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RESEARCH ARTICLE

Biofungicides derived from indigenous microorganisms fermented on *Tagetes erecta* L. flowers for the control of anthracnose in chili pepper (*Capsicum annuum* L.)

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Abstract

Local microorganisms (LMo) are native microbial consortia that colonize specific substrates and ferment them when supplemented with nutrients. LMo-based products are emerging as eco-friendly alternatives for plant disease management. In this study, *Tagetes erecta* L. flowers were used as a substrate to cultivate LMo, which were then cross-applied to chili peppers (*Capsicum annuum* L.) to control anthracnose disease caused by *Colletotrichum capsici*. The research aimed to evaluate the antifungal activity, minimum fungicidal concentration (MFC) and overall effectiveness of LMo derived from *T. erecta* flowers against *C. capsici*. The study was conducted in 3 stages: first, assessing the antifungal activity of LMo using the agar dilution method, second, determining the MFC of the LMo solution and third, evaluating the *in planta* efficacy of LMo in controlling anthracnose. Results demonstrated that LMo formulated from *T. erecta* exhibited strong antifungal activity, with a 15.99 mm inhibition zone and an MFC of 40%. In the field trial, a 50% LMo concentration significantly suppressed anthracnose, improving chili pepper condition and appearance compared to both negative and positive controls. These findings suggest that LMo based on *T. erecta* flowers offers a cost-effective, abundant and eco-friendly solution for anthracnose management, particularly for chili farmers in Southern Sulawesi.

Keywords

anthracnose; antifungal; botanical pesticide; *Colletotrichum capsici*; local microorganisms; *Tagetes erecta* L.

Introduction

Chili pepper (*C. annuum*) is an important horticultural crop cultivated commercially due to its nutritional content and high economic value. It is widely incorporated in the food sector and for domestic usage. Chili peppers have garnered interest for their positive pharmacological effects on inflammation, pain, obesity, heart health and cancer (1–3). However, the productivity of chili peppers has shown a declining trend over years. According to data from the Ministry of Agriculture (4), chili pepper production from 2011 to 2015 was unstable. Furthermore, the Statistics Center for Southeast Sulawesi reported an 80% decrease in chili pepper production from 2016 to 2017. This decline was attributed to factors such as

pest attacks and diseases, with anthracnose being the most common disease affecting the chilli plants (5).

Anthracnose is a major disease of chili pepper, alongside bacterial wilt and geminivirus. Various *Colletotrichum* species can cause anthracnose disease (6). This disease significantly reduces the marketability of chili peppers and leads to a substantial yield loss (7). In Indonesia, the chili anthracnose disease (CAD) caused by *Colletotrichum* has led to an approximate loss of 35% in the commercial yield of chilies annually (8). The mold species, *C. capsici*, attacks chili peppers, presenting symptoms such as blackish-brown spots on the peppers that spread until the fruits become withered and rotten. The central part of these spots often contains black points, which are fungal colonies.

Traditionally, farmers have controlled CAD using synthetic fungicides. However, the regular use of these fungicides has adverse effects on the environment, plants and human health by leaving residues and it also promotes disease resistance (9). Therefore, natural fungicides offer a viable alternative to counteract these detrimental effects with several benefits: they are less harmful to the environment and human health due to their natural ingredients and they are inexpensive, biodegradable, readily available and straightforward to use (10, 11). Botanical pesticides, known as phyto pesticides, are derived from plant parts such as flowers, fruits, leaves, seeds and roots (12–14). Phyto pesticides contain a multitude of phytochemical substances that enable them to function through diverse mechanisms, posing a lower risk to human health than synthetic pesticides and not contributing to greenhouse gas emissions (15). These attributes support the use of plants as substrates for producing microbial-based products such as bio fungicides. The combination of plant substrates containing various phytochemical compounds with microbial consortia, which may include hidden antagonistic microbes that inhibit the growth of pathogenic microorganisms, presents an interesting research approach (16). LMo-based products refer to any bioproduct resulting from the natural fermentation of specific substrates by stimulated indigenous microorganisms. Their application is commonly associated with fertilizers or agricultural practices (17). The uniqueness of LMo-based products lies in their use of diverse local ingredients as raw materials, which contributes to their distinctiveness and potential efficacy. The microbial community in LMo accelerates the decomposition process of organic materials, acts as a growth stimulant and serves as pest control agent. Based on these beneficial properties, LMo can be used as an organic fertilizer or pesticide, including fungicide. The use of liquid fertilizer made from chicken and goat droppings, leaf litter or banana saplings as organic material sources requires effective degradation facilitated by LMo as a microbial source (18).

T. erecta, commonly known as marigold, is a versatile plant with considerable potential in Indonesia, particularly for its wide use in medicinal applications. However, the use of *T. erecta* flowers as a substrate for the production of LMo has not yet been reported. Researchers found that *T. erecta* flowers contain alkaloids, flavonoids, amino acids and tannins (19, 20). The essential oil content of these flowers exhibits antioxidant, antimicrobial and cytotoxic activities (21). Owing to these biological properties, *T. erecta* flowers hold potential as natural biofungicides. Their phytochemical components are biodegradable, making them environmentally friendly and non-polluting. Previous study reviewed those flavonoids can inhibit fungal growth through mechanisms such as plasma membrane disruption, induction of mitochondrial dysfunction, inhibition of cell wall formation, cell division, RNA and protein synthesis and the efflux-mediated pumping system (22). There has been no initiative or effort to formulate bioactive ingredients in *T. erecta* flowers and link them with LMo production. Therefore, this research aims to evaluate the fungicidal activities of LMo derived from *T. erecta* on the *in vitro* growth of *C. capsici* and assess the *in vivo/in planta* effectiveness in chili pepper.

Materials and Methods

Sample collection

T. erecta flowers were collected from Ladongi District, East Kolaka Regency, Southeast Sulawesi, Indonesia. The flowers were freshly collected and checked to ensure they showed no signs of disease or mechanical damage from insect bites. Species authentication was conducted by plant taxonomists through the submission of a duplicate specimen to the Laboratory of Plant Taxonomy at Universitas Halu Oleo, Kendari, Indonesia (Reference No.: 299/IX/UN29.9.1.2/PP/2021).

Preparation of *T. erecta* flowers for LMo production

LMo solution was obtained through natural fermentation by floral endophytes in the tissue of fresh *T. erecta* flowers (LMo×Te). Two kg of *T. erecta* flowers were cut into small pieces and ground using a blender. The ground flowers were then placed into a clean container, followed by the addition of 500 g of brown sugar and 2 L of rice washing water residue. The mixture was stirred until homogeneous, then covered and left to ferment for 14 days or until it emitted an alcohol-like aroma. Finally, the mixture was filtered and stored in a bottle (23). Before fermentation, the LMo solution was brownish -yellow, had an undecomposed substrate and emitted an unpleasant odor. After 14 days of fermentation, the solution's color changed to brown and it developed a sour aroma similar to fermented tapai. The turbidity decreased and the substrate showed signs of decomposition, indicating active microbial processing and the sign of the final product.

Preparation of *C. capsici* inoculum

Potato dextrose agar (PDA) was used to regrow *C. capsici* from its stock. The preparation process involved dissolving 2.4 g of potato dextrose broth (PDB, Difco™) and 2 g of agar into 100 mL of distilled water, followed by heating and homogenizing with a magnetic stirrer. The medium

was then sterilized using an autoclave for 30 min, at 15 psi and 121°C (24). The phytopathogenic mold, *C. capsici*, obtained from the Laboratory of Microbiology, Universitas Halu Oleo, Indonesia, was re-grown by transporting fungal spores using a tube needle on a slanted PDA medium and then transferring them to a fresh PDA medium. The culture was incubated for 48 hr at 37°C. The grown fungus was then made into a suspension by touching the aerial hyphae with a loop, placing it in a microcentrifuge tube containing 0.95% NaCl and vortex mixing. The number of spores in the inoculum was calculated by pipetting 100 µL of the spore suspension onto a hemocytometer and then counting the spores under a microscope at 400× magnification. The number of spores was calculated using the formula (25):

Number of spores = $n \times 4 \times 10^6 \times$ dilution factor (Eqn. 1)

Where n is the number of spores in the observed area (5×16 grid).

In vitro antifungal test of LMo×Te against *C. capsici*

Well-diffusion method was used to assess the antifungal activity of LMo×Te against *C. capsici*. The initial step involved pouring Penassay base agar into a petri dish. A sterile probe was then aseptically placed on top of the solidified agar base medium. Next, the Penassay seed agar, containing 1 mL of spore suspension, was poured into the petri dish. Once the seed agar had solidified, the sterile probe was removed, creating wells in the media. These wells were then filled with LMo solution at varying concentrations, as well as with negative and positive controls as listed in Table 1.

Each treatment was repeated 5 times. The plates were incubated at 37°C for 24–48 hr. The inhibition or clear zone was measured using Vernier calipers. The diameter of the inhibition or clear zone was calculated using the following formula:

Table 1. Concentration of solution volume for *in vitro* test

Treatment(s)	Concentration and composition
Control (+)	0.1% of Amistar®Top in SDW
Control (-)	SDW
K1	10% of LMo×Te in SDW
K2	20% of LMo×Te in SDW
K3	30% of LMo×Te in SDW
K4	40% of LMo×Te in SDW

SDW = Sterile distilled water

$$Z = \frac{(D_1 - D_S) + (D_2 - D_S) + (D_3 - D_S)}{3} \quad \text{(Eqn. 2)}$$

Where Z is the clear zone, D_1 is the vertical diameter, D_2 is the horizontal diameter, D_3 is the diagonal diameter and D_S is the diameter of the wells. The clear zones were categorized as very strong (diameter more than 20 mm), strong (diameter 11–20 mm), moderate (diameter 6–10 mm) and weak (diameter less than 5 mm) (26).

Determination of minimum fungicidal concentration (MFC) of LMo×Te

The agar dilution method was used to determine the MFC of LMo×Te that inhibits the absolute growth of *C. capsici*. A range of concentrations of LMo×Te (displayed in Table 2) was incorporated into the molten growth medium of *C. capsici* and allowed to solidify. The *C. capsici* colony was then inoculated onto the solidified agar medium using the point method and incubated for 24 hr. Minimum inhibitory concentration (MIC) is defined as the lowest concentration of a solution that inhibits fungal growth, indicated by the stagnancy of colony growth on agar. MFC, analogous tominimum bactericidal concentration (MBC), represents the lowest concentration of a solution that showed no growth of the indicator within a specified time (27).

Table 2. Total concentration of LMo×Te used for MFC test

Concentration (%)	LMo solution volume (mL)	Media volume (mL)	Total (mL)
40	6	9	15
50	7.5	7.5	15
60	9	6	15
70	10.5	4.5	15

In planta test of LMo×Te antifungal efficacy against *C. capsici* in chili plants

Preparation of planting media and *C. annuum* seeds

The planting media was prepared by sieving soil to remove any residue. Roasted husks were added to the soil in a 2:1 ratio (3 kg) in each polybag. Red chili seeds used in the experiment were sourced from Lambuya District, Konawe Regency, Southeast Sulawesi, Indonesia. Thirty-day-old chili seedlings were transplanted into polybags filled with the soil and roasted husk mixture. The seeds were selected for their homogeneity in physical traits such as having 6–7 leaves, reaching a plant height between 22–25 cm and stem width ranging from 0.3–0.5 cm. In the field trial (*in planta*), the concentration of LMo solution included was 40% and 50%, as well as positive and negative controls (as displayed in Table 3). The volume of solutions utilized for *in vivo* testing was 240 mL LMo per treatment at all concentrations. This test was carried out once a week, resulting in a total volume of 960 mL LMo used for all treatments over one month (Table 3). For the spraying treatment, 120 mL of LMo solution was used before *C. capsici* infection and an additional 120 mL was used after the infection. Finally, the total LMo solution prepared for this study was 2500 mL. The remaining LMo solution was stored as stock. Before treatment initiation, the plants were regularly watered twice daily (morning and evening). Weed control was performed weekly by manually removing weeds around the chili plants.

Table 3. The concentration of solution volume for *in planta* test

Treatment(s)	Concentration and composition
Control (+)	0.1% of Amistar®Top in SDW
Control (-)	SDW
K1	40% of LMo×Te in SDW
K2	50% of LMo×Te in SDW

SDW = Sterile distilled water

Application of LMo×Te solution on *C. annuum* plants

The application of LMo×Te solution on *C. annuum* plants involved 2 different spray time treatments: before and after infection with *C. capsici*. Prior to *C. capsici* infection, the LMo×Te solution, sterile distilled water and synthetic fungicide were sprayed on chili plants at a rate of 20 mL per plant. This treatment was applied one week before the infection with *C. capsici*. Following the spraying, *C. capsici* infection was initiated on the plants at a rate of 2 mL per plant. The plants were covered with plastic for a day to prevent external contamination and the spread of infection to other plants. Then, LMo spraying was continued once a week for a duration of 4 weeks. After the initial infection with *C. capsici*, the LMo×Te solution, sterile distilled water and synthetic fungicide were sprayed on the *C. annuum* plants at a rate of 20 mL per plant. This treatment was applied one week after the infection with the same procedures as before the infection.

Field observation

After one week of LMo spraying, observations were made to detect symptoms of anthracnose, characterized by spots on the fruit. The effectiveness of the LMo solution was assessed by observing the ability to prevent infection and reduce the severity of disease on *C. annuum* peppers, by measuring the area of spots appearing during the first and final week of observation. The area of these spots was measured by using a ruler. The disease intensity was calculated using the following formula (28):

$$DI = \sum \frac{ni \,.\, vi}{N \,.\, V} \times 100\%$$

(Eqn. 3)

Where DI is disease intensity (%), vi is a score of each category, V is the number of chilies observed, N is the highest score for each category and ni is the number of chilies in each category.

Data analysis

Analysis of variance (ANOVA) was used to analyze the effect of LMo×Te treatments on the growth of *C. capsici*. Descriptive analysis was conducted to gather, process and present the data, while inferential analysis was employed to conclude the data. If the LMo treatment showed a significant effect on *C. capsici*, further testing or a *posthoc* Tukey's Honest Significant Difference (HSD) test would signify a similar interaction among treatments. Data analysis was performed using SPSS version 20.0.

Results and Discussion

Fermentation of *T. erecta* flowers as substrates for LMo production

The fermentation result of LMo solution of *T. erecta* flower is presented in Fig. 1. The successful fermentation of LMo was evidenced by the changes in color, aroma and substrate shape (Fig. 1B), which is consistent with the previous findings (29). Other researchers also added that fermentation improves food properties, including vitamins, essential amino acids, proteins, appearance, flavors and aroma (30).

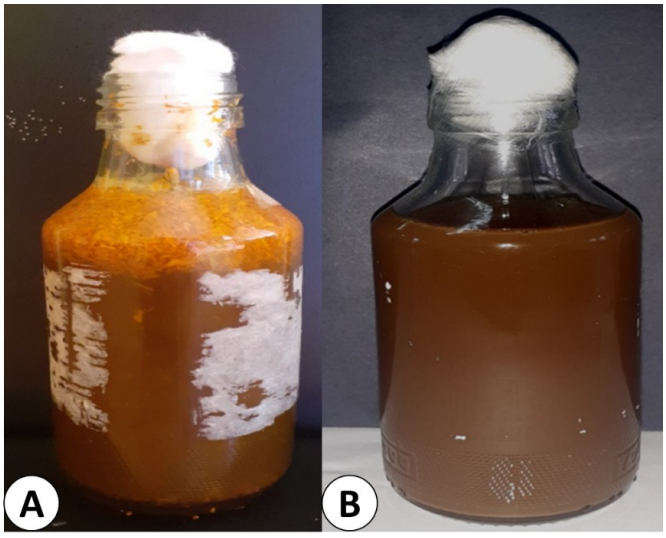

Fig. 1. Fermentation of *T. erecta* flowers into LMo products at (**A**) 0 days and (**B**) 14 days.

Antifungal activities of LMo×Te against *C. capsici*

The antifungal activities were determined using the agar well diffusion test with concentrations of 10%, 20%, 30% and 40% of LMo×Te, alongside a positive control (Amistar®Top) and a negative control (sterile distilled water). The results are illustrated in Fig. 2, which shows the inhibition zones formed around the wells after 15 hr of incubation.

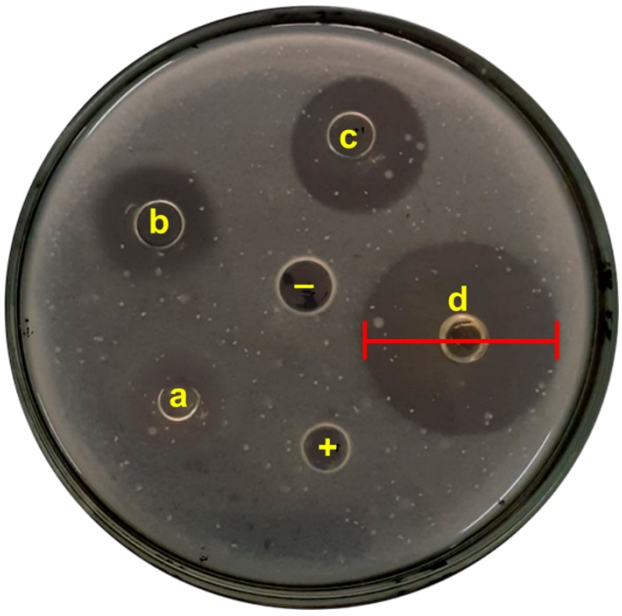

Fig. 2. Antifungal activity of LMo×Te against *C. capsici* based on inhibitory zones (red line): (**+**) commercial pesticide or Amistar®Top, (**–**) sterile distilled water, (**a**) 10%, (**b**) 20%, (**c**) 30%, (**d**) 40%. White arrows show the fungal lawn of *C. capsici*.

These zones of growth inhibition indicate that LMo×Te exhibited antifungal activity against *C. capsici*, in a dose-dependent manner (Table 4). Higher concentrations led to larger inhibition zones, reflecting greater antifungal efficacy. Conversely, both positive and negative control treatments did not show any inhibition zones. The inability of the positive control (Amistar®Top) to inhibit *C. capsici in vitro* may be attributed to the insufficient test concentration. In contrast, other studies have demonstrated that Amistar®Top exhibits efficacy as an antifungal agent at a concentration of 200 ppm against *Rhizoctonia solani* (31). Previous research demonstrated that the formation of clear zones indicates the ability of active compounds to inhibit fungal growth, whereas larger zones denote higher antifungal activity (32). Furthermore, another study emphasized that increased concentration correlates with higher levels of active substance components, thus contributing to stronger antifungal effects (33). The antifungal properties of *T. erecta* flowers are attributed to bioactive compounds such as flavonoids and their derivatives (34).

Table 4. Antifungal activities of LMo×Te on the *in vitro* growth of *C. capsici*

No	Concentration (%)	Average diameter of inhibition zone	Description
1.	Positive control	0	W
2.	Negative control	0	W
3.	Concentration of 10%	8.07^{ab}	M
4.	Concentration of 20%	11.72^{ab}	S
5.	Concentration of 30%	11.93^{ab}	S
6.	Concentration of 40%	15.99^{b}	S

Values within a column having the same letters are not significant using Tukey's HSD ($p \leq 0.05$).
M = moderate, S = strong, W = weak (35).

The results indicate that the highest inhibition zone diameter was observed at a concentration of 40%, measuring 15.99 mm, categorized as a strong inhibition zone. Conversely, the lowest diameter was recorded at a concentration of 10%, with a diameter of 8.07 mm, categorized as moderate inhibition zone strength (Table 4). Statistical analysis of the inhibition zone measurements from the diffusion test was conducted using ANOVA to determine the significant effect of LMo×Te on inhibiting the growth of *C. capsici*, as presented in Table 5. The ANOVA results show a significant effect of LMo solution concentration on *C. capsici* growth ($p=0.018<\alpha$), prompting further testing using Tukey's HSD test at a significance level of 10%. The results of the HSD test are shown in Table 6, indicating no significant difference among concentrations of 10%, 20% and 30%, but a significant difference at the 40% concentration. These findings suggest that the LMo×Te at a concentration of 40% is the most effective in inhibiting the growth of *C. capsici*.

Table 5. Analysis of Variance of ANOVA on the inhibition strength of IMO×Te on *C. capsici*

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1105.64	5	221.12	3.40	0.01
Within Groups	1559.05	24	64.96		
Total	2664.70	29			

Table 6. Multiple comparisons of treatment results using Tukey's HSD test

X (Treatments)	N	Subset for alpha = 0.05	
		1	2
K+	5	0.00	
K-	5	0.00	
10%	5	8.07	8.07
20%	5	11.72	11.72
30%	5	11.93	11.93
40%	5		15.99
Sig.		0.217	0.635

Determination of MFC of LMo×Te against *C. capsici*

Determination of the MFC was carried out using an agar dilution method to assess the ability of LMo×Te to inhibit the growth of *C. capsici* by applying different concentrations (40%, 50%, 60% and 70%), as well as PDA media without LMo×Te or positive control. The concentration in the agar was obtained from the MIC in the agar well diffusion test, which was 40%. The MFC of LMo×Te against *C. capsici* is presented in Fig. 3.

Fig. 3. Results of agar dilution test of LMo×Te against *C. capsici*. Control plate using Amistar®Top. Treatment plates using LMo×Te.

Fig. 3 shows no growth of *C. capsici* after 7 days of incubation, whereas *C. capsici* growth is observed on PDA medium without LMo treatment. The results indicate that LMo×Te effectively killed *C. capsici* colony. The results showed that the MFC of LMo×Te solution at 40% and 50% concentrations were optimal in eliminating *C. capsici*. This demonstrates that higher concentrations of LMo×Te correspond to increased efficacy against *C. capsici*. Researchers stated that a compound with antifungal properties will increase its activity from fungistatic to fungicidal if the concentration of the compound is increased (36). The higher the concentration level of an antifungal substance, the stronger its working activity and the higher its killing power.

In planta efficacy of LMo×Te as a biofungicide against anthracnose disease on chili plants

The efficacy of LMo×Te as a biofungicide was assessed in the field through 2 stages: application before and after *C. capsici* infection. The first stage, application of LMo×Te before *C. capsici* infection, aimed to prevent anthracnose disease in *C. annuum* peppers. The second stage, application of LMo×Te after *C. capsici* infection, evaluated the effectiveness of LMo in reducing the severity and spread of fungal colonization. Various concentrations were tested, including 40%, 50%, a positive control (Amistar®Top) and a negative control (sterile distilled water).

Preventive efficacy of LMo×Te against anthracnose disease on chili plants

The effectiveness of LMo×Te in mitigating anthracnose disease by *C. capsici* is shown in Fig. 4. The data indicates that at concentrations of 40% and 50%, no symptoms of anthracnose were observed from the first to the last week of observation. Physically, the chili plants remained in good health, as evidenced by the absence of black spots and wrinkles on the pepper. This suggests that LMo×Te effectively prevented the growth of *C. capsici*. In contrast, anthracnose was observed in both the positive control (synthetic fungicide) and negative control (sterile distilled water) treatments, indicated by the presence of typical symptoms of *C. capsici* colonization on the pepper. According to previous research, the bioactive compounds in *T. erecta* flowers contain essential oils, which are easily degraded and environmentally friendly (37). These essential oils exhibit biological activity that can inhibit the growth of pathogenic molds, viruses, insects and bacteria. The ability of LMo×Te to prevent anthracnose attacks on *C. annuum* was further determined by measuring the area of distribution of anthracnose on chilies as presented in Fig. 5.

Fig. 4. Visual appearance of chili peppers in each treatment for the first week (**A–D**) and the fourth week (**E–H**) of observation in preventive field assay: (**A, E**) negative control using sterile distilled water; (**B, F**) positive control using Amistar®Top; (**C, G**) LMO×Te at 40% and (**D, H**) LMO×Te at 50%.

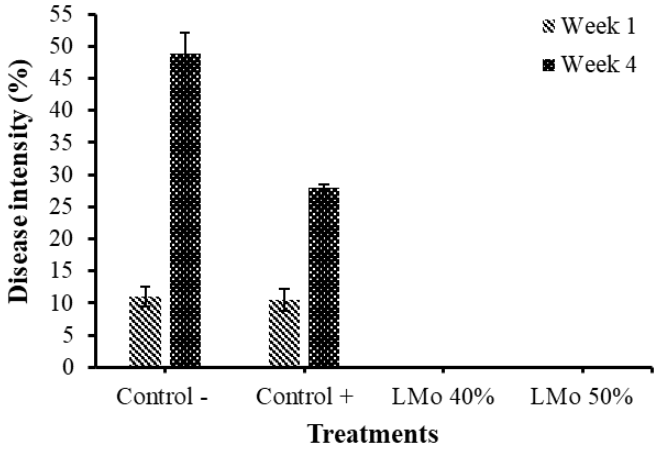

Fig. 5. Severity level of anthracnose disease through LMo×Te spraying treatment before *C. capsici* infection on chili plants. LMo = LMo×Te.

Fig. 5 shows the spread of anthracnose disease on chili peppers. The negative control (sterile distilled water) exhibited the highest disease spread, with 11.04% in the first week and 48.81% in the last week. The positive control (synthetic fungicide, Amistar®Top) showed a disease spread of 10.52% in the first week and 28.04% in the last week. In contrast, the concentrations of 40% and 50% LMo×Te showed no spread of anthracnose disease throughout the observation period. These results indicate that both positive and negative control treatments resulted in a significant increase in the severity of disease over 4 weeks, highlighting the limited efficacy of commercial pesticides in inhibiting *C. capsici* growth. Previous research supported this finding by stating that Amistar®Top fungicide, which contains active difenoconazole and azoxystrobin as active ingredients, can inhibit fungal mycelium growth, but may not be as effective as the LMo treatment (38).

Curative efficacy of LMo×Te against anthracnose disease on chili plants

Symptoms of anthracnose on *C. annuum* pepper after being inoculated with *C. capsici* and covered for one day were illustrated in Fig. 6. The symptoms observed included black spots around the fruit, a slightly concave shape and the pepper becoming dry and wrinkled. These observations align withprevious findings, which described anthracnose symptoms as water-soaked blackish spots on the pepper surface, accompanied by sunken lesions with concentric rings of acervuli (39). Fig. 7 demonstrates that the LMo×Te treatments effectively inhibited and suppressed the severity of anthracnose disease, as evidenced by the absence of *C. capsici* colonization on other peppers and no increase in the colonized area. This indicates that LMo×Te can inhibit the growth of *C. capsici*. Conversely, in both the negative and positive control treatments, continual infections were observed, highlighting their ineffectiveness in inhibiting *C. capsici* growth. The extent of anthracnose disease on red chilies was measured to determine the efficacy of LMo×Te in

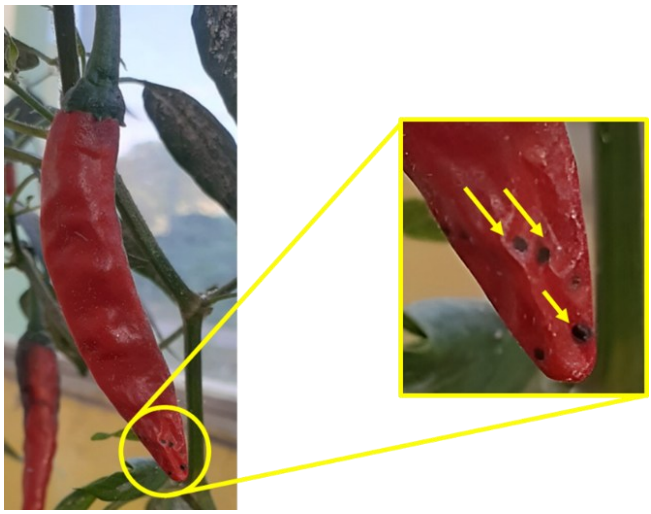

Fig. 6. Signs of *C. capsici* colonization or a symptom of anthracnose disease on chili peppers as indicated by the pointed arrows.

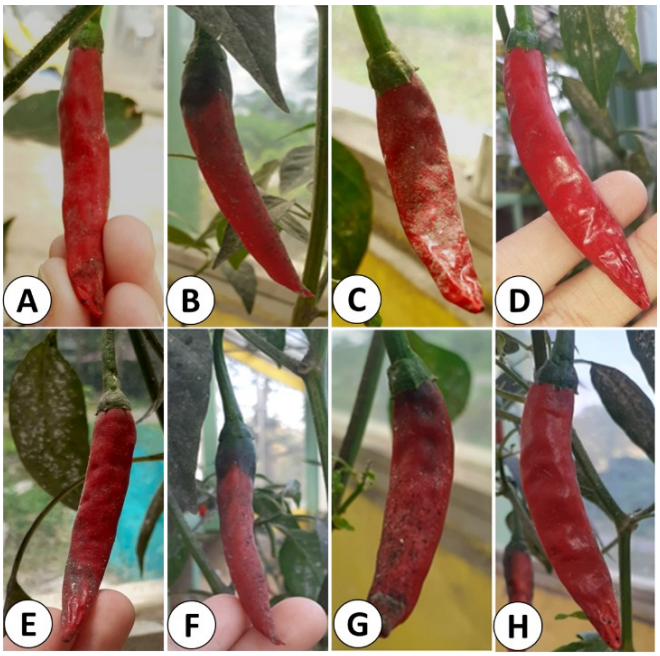

Fig. 7. The visual appearance of chili peppers in each treatment for the first week (**A–D**) and the fourth week (**E–H**) of observation in curative field assay: (**A, E**) negative control using sterile distilled water; (**B, F**) positive control using Amistar®Top; (**C, G**) LMO×Te at 40% and (**D, H**) LMO×Te at 50%.

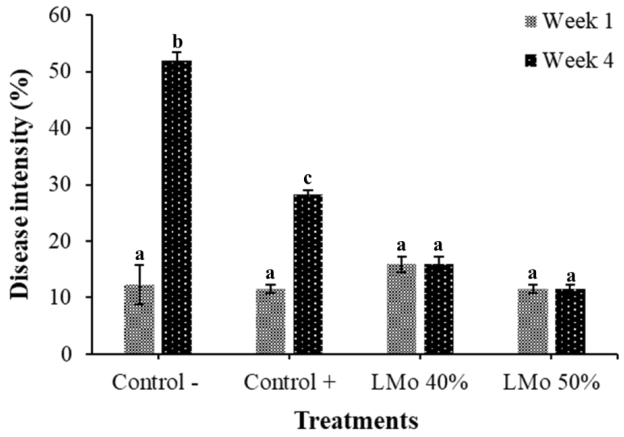

Fig. 8. Severity level of anthracnose disease through LMo×Te spraying treatment after *C. capsici* infection on chili plants. LMo = LMo×Te. Bars having the same letters in the same observation week are not significant using Tukey's HSD and vice versa (p≤0.05).

reducing the severity of anthracnose, as presented in Fig. 8.

Fig. 8 depicts the extent of anthracnose disease spread on chili peppers. The spread was highest in the negative control group (51.90%), followed by the positive control group (28%), the 40% LMo×Te concentration group (15.87%) and the lowest spread was observed in the 50% LMo×Te concentration group (11.57%). It is evident that at LMo×Te concentrations of 40% and 50%, there was no increase in black spots on the fruit surface from the first week to the last week of observation. This indicates that LMo×Te is also effective in mitigating the severity of anthracnose disease or in a curative way. Furthermore, the 50% concentration proved to be the most effective in inhibiting and suppressing the disease, whereas the highest severity levels were found in the positive and negative control treatments, both showing an increase in disease severity during the final week of observation. These results suggest that higher concentrations of LMo×Te may improve its efficacy to inhibit *C. capsici* colonization. Table 7 shows the result of ANOVA among treatments.

Table 7. Results of variance analysis of ANOVA

	Sum of	Df	Mean Square	F	Sig.
Between Groups	2949.67	3	983.22	728.16	0.00
Within Groups	10.80	8	1.35		
Total	2960.47	11			

Several researchers reported that in their study on *Colletotrichum* spp. infection in *C. annuum* in Sleman and Bantul Regency, Yogyakarta, the infection began 5 days after inoculation and continued until the fruit dried (40). This infection might be due to the presence of genes encoding pathogenicity. It was identified the ChEC3 gene as related to appressorium initiation (41). Furthermore, another study stated that the function of the CEC3 gene is conserved in the genus *Colletotrichum*, with homologs from 4 distinct *Colletotrichum* species promoting nuclear enlargement and cell death upon infection (42). The findings highlight the potential of LMo×Te as a biofungicide for managing *Colletotrichum* infections in chili plants. These insights may spur further research into the role of *Colletotrichum* pathogenicity genes and the application of LMo-based biofungicides in Southeast Sulawesi and other regions.

Conclusions

The findings of this study demonstrate that microbial consortia residing in *T. erecta* flowers can ferment to produce biofungicides based on LMo. The LMo×Te formulation exhibited significant antifungal activity against *C. capsici*, with the highest inhibition observed at a concentration of 40%, producing a notable inhibition zone of 15.99 mm. The MFC required to effectively inhibit *C. capsici* growth was determined to be 50%. Additionally, field trials showed that LMo×Te significantly reduced the severity of anthracnose disease in chili plants, with optimal suppression of *C. capsici* growth also observed at a concentration of 50%. These results underscore the potential of LMo derived from *T. erecta* flowers as a promising, environmentally friendly alternative for managing anthracnose disease in agricultural settings.

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Authors' contributions

SA: Conceptualization, Methodology, Investigation, Writing – Original Draft. NAY: Supervision, Validation, Writing – Review & Editing, Funding acquisition. JM: Supervision, Formal analysis, Software, Data curation. EYA: Resources,

Visualization, while A Methodology, Software, and Validation.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

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Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the authors used Quillbot and Grammarly in order to improve the language and readability. After using this tool/service, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

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