



RESEARCH ARTICLE

The efficacy of *Fusarium* spp. as an entomopathogen against *Aphis gossypii* (Homoptera: Aphididae) and the influence of mancozeb on *Fusarium* spp. in chilli (*Capsicum annuum* L.)

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Abstract

Chilli pepper (*Capsicum annuum* L.) is a valuable horticultural crop whose productivity is significantly hindered by aphid infestations, particularly *Aphis gossypii* (Homoptera: Aphididae). This pest inflicts direct damage through sap extraction and indirectly diminishes yield by transmitting deleterious viruses, such as pepper yellow leaf curl virus (PYLCV), which can cause substantial yield losses or complete crop failure. Currently, aphid management relies predominantly on synthetic insecticides, raising concerns about environmental sustainability, human health and the development of resistance. Therefore, there is an urgent need for environmentally sustainable alternatives that are based on biological control agents. Entomopathogenic fungi represent a promising solution and *Fusarium* spp. have been identified as potential aphid pathogens. This study investigated the potential of *Fusarium* spp. as a biological control agent against *A. gossypii* on chilli plants and evaluated its efficacy under varying fungicide treatments and conidial concentrations. Laboratory experiments were conducted at the Laboratory of Pests and Diseases, Faculty of Agriculture, Hasanuddin University, using a completely randomised design. Six conidial concentrations (120000; 12000; 1200; 0.120; and 0.012 ppm) and a control were applied, with five replications per treatment. Aphid mortality and infection symptoms were recorded to assess pathogenicity. These results indicate that *Fusarium* spp. exhibited significant pathogenicity against *A. gossypii*, with aphid mortality increasing in a concentration-dependent manner. Fungicide exposure affects fungal efficacy, underscoring the importance of compatibility in integrated pest management systems. Overall, *Fusarium* spp. demonstrated strong potential as an effective biological control agent to support sustainable chilli production and enhance the quality of chilli commodities in Indonesia.

Keywords: *Aphis gossypii*; biological control; *Capsicum annuum*; entomopathogenic fungi; *Fusarium* spp.

Introduction

The chilli pepper (*Capsicum annuum* L.) is a crucial horticultural crop with significant economic value, playing a key role in meeting global food demand (1). This species is a key ingredient in chilli paste, pickles and various culinary seasonings and is also highly appreciated as a fresh vegetable for direct consumption (2). Chilli peppers are valued not only for their role in cooking but also for providing a wealth of vital nutrients important for human health. These include proteins, lipids, carbohydrates, calcium, phosphorus, iron and a wide range of vitamins (3). The plant is rich in various bioactive compounds, including alkaloids, flavonoids and essential oils, which enhance its potential in pharmacology and nutraceuticals (4). The distinct spiciness of chilli peppers is mainly due to capsaicin (C₁₈H₂₇O₃N), a phenolic alkaloid that has been widely studied for its significant biological effects, including pain relief, anti-inflammatory, antioxidant and antimicrobial properties (5).

Economically, fresh red chilli is one of Indonesia's six leading vegetable export commodities. Chilli production is spread across regions from North Sumatra to South Sulawesi, with Java Island

being the largest contributor. However, the national productivity of chilli remains quite low compared to its potential yield. This reduced productivity is due to several biotic and abiotic factors that impact production stability and diminish the competitiveness of Indonesian chilli in the international market (6).

Fresh red chilli ranks among Indonesia's top six vegetable export commodities, cultivated across production centres from North Sumatra to South Sulawesi, with Java Island as the largest contributor. Despite this extensive cultivation, national productivity remains far below its genetic and agronomic potential, constrained by a range of biotic and abiotic factors that undermine production stability and weaken the competitiveness of Indonesian chilli in international markets (6). Among the biotic constraints, *Aphis gossypii* Glover (Homoptera: Aphididae) stands out as a major pest whose management has historically relied on frequent applications of synthetic insecticides. This reliance has generated a cascade of ecological and agronomic problems, including the emergence of insecticide-resistant aphid populations, disruption of natural enemy communities, environmental contamination and pesticide residue

accumulation in harvested chilli, all of which pose potential health risks to consumers (7). These compounding issues highlight the pressing need for pest management strategies that are more sustainable, efficacious and environmentally sound, in line with the principles of integrated pest management, sustainable agriculture and food safety.

Within this context, entomopathogenic fungi offer a promising biological alternative and emerging evidence suggests that beyond well-characterised genera such as *Beauveria* and *Metarhizium*, several *Fusarium* spp. also possess insect-pathogenic potential. *Fusarium moniliforme*, for instance, has been documented to infect *Ostrinia nubilalis*, while *Fusarium solani* produces fusaric acid capable of inhibiting enzymatic activity in insect larvae and *Fusarium reteritum* has been reported as a pathogen of shield bugs. Despite these findings, most existing reports remain limited to incidental observations of pathogenicity, with a notable absence of comprehensive quantitative evaluations addressing infection efficacy, optimal inoculum dosage and compatibility with other control inputs in integrated cropping systems (8). The present study addresses this gap by systematically evaluating the entomopathogenic potential of *Fusarium* spp. against *A. gossypii* on chilli through a quantitative framework that links conidial concentration to aphid mortality and examines its interaction with fungicide application. By elucidating both the mechanistic basis and field-relevant efficacy of *Fusarium* spp. as biocontrol agents, this approach bridges laboratory-scale findings and practical field applications, thereby offering a meaningful scientific contribution toward sustainable integrated pest management and reduced dependence on synthetic insecticides in chilli agroecosystems (9).

Materials and Methods

Seedling preparation and transplanting of *Capsicum annum* L. (Arimbi cultivar)

These chilli pepper (*Capsicum annum* L.) seeds of the Arimbi cultivar were sown in plastic trays (20 cm × 30 cm) containing sterile sand as the growth medium. Seeds were planted at a depth of 0.5 cm with a spacing of 2 cm between seeds. Seedlings were irrigated twice daily (morning and afternoon) using a hand sprayer to maintain adequate moisture for germination and early growth. After establishment, healthy seedlings were transplanted into polybags (20 cm × 30 cm) filled with a soil and well-decomposed farmyard manure mixture at a ratio of 1:3 (v/v) (10).

Aphis gossypii rearing

Aphid rearing was conducted to evaluate the effectiveness of *Fusarium* spp. as a biological control agent. Rearing cages were constructed using polyvinyl chloride (PVC) pipes with a diameter of 0.5 inches and a height of 75 cm. The pipes were assembled into cylindrical cages and covered with fine-mesh gauze, cut to fit the cage dimensions, to prevent insect escape and contamination. Adult aphids (*Aphis gossypii* Glover) were collected from chilli pepper fields and introduced into prepared cages containing healthy chilli plants as hosts. The aphids were maintained under controlled conditions to establish a stable colony for subsequent bioassays (11).

Determination of lethal concentration and lethal time of *Fusarium* spp. against *Aphis gossypii* on chilli plants

To determine the median lethal concentration (LC₅₀) of *Fusarium* spp., which is defined as the conidial concentration required to

cause 50 % mortality of the test insects, fungal colonies were harvested from actively sporulating cultures and suspended in sterile distilled water containing a surfactant (0.03–0.1 % Tween 80) to reduce conidial clumping and ensure homogeneous dispersion a method consistent with standard protocols in which conidia are dislodged using a sterile pestle and Tween 80 (0.03 % v/v) before being agitated vigorously on a vortex mixer. The resulting suspension was subsequently filtered through sterile cheesecloth or muslin to remove residual mycelial fragments and agar debris, yielding a homogeneous conidial stock free of hyphal contamination following established procedures in which conidial suspensions are prepared by scraping conidia from culture plates into distilled water and filtering through several layers of cheesecloth to remove mycelial mats (12). Conidial density in the stock suspension was quantified using a Neubauer improved hemocytometer under light microscopy (× 400 magnification) and the stock concentration was subsequently adjusted through a serial dilution series to obtain the target concentrations of 10⁴, 10⁵, 10⁶, 10⁷ and 10⁸ conidia mL⁻¹ consistent with the practice of determining stock suspension concentration through counts of serially diluted suspensions in a hemocytometer chamber. Prior to use in bioassays, conidial viability was verified through a germination assay, in which an aliquot of the suspension was spread on solid agar medium and incubated for 24 hr. Only suspensions exhibiting germination rates exceeding 95 % were used for subsequent inoculation in accordance with standard viability-testing procedures requiring germination rates above 95 % following 24 hr of incubation at 28 °C prior to bioassay use (13).

This tiered, haemocytometer-based approach to conidial standardisation is essential to ensure that observed differences in *Aphis gossypii* mortality across treatments can be attributed specifically to conidial dose-response relationships rather than to confounding variation in inoculum viability or conidial clumping. This serves as a methodological safeguard that is increasingly emphasised in entomopathogenic fungal bioassays, given the known operator-to-operator variability in manual counting. Conventional methodologies, such as haemocytometer counting and plate dilution assays, while foundational, are time-consuming and subject to significant operator-to-operator variability (14).

Ten aphids (*Aphis gossypii*) were transferred onto two-week-old chilli seedlings, each placed individually in 500 mL plastic cups containing 10 mL of soil-free nitrogen, phosphorus and potassium (NPK) nutrient solution. After 48 hr, adult aphids were removed and 20 nymphs were retained on each plant. When the nymphs reached the second and third instar stages (approximately three days after placement), 1 mL of the respective conidial suspension was applied to the aphids. Each conidial concentration was replicated five times, with each plant serving as one replicate (15), modified.

For the determination of the median lethal time (LT₅₀), the time in days required to cause 50 % mortality of the test insects conidial suspensions at concentrations of 10⁴, 10⁵, 10⁶ and 10⁷ conidia mL⁻¹ were applied to second- and third-instar *A. gossypii* nymphs following the same procedures described for the LC₅₀ bioassay. Mortality was recorded at predetermined time intervals and the data were used to estimate LT₅₀ (16).

Effect of *Fusarium* spp. application on the population development of *Aphis gossypii*

One-month-old chilli plants were allocated to each rearing cage and ten adult *Aphis gossypii* were introduced onto each plant. After one

week of post-infestation, three cages were treated with a *Fusarium* spp. suspension at 5 mL per plant, applied with a hand sprayer. The fungal suspension was prepared at a concentration of 10^5 conidia mL^{-1} , while the remaining cages served as controls and were sprayed with sterile distilled water. Observations were conducted weekly over a three-week period, beginning one week after treatment, to quantify the numbers of live and dead aphids.

Effect of mancozeb on *Fusarium* spp. colony growth

Mancozeb, a commonly used fungicide by farmers (mancozeb), was evaluated for its effect on the radial growth of *Fusarium* spp. The fungicide was initially prepared according to the recommended field dose by dissolving 3 g of mancozeb in 1000 mL of sterile distilled water. From this stock solution, 100 mL was prepared using 0.3 g of fungicide. The stock solution was subsequently diluted to obtain a range of fungicide concentrations.

For each treatment, 19 mL of molten potato dextrose agar (PDA) medium was dispensed into sterile bottles and 1 mL of the respective fungicide solution was added to obtain final concentrations of 0.012, 0.120, 1.200, 12.000 and 120.000 ppm. The PDA without fungicide (0.000 ppm) served as the control. Each treatment consisted of five replicates. The fungicide-amended PDA was poured into petri dishes and allowed to solidify. A mycelial plug (1 cm diameter) was excised from the margin of an actively growing *Fusarium* spp. colony cultured on PDA using a sterile corkborer and placed at the centre of each petri dish. Plates were incubated under laboratory conditions and colony diameter (cm) was measured at 24 hr intervals for 10 days.

Effect of mancozeb fungicide on the percentage inhibition of *Fusarium* spp. colony growth

Colonies of *Fusarium* spp. treated with mancozeb at five different concentrations, each with five replicates, were evaluated for growth inhibition. The percentage of growth inhibition was calculated using the following formula (17):

$$\text{Inhibition (\%)} = \frac{C - T}{C} \times 100 \text{ (Eqn. 1)}$$

C represents the mean colony diameter (cm) of the control treatment and T represents the mean colony diameter (cm) of the fungicide-treated colonies.

Effect of mancozeb fungicide on spore development of *Fusarium* spp. at 10^6 mL^{-1}

Fungal colonies treated with mancozeb at five different concentrations, each with five replicates, were evaluated for spore production. Spore counts were determined by excising a mycelial plug (1 cm diameter) from each colony using a sterile corkborer. The plug was suspended in 1 mL of sterile distilled water in a beaker and homogenised thoroughly. Spore density was then quantified using a hemocytometer under a light microscope for each replicate.

The spore concentration was calculated using the following equation (18):

$$K = \frac{t}{n \times 0.25} \times 10^6$$

K denotes the spore concentration (spores mL^{-1}), t is the total number of spores counted, n is the number of hemocytometer squares counted and 0.25 represents the volume correction factor of the hemocytometer chamber.

Results

Determination of lethal concentration and lethal time of *Fusarium* spp. against *Aphis gossypii* on chili plants

The relationship between log-transformed treatment concentration and the mortality response of *Aphis gossypii* nymphs was analysed using probit regression, as presented in Table 1. The horizontal axis represents the log-transformed treatment concentration (+1), while the vertical axis indicates probit mortality values derived from percentage mortality of the test insects. Probit transformation was applied to obtain a linear relationship between treatment concentration and biological response, as described in Eqn. (1). The LC_{50} value was estimated from the intersection of the probit regression line with the probit corresponding to 50 % mortality and is indicated by the dashed line in Fig. 1.

Empirical probit values increased linearly with increasing treatment concentration, indicating a positive dose mortality relationship for *Aphis gossypii* nymphs. Probit regression was described by $y = 3.214 + 0.285x$, showing that each unit increase in log-transformed concentration increased mortality by 0.285 probit units. The LC_{50} was estimated at probit = 5, corresponding to 50 % mortality, yielding a log concentration of 6.27, which represents the concentration required to cause 50 % mortality in the tested nymph population (Fig. 1).

The median lethal concentration (LC_{50}) of *Fusarium* spp. against *Aphis gossypii* nymphs was 1.8×10^4 , reached after 6 days of exposure, indicating 50 % mortality at this concentration (Appendix 6a). Probit regression analysis yielded the equation $y = 3.22 + 0.284x$, while the median lethal time (LT_{50}) was 4.23 days, demonstrating that *Fusarium* spp. required 4.23 days to induce 50 % mortality in *A. gossypii* nymphs (Table 1 and Fig.1).

Growth suppression of *Fusarium* spp. under different mancozeb concentrations

Based on the observations of the effect of the fungicide mancozeb on *Fusarium* spp., no visible colony development was observed during the first 24 hr after inoculation. In subsequent observations, mancozeb applied at a concentration of 120000 ppm (equivalent to the recommended field dose) markedly suppressed fungal growth, with several replicates showing no detectable colony formation. In contrast, the control treatment and lower concentrations exhibited a progressive increase in colony diameter over time across all replicates, as presented in Table 2.

Table 1. Lethal concentration (conidia mL^{-1}), lethal time (days) and probit regression equations of *Fusarium* spp. against *Aphis gossypii* nymphs

Bioassay insect	LC_{50}	Probit regression equation	LT_{50} (Days)	Probit regression equation
<i>Aphis gossypii</i> nymphs	1.84×10^4	$y = 3.22 + 0.284x$	4.23	$y = -7.91 + 4.293$

LC_{50} : lethal concentration; LT_{50} : lethal time.

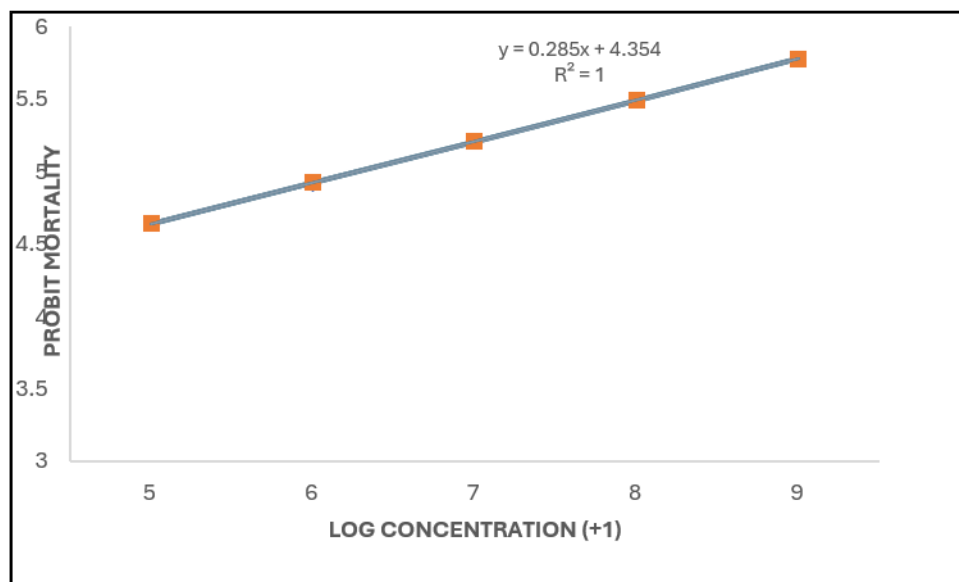


Fig. 1. Probit regression showing the relationship between log-transformed treatment concentration (+1) and mortality of *Aphis gossypii* nymphs ($y = 0.285x + 4.354$; $R^2 = 1.00$). Increasing concentrations resulted in higher mortality and the lethal concentration (LC_{50}) was estimated at probit = 5.

Table 2. Growth inhibition of *Fusarium* spp. colony diameter under graduated antagonist concentrations, demonstrating dose-dependent biocontrol efficacy and quantitative assessment of treatment-induced growth suppression

Ppm	Colony diameter of <i>Fusarium</i> spp. (cm)								
	II	II	IV	V	VI	VII	VIII	IX	X
120	0	0	0	0.1	0.32	0.50	0.64	0.74	1.02
12	0.12	1.04	1.9	2.58	3.32	4.16	4.86	5.32	5.98
1.2	1.54	2.14	2.74	3.78	4.58	5.2	5.64	6.12	6.92
0.12	1.52	2.24	2.86	3.88	4.46	5.2	5.7	6.2	6.82
0.012	1.58	2.1	2.94	3.88	4.56	5.18	5.76	6.24	6.82
0	1.4	2.3	3.18	4	4.84	5.54	6.1	6.44	7.24

As shown in Fig. 2, statistical analysis revealed that the mean colony diameter in the control treatment reached 4.56 cm across all replicates. This value was significantly different from that observed at 120000 ppm (equivalent to the recommended field dose), which recorded a mean diameter of only 0.47 cm and at 12000 ppm, which showed a mean diameter of 3.27 cm. In contrast, no significant differences were observed at concentrations of 1200 ppm (4.30 cm), 0.120 ppm (4.34 cm) and 0.012 ppm (4.54 cm) when compared with the control (Fig. 2).

Growth suppression of *Fusarium* spp. under different mancozeb concentrations

In antifungal and biological control research, “inhibition rate” is a standard parameter used to express the percentage reduction in fungal growth relative to the control treatment. Statistical analysis indicated that 120000 ppm concentration (equivalent to the recommended dose) resulted in the highest inhibition rate, reaching 89.8 % and was highly significantly different from the 0.012 ppm treatment, which showed an inhibition rate of only 0.6 %. A

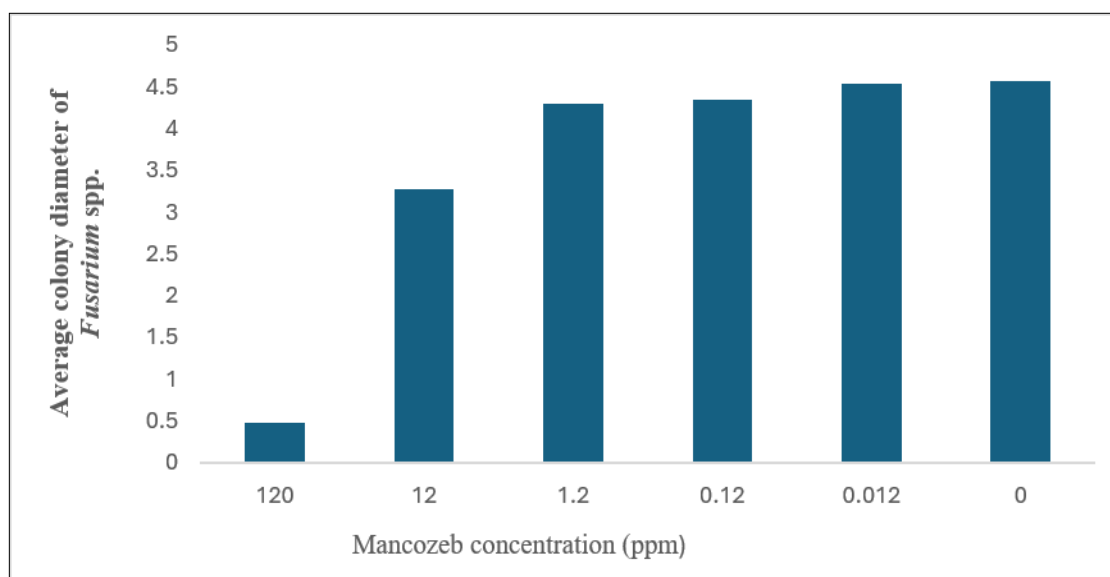


Fig. 2. Effect of different mancozeb concentrations (ppm) on the mean colony diameter of *Fusarium* spp.: Dose-response inhibition kinetics, mechanistic characterisation of fungicide-target interaction and quantitative assessment of phytopathological control efficacy under graduated dithiocarbamate exposure.

moderate inhibition rate of 28.3 % was observed at 12000 ppm. In contrast, no significant differences were observed for the 1200 ppm treatment, which exhibited an inhibition rate of 5.9 %, or for the 0.120 ppm concentration, which exhibited an inhibition rate of 5.4 % (Fig. 3).

Discussion

The results of the study demonstrated that the application of *Fusarium* spp. to *A. gossypii* nymphs resulted in observable mortality as early as one day after treatment. This effect is attributable to the differences in *Fusarium* spp. concentrations applied to the nymphs, whereby each concentration elicited a distinct response. This finding is consistent with a previous study (9) which reported that smaller insects allow active compounds to reach target sites more rapidly and at sufficient concentrations to induce toxicity compared with larger insects. In addition, variations in susceptibility to specific bioactive compounds may arise from differences in insects' barriers to compound penetration, such as cuticle thickness. The toxic compounds present in *Fusarium* spp. include fusaric acid and the pigment naphthazarin, which are classified as mycotoxins capable of inhibiting several enzymatic reactions (8).

The results presented in Table 1 show that, based on probit analysis, the LC_{50} value of *Fusarium* spp. against *A. gossypii* nymphs was 1.8×10^4 , with a regression equation of $y = 0.285x + 4.354$. This indicates that *Fusarium* spp. at a concentration of 1.8×10^4 is capable of causing 50 % mortality of the tested *A. gossypii* nymphs. Furthermore, probit analysis yielded an LT_{50} of 4.23 days, with the regression equation $y = 0.285x + 4.354$. This result indicates that the time required to kill 50 % of the test insects was 4.23 days. As illustrated in Fig. 4, treatment with *Fusarium* spp. resulted in a substantially higher mortality rate in *Aphis gossypii* than in the untreated control, indicating the entomopathogenic activity of the tested isolate against this aphid pest. Interestingly, the mortality pattern also revealed a density-dependent phenomenon, in which the number of dead insects tended to fluctuate in accordance with variation in aphid population density across replicates a pattern commonly attributed to density-dependent transmission dynamics, whereby the probability of conidial contact and subsequent infection per individual insect increases with higher host density due to greater

opportunities for inter-individual contact, conidial dispersal and secondary cycling of inoculum within the aphid colony (19).

Overall, the mortality data indicate that *Aphis gossypii* is most effectively suppressed when *Fusarium* spp. is applied at the appropriate timing and through repeated applications, rather than as a single inoculation. This repeated-exposure strategy is essential to sustain adequate conidial density on the aphid colony over time, thereby maintaining continuous infection pressure as the pest population fluctuates. The entomopathogenic activity of *Fusarium* spp. was further confirmed by re-isolation of the fungus from infected cadavers and by observation of characteristic mycosic symptoms on treated aphids (Fig. 5 and Fig. 6), thereby fulfilling Koch's postulates and confirming that the observed mortality was attributable to fungal infection rather than confounding factors (Fig. 7). These findings are consistent with the previous study (20), which reported that repeated fungal applications are highly effective in suppressing pest populations to levels below the economic injury threshold, provided that application intervals remain within limits that do not compromise conidial viability or field persistence.



Fig. 4. Macroconidia of *Fusarium* spp. isolated from *Aphis gossypii* cadavers collected from chilli pepper (*Capsicum annum* L.) plants: Morphological characterisation, diagnostic features and entomopathogenic potential assessment.

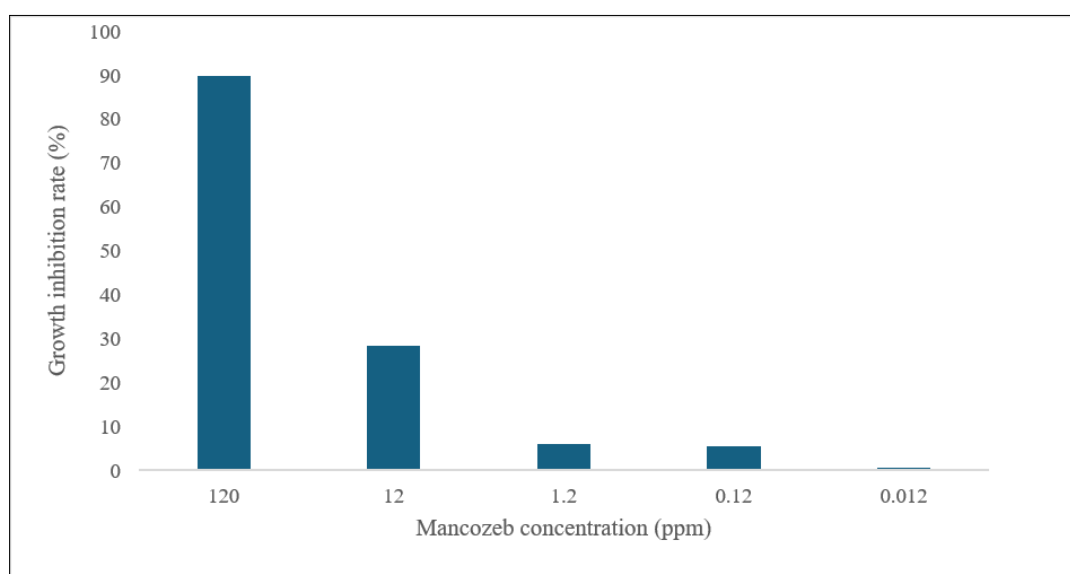


Fig. 3. Effect of mancozeb concentration on fungal growth inhibition rate (%), illustrating dose-response kinetics and mechanistic characterisation of dithiocarbamate-mediated multi-target enzymatic inhibition against *Fusarium* spp.

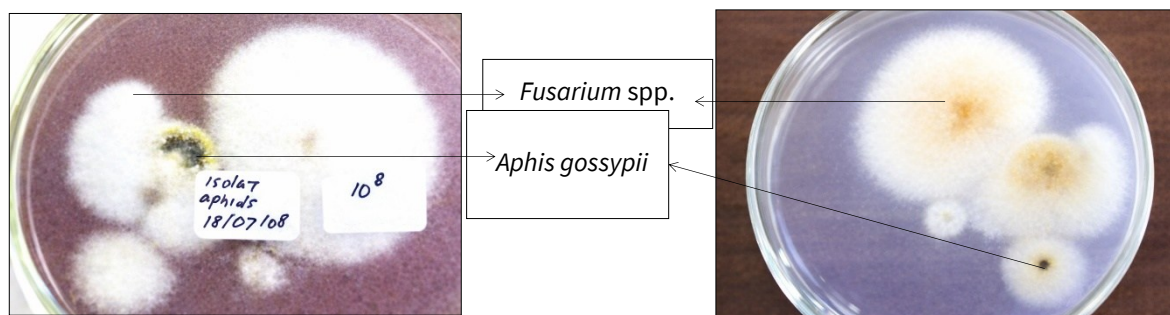


Fig. 5. Macroconidia of *Fusarium* spp. re-isolated from mycosed *Aphis gossypii* cadavers on chili pepper. Macroconidia are hyaline, multicellular, falcate to slightly curved with tapered ends, 4–7-septate, measuring $26.3\text{--}43.5 \times 3.2\text{--}4.8 \mu\text{m}$. Structures are characteristic of entomopathogenic *Fusarium* spp. (PDA, 7 days, 25 °C; lactophenol cotton blue staining; 400 ×). Scale bar = 20 μm .

PDA: potato dextrose agar.



Fig. 6. *Fusarium* spp. Macroconidia cultured on mancozeb-amended PDA retained normal morphology and virulence potential for *Aphis gossypii* biocontrol on chili pepper (scale bar = 20 μm).

IPM: integrated pest management.

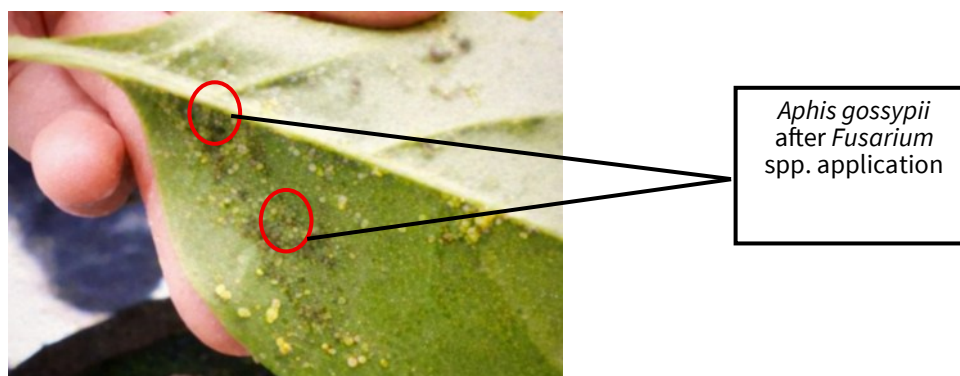


Fig. 7. Spatiotemporal infection dynamics of *Aphis gossypii* populations following *Fusarium* spp. biocontrol application: Abaxial leaf surface colonisation and mycosis progression.

The colony diameter of *Fusarium* spp. decreased progressively with increasing mancozeb concentration in the PDA medium, with the recommended dose of 120000 ppm producing the smallest mean colony diameter (0.47 cm) and the untreated control (0 ppm) producing the largest (4.56 cm), a difference that was statistically significant (Fig. 2). This inverse relationship between fungicide concentration and colony diameter corresponded directly to the pattern of growth inhibition, which peaked at 89.8 % under 120000 ppm treatment and declined to only 0.6 % at the lowest concentration tested (0.012 ppm), confirming a clear dose-dependent inhibitory response of mancozeb against *Fusarium* spp. (Fig. 3). This inhibitory pattern is consistent with earlier findings on a related species, where mancozeb tested against *Fusarium oxysporum* f. sp. *melongenae* at four concentrations achieved its highest inhibition of 62.2 % at 0.20 %, a concentration that also

enhanced seed germination, seedling vigour, vegetative growth and hypersensitive response while conferring 51 % disease protection, underscoring the fungicide's efficacy across both *in vitro* and greenhouse conditions (21).

These results indicate a clear effect of mancozeb on the colony diameter growth of *Fusarium* spp., as presented in Fig. 3, while the re-isolation of aphids following the application of *Fusarium* spp. is shown in Fig. 8. This effect is attributed to the active ingredients in mancozeb, which inhibit fungal respiration, thereby suppressing hyphal growth. This finding is consistent with previous research (22), which reported that mancozeb acts as a non-specific, thiol-reactive compound that inhibits fungal respiration. The same study also evaluated the effects of mancozeb (0–2000 ppm) on soil biology in apple orchard soils. It was found that lower doses suppressed fungi and actinomycetes, whereas higher doses

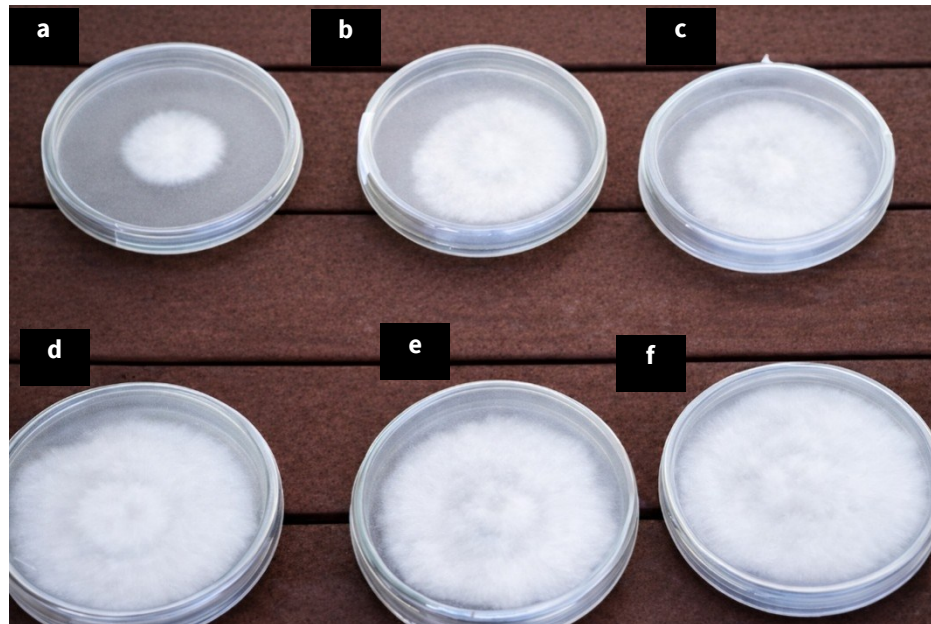


Fig. 8. Concentration-dependent inhibition of *Fusarium* spp. growth kinetics and macroconidial morphogenesis across six treatment levels: (a) 120000 ppm, (b) 12000 ppm, (c) 1200 ppm, (d) 120 ppm, (e) 12 ppm, and (f) 0 ppm (untreated control). Panels illustrate the gradual phenotypic plasticity of fungal colonies, putative inhibitory mechanisms across the concentration gradient and evidence supporting the optimization of antagonist-mediated biocontrol.

inhibited bacterial populations. Additionally, elevated concentrations impaired ammonification and nitrification processes, disrupted soil enzyme activities, altered phosphorus solubilisation, and reduced overall microbial biomass. These results indicate the potential ecological risks associated with the application of mancozeb. The number of *Fusarium* spp. Microconidia produced across the tested mancozeb concentrations showed a clear concentration-dependent inverse relationship with colony diameter (Fig. 4). Microconidial density decreased progressively with increasing mancozeb concentration, reaching its lowest level at the recommended dose (120000 ppm) and its highest in the untreated control (0 ppm). This pattern corroborates the dose-dependent suppression of both the colony diameter of *Fusarium* spp. and the growth inhibition rate of the colony of *Fusarium* spp. (Fig. 2 and 3). The highest spore density, reaching $1.57 \times 10^5 \text{ mL}^{-1}$, was observed at a concentration of 120000 ppm (equivalent to the recommended dose), whereas the lowest spore density was recorded in the control treatment, at $0.60 \times 10^0 \text{ mL}^{-1}$. These findings indicate that increasing fungicide concentrations in PDA medium reduced colony diameter, an effect inversely proportional to both the inhibition rate and spore production (15). Earlier research (2023) optimised submerged culture conditions for *Fusarium equiseti* JMF-01 using single-factor and Box-Behnken designs, significantly increasing mycelial biomass and sporulation. Optimised cultures enhanced virulence, resulting in 95 % mortality in *Myzus persicae*, highlighting strong potential for large-scale biocontrol applications. Following application under greenhouse conditions, infected aphid colonies exhibited extensive mycelial development covering the entire body surface, forming a characteristic “white blanket” typical of entomopathogenic fungal infection. The mycelium appeared as a dense white layer enveloping the insect integument, indicating effective host colonization and tissue penetration. Subsequent laboratory observations revealed that the pathogen produced elongated, fusiform macroconidia with tapered ends, consisting of 4–7 septa, which are key diagnostic features for identifying specific entomopathogenic fungal species, as shown in Fig. 4. Optimised submerged culture conditions of *Fusarium equiseti* JMF-01 demonstrated high virulence toward

Myzus persicae, evidenced by infection efficacy observable at both macroscopic and microscopic organisational levels, thereby establishing its viability as a potent biological control agent within an integrated pest management (IPM) framework under greenhouse-protected cultivation systems (23).

Under greenhouse conditions, the optimised *F. equiseti* JMF-01 inoculum successfully colonised *M. persicae* populations, as evidenced by extensive mycelial proliferation and tissue invasion. Infected aphid colonies exhibited dense white mycelial coverage enveloping the entire insect body surface, a characteristic phenotype indicative of active fungal pathogenesis and complete host encasement, a hallmark feature of entomopathogenic fungi in natural transmission scenarios. Microscopic examination of fungal morphology confirmed species identity and inoculum quality by observing elongated, fusiform macroconidia bearing tapered apical cells and characteristic foot-shaped basal cells with 4–7 transverse septa ($18.1\text{--}41.6 \times 2.5\text{--}4.9 \mu\text{m}$), morphological features diagnostic of *F. equiseti* within the *Fusarium incarnatum-equiseti* complex. Following application under greenhouse conditions, *M. persicae* populations exhibited typical entomopathogenic infection symptoms. Macroscopic observations revealed extensive white mycelial coverage (the characteristic “mycelial blanket”) completely enveloping infected aphid bodies, confirming successful host colonisation and systemic tissue invasion. Microscopic characterisation at $400 \times$ magnification revealed diagnostic fungal features consistent with *F. equiseti* taxonomy. The macroconidia exhibited the characteristic morphotype of the *Fusarium incarnatum-equiseti* species complex (FIESC), with an elongated, fusiform shape, distinctly tapered apical cells, prominent foot-shaped basal cells, and 4–7 transverse septa. The conidial dimensions ranged from $24.0\text{--}40.0 \times 4.0\text{--}6.0 \mu\text{m}$. These morphological determinants, combined with isolation of the pathogen from aphid cadavers and successful re-isolation under axenic conditions, confirm species identity and validate the genomic stability of the post-optimisation inoculum. These findings confirm that the biocontrol agent is highly effective against aphids at both macro- and microscopic levels, demonstrating its strong potential as a biological control option within integrated

pest management (IPM) systems in greenhouses. According to the previous research (24) *Fusarium keratoplasticum* TKKS-1 significantly outperforms *Beauveria bassiana* in controlling *Spodoptera litura* larvae, achieving 100 % mortality within 6 days compared to 9 days for *B. bassiana*, with an LT_{50} of 2.96 days at 1×10^7 conidia/mL and an LC_{50} of 2.74×10^6 conidia/mL. Semi-field validation on mustard plants confirmed that *F. keratoplasticum* maintains superior effectiveness under field-proximate conditions, establishing strong potential for biopesticide formulation development within IPM systems.

Conclusion

This study provides novel evidence highlighting the dual role of *Fusarium* spp. in sustainable pest management, demonstrating their efficacy as entomopathogenic agents against *Aphis gossypii* and revealing that their biological performance is significantly influenced by fungicide exposure. The low LC_{50} (1.8×10^4) and relatively short LT_{50} (4.23 days) demonstrate the high pathogenicity of *Fusarium* spp. against aphid nymphs, highlighting their promise as an alternative to synthetic insecticides. Importantly, this study reveals a dose-dependent response of *Fusarium* spp. to mancozeb, with higher fungicide concentrations significantly reducing colony growth while simultaneously increasing inhibition rates and spore production. These findings advance current understanding of fungicide interactions and provide practical insights for integrating entomopathogenic fungi into integrated pest management (IPM) programs. Strategically timed and repeated applications of *Fusarium* spp., combined with careful fungicide management, may enhance aphid control efficacy while minimising negative impacts on biological agents, thereby contributing to more environmentally sustainable crop protection strategies.

Authors' contributions

DSF was responsible for developing the research direction and preparing the manuscript. ANS conceived the study and participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

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During the preparation of this work the authors used GRAMMARLY in order to improve the language quality. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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