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Biocontrol potential of *Fusarium equiseti* and *Cladosporium cladosporoides* against *Aphis fabae* under controlled conditions

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Bean aphid is a significant insect pest that limits achievement of maximum yields and quality of beans. Overreliance on synthetic insecticides has led to the development of insecticide resistance, negatively impacting both human and environmental health. Use of entomopathogenic fungi is considered safer and environmentally friendly for managing bean aphids. A study was conducted to evaluate the effectiveness of three different conidial concentrations 1×10^7, 1×10^8, and 1×10^9 spores/ml from two fungal isolates, *Fusarium equiseti* (JD02) and *Cladosporium cladosporioides* (JD07) along with distilled water containing 0.1% Triton x-100 and Imidacloprid as controls, against the *Aphis fabae*. A completely randomized factorial experiment was used in which petri dishes containing live aphids were sprayed with suspensions of the fungi concentrations. Mortality rate was recorded at the 3rd, 5th, and 7th days after inoculation. At the 7th day, the results showed that Imidacloprid treatment caused high mortality rates of 100%. A concentration of 1×10^9 spores/ml resulted in mortalities of 93.65% and 72.9%, followed by 1×10^8 spores/ml, which resulted in mortalities of 78.57% and 57.38% for JD-02 and JD-07, respectively. Meanwhile, a concentration of 1×10^7spores/ml led to lower mortalities of 62.02% and 40.24% for JD-02 and JD-07, respectively. Additionally, the LT50 and LT90 of *F.equiseti* and *C.cladosporioides* differed significantly at $P<0.05$; however, among the tested

concentrations, 1×10^9 spores/ml of both *F.equiseti* and *C.cladosporioides* took 5.09 and 5.23 days to kill 50%, and 6.92 and 7.52 days to kill 90% of bean aphids, respectively. The isolate *F.equiseti* caused higher mortality compared to *C.cladosporioides*; additionally, a concentration of 1×10^9 spores/ml of *F.equiseti* resulted in higher mortality > 90% at the seventh day. Therefore, *Fusarium equiseti* demonstrated significant potential for controlling bean aphids.

KEYWORDS

Entomopathogenic fungi, *Fusarium equiseti*, *Cladosporium cladosporioides*, *Aphis fabae*, *Phaseolus vulgaris*

1 Introduction

The black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae), is a complex species that prefers the new growing parts of plants to suck the sap and to deposit nymphs (Esmaeili-Vardanjani et al., 2013; Stoddard et al., 2010). It's estimated that bean aphids are capable of causing yield losses of 70% in various crops, especially vegetables (Nordey et al., 2017). The losses are due either to direct damage by feeding of the plant sap from the plant vascular tissue or to indirect transmission of plant pathogenic virus (Boni et al., 2021). Additionally, bean aphids contaminate the leaves through honeydew deposits on the surface of the plant foliage, thus favoring the growth of the sooty molds, which hampers the photosynthetic efficiency of the plants (Wamonje et al., 2020).

Management measures for bean aphids, such as cultural, mechanical, and chemical control, have been implemented to reduce their population; however, the use of synthetic insecticides, especially pyrethroids, neonicotinoids, organophosphates and carbamates, outweighs other control measures (Zanic et al., 2013). This is because they are easy to apply (labor-saving) and accessible compared to other methods. Consequently, indiscriminate and extensive use of synthetic insecticides in the management of bean aphids has resulted in environmental pollution (bio-magnification), build-up of insect resistance, threats to natural beneficial organisms (pollinators, parasitoids, prey and predators) (Bass et al., 2015; Sharma et al., 2019).

Therefore, the negative effects of synthetic chemicals have prompted agricultural producers and scientists to seek environmentally safe and acceptable control measures against bean aphids while safeguarding the ecosystem and human health. One promising alternative avenue is the use of microbes, especially entomopathogenic fungi (EPF).

Entomopathogenic fungi (EPF) have been reported to be effective against many species of insect pests in agricultural settings presenting an opportunity to be used in the management of bean aphids (Maina et al., 2018; Mweke et al., 2018). The mode of action of entomopathogenic fungi is through enzymatic degradation of insect bodies, release of toxic proteins and bioactive secondary metabolites upon penetration, which results in paralysis, disrupting physiological processes like the immune system and nerve conduction that leads to abnormal behaviors, dehydration and eventually death (Dauda and Maina, 2018; Litwin et al., 2020). Several species of entomopathogenic fungi (*Beauveria* spp, *Metarhizium* spp *and Isaria* spp) are well known and currently commercially marketed as bioinsecticides to control bean aphids (McGuire and Northfield, 2020; Zimmermann, 2008). However their utilization has been increasing in the market of the United States, Europe and Asia for several decades (Dauda and Maina, 2018). Their use in East Africa, especially in Tanzania, is limited due to a lack of suppliers and information about suitability for the local climate (Boni et al., 2021). Consequently, this situation necessitates testing of locally available fungi species which could be potentially entomopathogenic to bean aphids. This strategy can help to support efforts to identify more virulent species that can be used in the management of bean aphids. This study was therefore set to assess effectiveness of *Fusarium equiseti* and *Cladosporium cladosporioides* species naturally sourced from the local environment against *Aphis fabae*.

2 Materials and methods

2.1 Description of the study area

This study was conducted between April 2025 to July 2025 in the Mycology laboratory and screen houses of the Department of Crop Science and Horticulture (DCSH) located at Sokoine University of Agriculture (SUA), Tanzania (6.8520°S and 37.6576°E). Morogoro municipality is located at the foothills of the Uluguru Mountains. It is characterized by a tropical-sub humid climate with bimodal rainfall ranging from 800 to 1,200mm, average annual temperature ranging from 18°C to 30°C, with

cooler conditions during the rainy season and warmer conditions during the dry season (Kacholi, 2020).

2.2 Establishment of common bean and rearing of bean aphids

Common Beans, *Phaseolus vulgaris L* (one plant per pot) (Fabaceae) were grown to feed *Aphis fabae*. The plants were established in 20 plastic pots of 1 L, then placed on top of tables in the screen house. Standard management practices were applied to the common bean plant. Bean aphids were collected from unsprayed bean plants within and around the SUA crop museum located in Morogoro, Tanzania (6.8520°S and 37.6576°E). Bean aphids were collected by plucking the aphid-infested leaves from a bean plant and placed inside a plastic container, then transferred to the mycology lab and identified under a light microscope by using polyphagous aphid keys (Blackman and Eastop, 2000). Identified bean aphids were inoculated onto insect-free bean plant leaves in clip cages (2.5–4 cm) for multiplication. Plants were watered twice a day, depending on the moisture content of the soil detected by the feel method. Temperature and relative humidity (RH) were 26 ± 3°C, 65 ± 5%, respectively and a photoperiod of 12:12h (L: D).

2.3 Isolation, identification and preparation of fungal isolates

The two fungal isolates were isolated from cadavers of bean aphids and then molecularly identified as *F.equiseti* and *C.cladosporiodes* through the use of ITS gene region (ITS1 (F5'TCCGTAGGTGAACCTGCGG-3 ') and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (Hoggard et al., 2018). After identification the purified fungal species were stored in a slant at the mycology lab of the Department of Crop Science and Horticulture, Morogoro, Tanzania. Before performing the pathogenicity and bioassay test the fungal species were cultured on PDA for 10 days at 28 ± 2 ^{0}C and 75% RH until mycelia grew in full; the obtained pure cultures were used for bioassay experiments.

2.4 Inoculum of isolates of entomopathogenic fungi

One isolate of *F.equiseti* and *C.cladosporioides* were initially screened for their pathogenicity against the 10 apterous adults of bean aphids using one concentration of 1×10^{8} spores/mL. The spore suspension was prepared from 12-day-old cultures grown on PDA plates at 24 ± 2°C. The colony surface was scraped with a sterile inoculating needle and harvested into 20 mL of sterile distilled water as per Zekeya et al. (2019). The suspensions were filtered using a double-layer muslin cloth and transferred to a conical flask containing distilled water with a drop of Tween-80 to keep the conidia dispersed uniformly, and then shaken thoroughly for 10 min (Boni et al., 2021). The conidia spores were counted in the suspension with the aid of a light microscope and adjusted with a hemocytometer (Swastik Scientific Company, India). A hemocytometer is essentially a microscope slide bearing a small well of known depth, the base of which is marked with squares of known dimensions. During use, the well is covered with a special coverslip (usually 0.4 mm thick). The other three working concentrations (1 ×10^{7}, 1×10^{8}, and 1×10^{9} spores/mL) were prepared by diluting the stock inoculum with sterile distilled water as per Zekeya et al. (2019).

2.5 Experimental design

The bioassay factorial experiment was set under a completely randomized design with the two factors (Fungal isolate and conidia concentration) by using *F.equiseti* (JD 2) and *C. cladosporoides* (JD 7) set at 1 ×10^{7}, 1×10^{8}, and 1×10^{9} spores/ml as serial conidial concentrations. Bean leaves free of aphids and disease symptoms were collected and rinsed with distilled water, then placed in a Petri dish (120×20 mm) on top of sterile filter paper for bean aphid feeding. These leaves were changed daily up to seven days. Then, 50 adult bean aphids were transferred to each bean leaf using a fine brush. Each Petri dish containing 50 adult bean aphids was sprayed with an aliquot of 1 ml of a suspension of conidia of the entomopathogenic fungus with concentrations of 1 ×10^{7}, 1 ×10^{8} and 1 × 10^{9} conidia ml^{-1}. Each treatment was replicated three times. The controls were sprayed with distilled water with 0.1% Tween-80. All the treated bean aphids were kept at lab temperature (26 ± 2°C).

2.6 Data collection and confirmation the cause of aphids' mortality

Cumulative aphid mortality was recorded daily for up to seven days after application of the three different concentrated conidial suspensions of each fungus, besides negative and positive controls. The dead aphids were surface sterilized with 1% Sodium hypochlorite solution, and washed with distilled water before being placed in Petri dishes (60x10 mm) with lined and wet blotter paper for facilitating mycelial growth. The petri dishes were sealed and incubated at 28 ± 2°C and 75% RH as per Thaochan and Sausa-Ard (2017). On the fifth day of incubation, cadavers were checked for mycosis signs with a dissecting microscope (Leica Zoom 2000 No Z45V). Emerging mycelia were harvested using a sterile inoculating needle and transplanted onto plates made up of PDA for confirmation procedures. After 7 days, the mycelia were examined under the microscope. If a dead aphid did not show fungal outgrowths of similar characteristics to those of the one applied as the treatment, its death was considered as caused by another factor, or factors, and was therefore discarded.

2.7 Statistical data analysis

Cumulative mortality rates data were corrected on the 3rd 5th and 7th day after inoculation by using Abbott's formula (Abbott,

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TABLE 1 Percentage mean mortality of bean aphids (*Aphis fabae*) under varying concentrations of spores of *Fusarium equiseti* and *Cladosporium cladosporioides*, along with positive and negative control at 26± 2°C.

Day	Concentration of spores	% Mortality ± SE	
		F.equiseti	*C.cladosporioides*
	1×10^7	6.67 ± 0.67	2.03 ± 1.16
3	1×10^8	5.33 ± 0.67	3.36 ± 1.32
	1×10^9	9.33 ± 0.67	9.39 ± 4.34
	C+	90.67 ± 1.33	92.6 ± 0.73
	C-	0	1.33 ± 1.33
	1×10^7	17.04 ± 1.49	18.63 ± 1.36
5	1×10^8	25.19 ± 3.23	27.6 ± 3.55
	1×10^9	35.56 ± 5.88	41 ± 12.1
	C+	98.52 ± 1.48	100 ± 0
	C-	10 ± 0.00	10.7 ± 0.67
	1×10^7	62.03 ± 3.68	40.2 ± 2.68
7	1×10^8	78.57 ± 3.04	57.4 ± 0.12
	1×10^9	93.66 ± 0.91	72.9 ± 3.97
	C+	100 ± 0	100 ± 0
	C-	15.33 ± 2.40	18.7 ± 1.33

1925). Percentage Cumulative mortality data from bioassay post-inoculation were subjected to analysis of variance(ANOVA) by using R-studio version (2025.05.1 + 513 exe) under Car package. The residuals from the analysis of variance table were tested for normality by using the Shapiro-Wilk test. Serial time-mortality data from bioassay were analyzed by Probit analysis using R-studio to calculate Median lethal time 50% (LT50) and 90% (LT90%). Mean separation was performed by a Tukey *post-hoc* test at a significance level ($P< 0.05$).

FIGURE 2
Typical mycosis observed externally on the bean aphid cadaver due to fungal infection after the cadaver was incubated for 5 days at 28 ± 2°C.

3 Results

3.1 Efficacy of *Fusarium equiseti* and *Cladosporium cladosporioides* fungi against the black bean aphid

Findings have shown that the three spore concentrations of *F. equiseti* and *C. cladosporioides* were suppressive to bean aphids (Table 1 and Figure 1). Mortalities of bean aphids gradually increased with the increase in concentration of spores and time of exposure.

The pathogenic activity of fungi began showing effects after 2 days post-infection, and the bean aphids were observed not moving from one place to another Figure 2.

The colony characteristics and macroconidia from the cadavers revealed similarity to the ones that were used during the infecting process Figure 2.

Regardless of the isolates used, the average cumulative mortality (%) increased with an increase in concentrations. Bioassay results showed that the two fungi species, *F. equiseti* and *C. cladosporioides*, do not differ significantly at $P = 0.531$ and 0.434 at day 3 and day 5,

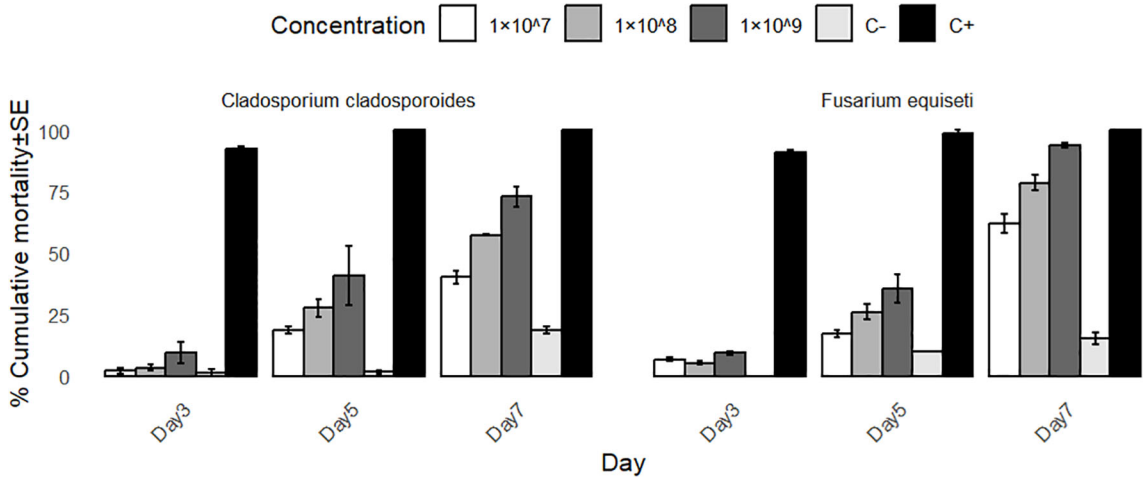

FIGURE 1
Mean aphid mortality % caused by different conidial concentration of entomopathogenic fungi at 3th, 5th and 7th days.

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respectively (Table 2). However, the tested concentration of 1×10^7, 1×10^8, 1×10^9 spores/ml differ significantly in causing mortality of bean aphids (P<0.00001) across all days of post infection (Table 2). The concentration of 1×10^9 spores/ml for both *Cladosporium cladosporioides* and *Fusarium equiseti* resulted in high mortalities of 72.09% and 93.86%, respectively, followed by 1×10^8 spoes/ml with mortalities of 57.38% and 78.57% spores/ml. However, 1×10^7 spores/ml had lower mortalities of 62.02% and 40.24%, respectively (Table 1).

3.2 Pathogenicity comparison of the isolates

At 7 days post-infection, statistical analysis revealed a highly significant difference (P<0.0001) in mortality of bean aphids exposed to *F. equiseti* (JD2) and *C.cladosporioides* (JD7), at varying spore concentrations (1×10^7,1×10^8,1×10^9 spores/ml). Notably, *F.equiseti* consistently induced higher mortality compared to *C.cladosporioides* Table 3 and Figure 3.

3.3 Median lethal time (LT50) and (LT90) of *Fusarium equiseti* and *Cladosporium cladosporoides* at different concentrations

Median lethal time(LT50) and (LT90) results between *F.equiseti* and *C.cladosporioides* (The time each concentration took to cause 50% and 90% of aphids' mortality) differed significantly at P<0.05. The 1×10^7 spores'/ml concentrations of both *F.equiseti* and *C. cladosporioides* took the longest time of about 6.20 and 6.68 days to kill 50%, 8.78 and 9.59 days to kill 90% of the bean aphids (Table 4, Figures 4, 5) respectively followed by 1×10^8 spores/ml which took 5.67 and 5.97 days to kill 50%, 5.67 and 5.97 days to kill 90% of bean aphid, additionally, 1×10^9 spores/ml of both *F.equiseti* and *C.cladosporioides* took shorter time of about 5.09 and 5.23 days to kill 50% and 6.92 and 7.52 days to kill 90% of bean of aphids (Table 4, Figures 4, 5) respectively. However, positive control (Imidacloprid) took the shortest time of about 0.38 to 0.66 days to kill 50% and 2.9 to 3.7 days to kill 90% of the aphids' population (Table 4, Figures 4, 5).

TABLE 3 The Tukey multiple comparison of means mortalities for grouped fungal isolates at 95% family- pairwise confidence level.

Day	Fungal isolate	Diff	Lwr	Upr	Adj P
3	*F. equiseti and C.cladosporioides*	0.67	-1.51	2.85	0.53
5		-2.31	-8.35	3.72	0.433
7		12.081	9.02	15.15	1e-07*

*Indicate a significant difference

4 Discussion

4.1 Bioefficacy of *Fusarium equiseti* and *Cladosporium cladosporioides* against *Aphis fabae*

The use of entomopathogenic fungi species in managing black bean aphids has the potential to reduce economic losses caused by these pests, as well as decrease reliance on synthetic insecticides. There is potential to exploit locally available entomopathogens associated with bean aphids. Findings of this study have demonstrated that all fungal isolates (*F.equiseti* and *C. cladosporioides*) had a pathogenic effect, on *Aphis fabae*. However, the effect of the isolated entomopathogenic fungal strains appeared to depend on time and spore concentrations. The results showed

TABLE 2 A two-way ANOVA revealed a significant difference between the fungal isolate and concentration of spores and their interaction on the mortalities of bean aphids, with the P< 0.05 at 7 days' post-infection.

Day	Source of variation	df	SS	MS	Fvalue	Pvalue	Code
3	FI	1	3	3	0.407	0.531	
	CS	4	36503	9126	1113.7	<2e-16	***
	FI×CS	4	43	11	1.306	0.302	
	Residuals	20	164	8			
5	FI	1	40	40	0.638	0.434	
	CS	4	30355	7589	120.715	1.05e-13	***
	FI×CS	4	21	5	0.0882	0.987	
	Residuals	20	1257	63			
7	FI	1	1095	1095	67.44	7.76e-08	***
	CS	4	24348	6087	374.98	<2e-16	***
	FI×CS	4	954	239	14.7	9.41e-06	***
	Residuals	20	325	16			

Significant codes: 0 '***'.

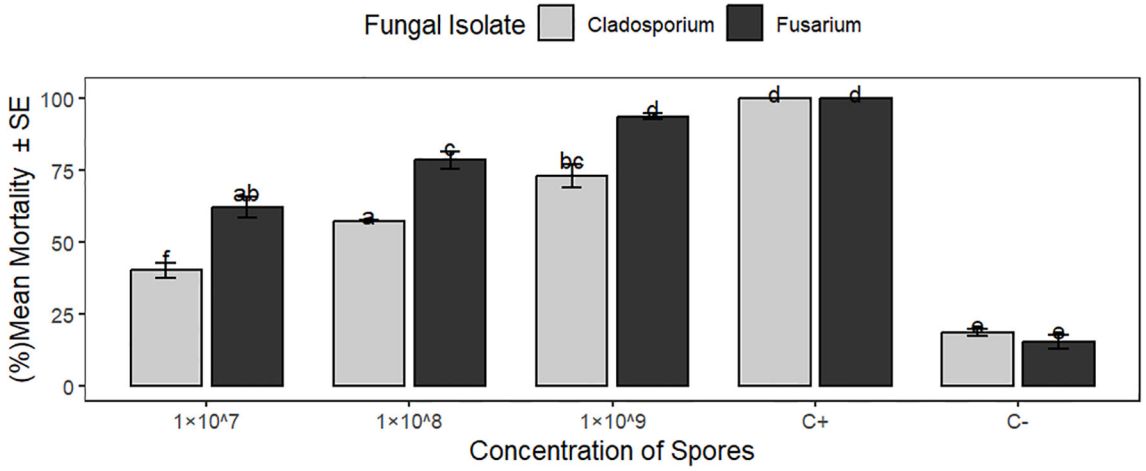

FIGURE 3
Bars represent the mean mortality (%) of *Aphis fabae* caused by *Fusarium equiseti* and *Cladosporium cladosporioides* at different concentrations. Means followed by similar letters are not significantly different by Tukey's HSD multiple range test at p<0.05.

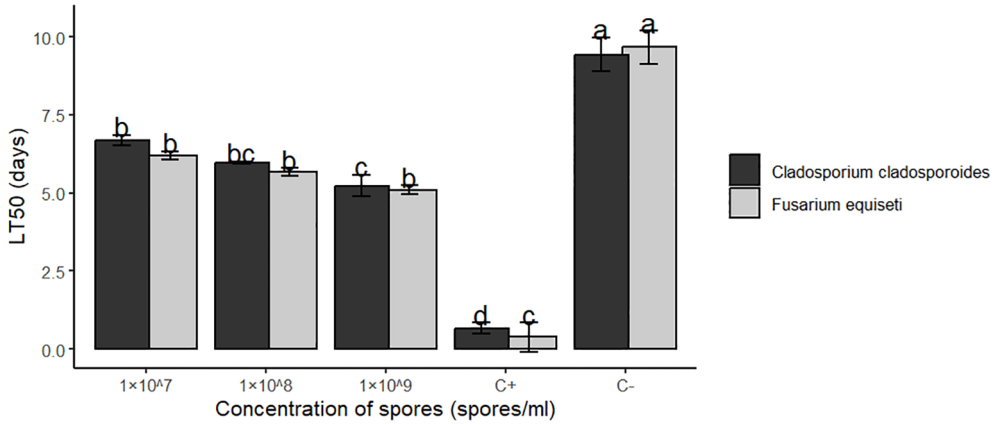

FIGURE 4
Median lethal time (LT50) of *Fusarium equiseti* and *Cladosporium cladosporioides* against *Aphis fabae* at different concentrations followed by the same small letter, do not differ significantly at (P<0.05).

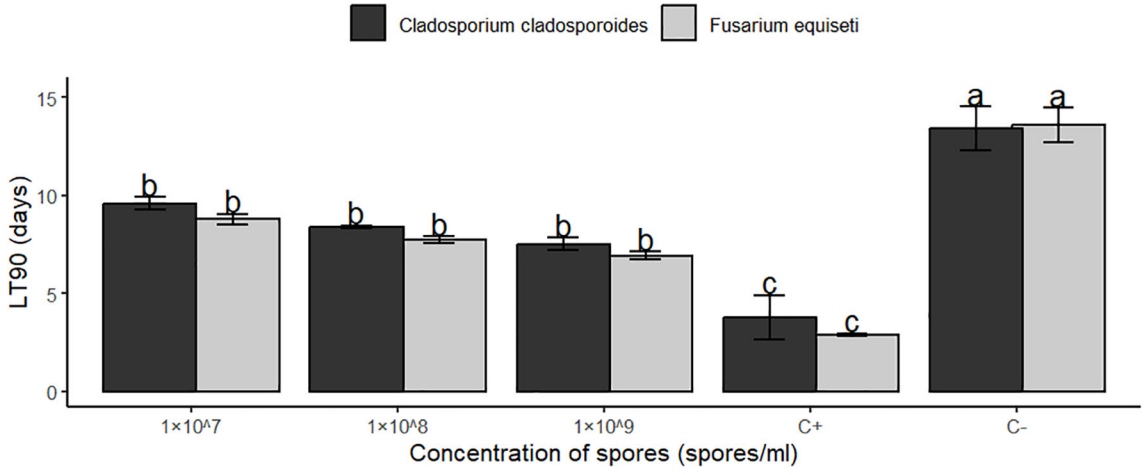

FIGURE 5
Median lethal time LT90 of *Fusarium equiseti* and *Cladosporium cladosporioides* against *Aphis fabae* at different concentrations means followed by the same small letter do not differ significantly at (P<0.05).

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TABLE 4 Median lethal time (LT50 and LT90) for *Aphis fabae* treated with two entomopathogenic fungi at different concentrations (1×10^7, 1×10^8 and 1×10^9 spores/ml) along with commercial insecticide (Imidacloprid) and distilled water containing 0.1% Triton X-100 as positive and negative control, respectively.

Concentration of spores (spores/ml)	*Fusarium equiseti* (LT50)	*Cladosporium cladosporioides* (LT50)	*Fusarium equiseti* (LT90)	*Cladosporium cladosporioides* (LT90)
1×10^7	6.20 ± 0.13b	6.68 ± 0.17b	8.78 ± 0.28b	9.59 ± 0.33b
1×10^8	5.67 ± 0.12b	5.97 ± 0.04bc	7.73 ± 0.17b	8.39 ± 0.08b
1×10^9	5.09 ± 0.15b	5.23 ± 0.35c	6.92 ± 0.2b	7.52 ± 0.34b
C+	0.38 ± 0.46c	0.66 ± 0.18d	2.91 ± 0.07c	3.79 ± 1.13c
C-	9.66 ± 0.53a	9.43 ± 0.54a	13.59 ± 0.89a	13.4 ± 1.13a
F(Value)	101	78.8	105.9	22.02
P(value)	4.83e-08 ***	1.67e-07 ***	3.84e-08 ***	6.05e-05 ***

Means followed by the same letter within a column are not significantly different according to the Tukey test ($p<0.05$).
Significant codes: 0 '***' 0.001.
Lower case letter indicates the statistical significance.

that at a concentration of 1×10^9 spores/ml of *F.equiseti* and *C.cladosporioides*, the cumulative mortality of bean aphids varied between 40.34%, (the lowest), and 93.65%, (the highest), at 7 days' post-infection. These findings align with previous studies, which reported a maximum mortality of 85.3% and a minimum of 60.0% at higher concentrations of *Metarhizium anisopliae* at eight days of post-infection (Trinh et al., 2020). Cumulative mortalities caused by (*Conidiobolus obscurus, Conidiobolus thromboides*, and *Basidiobolus ranarum*) have additionally been reported ranging from 51.2% to 91.7% against *Aphis fabae* after 72 hours of post-infection (Halimona and Jenkevica, 2011). In another study by Saranya et al. (2010), 96% and 80% of *Aphis craccivora* were affected by isolates of *Beauveria bassiana* and *Metarhizium anisopliae*, respectively, at 7 days post-treatment. Furthermore, research conducted by Arıcı et al. (2012) on the biological effectiveness of the entomopathogenic fungus *Fusarium subglutinans*, isolated from cotton aphids, against *Aphis fabae*, showed significant differences in aphid mortality rates at 25°C, even though no significant difference in mortality rate was observed between 1×10^7 and 1×10^8spores/ml.

4.2 The median lethal time (LT50 and LT90) of *Fusarium equiseti* and *Cladosporium cladosporioides*

The value of LT50 and LT90 of the two entomopathogenic fungi isolates (*F.equiseti* and *C.cladosporioides* against the adults *Aphis fabae* were also recorded in the present study. As reported in the study, the LT 50 value of *F. equiseti* and *C. cladosporioides* at 1×10^9 spores/ml was 5.09 and 5.23, (Table 4) respectively are close to findings of study by Saruhan (2018), who reported a mean lethal time(LT50) of 6 entomopathogenic fungi at a concentration of 1×10^9 spores/ml, ranging from 3.08 to 3.83 days. In another study, three entomopathogenic fungi were used to control *Aphis fabae*, and LT50 values ranged from 2.79 to 4.24 days (Yeo et al., 2003). At the concentration of 1×10^8 spores/ml of *Lamellicola* isolate and *Verticillium lecanii*, LT 50 values were 2.1 and 2.33 days, respectively (Saruhan et al., 2015). The current research findings are however, slightly higher with a difference of 1n to 2.5 days.

As of LT 90 values of the present study, *F. equiseti* proved to perform better as it took only 6.92 days to kill 90% of Aphids, while *C. cladosporioides*, took 7.52 days (Table 4). The synthetic insecticides took only 2.91 days. These results align with the results of Saruhan (2018) who used *Lecanicillium muscarium* to control *Aphis fabae*, where LT 90 values were found to range between 4.3 days to 6.06 days.

The number of days for *F.equiseti* and *C. cladosporioides* to cause 50% and 90% mortality varied slightly; this is due to the inherent ability of entomopathogenic fungi to induce pathogenicity in a targeted insect pest or host within a given period. In earlier findings of Mweke et al. (2018), who evaluated different entomopathogenic fungi for managing *Aphis crassivora*, some fungal strains took longer or shorter to cause harm to the target insect pest. Another study by Srinivasan et al. (2019) reported that mortality rates caused by *Mertahizium anisopliae* against bean aphids, could be attributed to the pathogen strain's ability to cause secondary infections in the targeted pest.

5 Conclusion and recommendation

The isolates obtained, belonging to the entomopathogenic fungi *F. equiseti* and *C. cladosporioides*, along with synthetic imidacloprid tested in this study, showed insecticidal effects against *Aphis fabae*. However, the comparison of results between *F.equiseti* and *C.cladosporioides* regarding the mortality of bean aphids indicated that *F.equiseti* was the more virulent at 7 days post-infection. These results suggest that *F.equiseti* has potential for development as a microbial biopesticide and could be included in integrated pest management programs aimed at controlling bean aphid populations. Moreover, further evaluation under field conditions is necessary before recommending these isolates for biological control of *Aphis fabae.*

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

JD: Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. MM: Conceptualization, Supervision, Writing – review & editing. DM: Conceptualization, Supervision, Writing – review & editing.

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Conflict of interest

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