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and leaf area compared to plants in the control group. Additionally, it suppressed root diseases caused by *R. solani* (Mimma *et al*., 2023).

In addition, Siqueira *et al*. (2020) found that both *M. robertsii* and *M. humberi* induced auxin-regulated gene expression in the roots of tomato plants and were able to produce indole-3-acetic acid (IAA), along with key compounds such as enzymes, hormones, and metabolites involved in promoting plant growth. IAA plays a crucial role in regulating plant growth by stimulating seed germination, enhancing root development, modulating vegetative growth processes, and influencing photosynthesis and the biosynthesis of various metabolites (Spaepen and Vanderleyden, 2011; Rana *et al*., 2019). Additionally, the presence of *M. anisopliae* increased both root and shoot dry matter of sugarcane by approximately 1.3 times compared to plants that were not inoculated, regardless of the NPK doses. While *M. anisopliae* promoted sugarcane growth, it did not result in a reduction in the use of NPK fertilizers for the crop (Oba *et al*., 2024).

However, Bozca *et al*. (2022) reported that the percentage of maize seed germination was not affected by *M. anisopliae* treatment. Nonetheless, maize plants treated with fungi exhibited higher root and stem biomass compared to plants in the control group. Conversely, *M. anisopliae* increased root-stem weight, root length, stem diameter, and biomass in sunflower samples compared to other EPF applications and the control group.

It is concluded that the nine isolates of EPF have the ability to both control aphids and promote the growth of green cos lettuce. The fungus NM*Met*_KTLS7/3 exhibited superior ability to control aphids, while NM*Met*_KBR5/1 showed the highest PSI. Additionally, NM*Met*_KBR5/1 and NM*Met*_KTLS7/3 yielded higher growth indices in terms of fresh weight (above-ground and total). Based on their ability to control aphids and yield,

isolates NM*Met*_KBR5/1 and NM*Met*_KTLS7/3 are recommended for further field tri

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Conflicts of interest

The authors declare no conflict of interest.

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