Insecticidal Potential of Emerging Entomopathogens Against Bemisia tabaci (Gennadius)

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The present study evaluated the insecticidal potential of two emerging entomopathogenic fungal genera, Penicillium and Cladosporium, against different life stages of the cotton whitefly (Bemisia tabaci). Pathogenicity assays were conducted on 4th instar nymphs and adults using two conidial concentrations (4×10^8 and 4×10^4 spores mL^{-1}), with each treatment performed in three independent replicates. Results showed that both fungal genera were more effective against nymphs than adults, with Penicillium species demonstrating higher virulence overall. Among them, P. expansum exhibited the highest mortality against both developmental stages. Similarly, Cladosporium sp. outperformed C. cladosporioides, showing more consistent efficacy across spore concentrations. The findings suggest that specific isolates of Penicillium and Cladosporium have promising potential as biocontrol agents against B. tabaci, particularly at the nymphal stage. These insights contribute to the development of environmentally friendly pest management strategies in cotton cultivation.

Keywords: Cotton whitefly, Biocontrol, Virulence Bioassay.

Introduction:

The whitefly (Bemisia tabaci) is a destructive polyphagous pest responsible for severe economic losses in a wide range of crops globally, both in open fields and greenhouse environments [1]; [2]. Damage results from direct feeding on phloem sap, excretion of honeydew that encourages sooty mold growth, and more critically, the transmission of over 100 plant viruses, including begomoviruses such as tomato yellow leaf curl virus (TYLCV), making B. tabaci one of the most significant agricultural pests worldwide [3]; [1]; [4].

Heavy infestations lead to stunted growth, chlorosis, leaf drop, and ultimately reduced vigor and yield of host plants [5]. While cultural practices such as crop rotation, intercropping, and the use of reflective mulches are employed to reduce pest pressure [6], chemical insecticides remain the most widely used method for managing B. tabaci. However, continuous and excessive use of insecticides has led to the development of resistance in B.

tabaci populations across various regions and also contributed to the resurgence of vector-borne viral diseases [4]. Given these limitations, biological control using natural enemies has gained interest. With the growing concern over pesticide residues and environmental impact, entomopathogens are being actively explored as biocontrol alternatives in integrated pest management (IPM) systems against B. tabaci [7]. Several predators (e.g., Delphastus catalinae) and parasitoids (e.g., Encarsia formosa) have been employed for B. tabaci management, with varying degrees of success [8][9]. However, natural enemy-based systems are often unreliable under field conditions due to environmental fluctuations, host specificity, and integration challenges with other pest management strategies [10]. Recent research has provided positive outcomes with the use of EPFs Beauveria bassiana, Metarhizium anisopliae, and Isaria fumosorosea that resulted in a substantial mortality of nymphal and adult stages in controlled and semi-field conditions [11]. The studies emphasize the growing role of investigating non-conventional EPFs as other biocontrol agents in the sustainable control of B. tabaci.

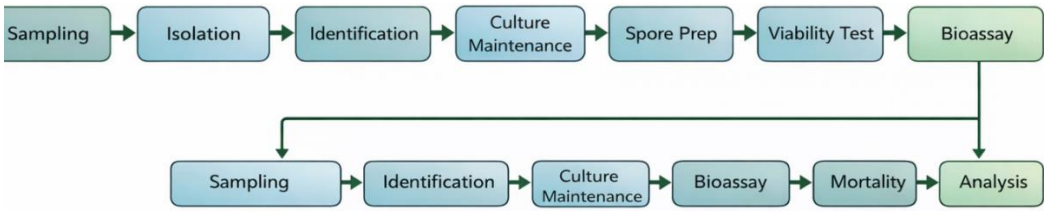
Entomopathogenic fungi (EPF) offer a promising alternative due to their broad host range, ease of application, and minimal non-target effects. Several fungal genera, such as Beauveria, Metarhizium, Isaria, and Lecanicillium, have shown effective control of whiteflies under both laboratory and field conditions [12][13][14]. EPFs can be directly applied to the leaf surfaces where nymphs and adults are primarily found, enhancing contact and infection efficacy. Despite the growing body of research on established entomopathogens, limited attention has been given to other potentially effective fungal genera. Notably, Penicillium and Cladosporium species, though traditionally studied for their secondary metabolites, have recently been isolated from insect hosts and demonstrated pathogenic effects against several soft-bodied insects. However, their potential as biocontrol agents against B. tabaci remains largely unexplored.

The objective of this study was to evaluate the pathogenicity of Penicillium and Cladosporium species against different developmental stages (4th instar nymphs and adults) of Bemisia tabaci, compare the virulence of different isolates within each fungal genus at low and high conidial concentrations, determine the relative susceptibility of nymphal and adult stages of B. tabaci to these emerging entomopathogenic fungi, and assess the potential of non-conventional fungal genera as eco-friendly biocontrol agents for the sustainable management of B. tabaci.

Novelty Statement:

The novelty of this work lies in the assessment of non-conventional fungi with promising bioactivity, which may offer eco-friendly, sustainable alternatives to chemical insecticides and broaden the spectrum of available biocontrol agents. Moreover, the findings could pave the way for the development of novel mycoinsecticides with unique modes of action and minimal ecological risks.

Materials and Methods:



Flow chart diagram of Methodology

Survey and Sampling:

A detailed survey of cotton fields was conducted to collect naturally infected dead insect samples across three agroecological zones of Punjab, Pakistan: (1) Hot Arid Zone, (2) Central Mixed Zone, and (3) Cotton Zone [15][16]. Insects, including Aphis gossypii (Aphididae), Bemisia tabaci (Aleyrodidae), and Phenacoccus solenopsis (Pseudococcidae),

exhibiting visible signs of fungal infection, were carefully collected from the upper leaves (3–4 feet above soil level). The infected specimens were immediately transferred to sterile sampling tubes and stored at 4°C until fungal isolation.

Isolation and Characterization of Entomopathogenic Fungi:

To isolate fungi, insect cadavers were first surface sterilized using 1% sodium hypochlorite (NaOCl) for 2 minutes, followed by rinsing three times with sterile autoclaved distilled water [17]. The sterilized samples were then placed on 1/4 strength Sabouraud Dextrose Peptone Yeast Extract Agar (SDAY/4) and incubated at 25°C for 3–5 days under controlled conditions. Emerging fungal colonies were sub-cultured on fresh SDAY/4 plates to obtain pure cultures. Fungal isolates were purified through hyphal tip transfer and repeated sub-culturing. Morphological identification was performed using light microscopy and standard taxonomic keys based on colony texture, spore morphology, and pigmentation. For molecular characterization, genomic DNA was extracted from 7-day-old fungal cultures using a standard CTAB protocol. The internal transcribed spacer (ITS) region was amplified using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). PCR products were sequenced and subjected to BLAST analysis for species-level identification. All confirmed isolates were submitted to the 1st Fungal Culture Bank of Pakistan (FCBP) and assigned accession numbers.

Selection Criteria for Pathogenicity Testing:

Fungal isolates selected for pathogenicity assays included those with confirmed entomopathogenic traits based on both morphological features and sequence similarity (>98%) with known entomopathogenic species. Isolates representing diverse genera were grouped for comparative bioassays.

Maintenance of Bemisia tabaci:

A colony of B. tabaci (biotype B) was maintained under controlled conditions. Cotton plants (height 25–30 cm with 10–15 leaves) were grown in a growth chamber at 25°C and 50% RH with a 12 h light/12 h dark photoperiod. Adult whiteflies were collected from healthy cotton plants using a hand-held aspirator and introduced onto fresh cotton plants in a glasshouse. After 3–4 weeks, high populations of eggs, nymphs, and adults were established. These infested plants were transferred to clip cages and maintained in an insectary at 23–25°C and 40–50% RH. Regular observations and maintenance were done using a hand lens.

Clip Cage Construction:

Clip cages were made using two transparent plastic rings (3 cm diameter) covered on both sides with fine muslin mesh. The rings were clipped together using binder clips to enclose the cotton leaf section. Each cage confined a known number of insects (nymphs or adults) on the leaf surface for treatment.

Spore Suspension Preparation and Viability:

Stored fungal cultures on SDAY/4 plates and incubated at 28°C for 7 days. Spores were harvested by gently scraping the culture surface and suspended in 5 mL autoclaved distilled water containing 0.05% Tween 20 as a surfactant. The suspension was vortexed for 2 minutes and filtered through sterile muslin cloth to remove mycelial debris. Spore concentration was determined using a Neubauer hemocytometer. The two concentrations (4×10^8 and 4×10^4 spores/mL) were selected based on standard dose-response ranges commonly used in entomopathogenic fungal bioassays to evaluate both high and low virulence effects, allowing comparison of isolate efficacy and dose-dependent mortality (Batta, 2012; Fan et al., 2014). These concentrations were selected to assess both high and low dose virulence response in B. tabaci. All suspensions were stored at 4°C until use. Spore viability was assessed before each bioassay. A 100 µL aliquot from each suspension was spread onto SDAY/4 plates and incubated at 28°C for 24 hours. Germination rate was calculated by

microscopic examination of 100 randomly selected spores. Only suspensions with >90% viability were used in bioassays.

Figure 1. Clip cages used in experimentation for the virulence bioassay of Bemisia tabaci

Pathogenicity Bioassay against B. tabaci:

Pathogenicity assays were conducted to evaluate the virulence of fungal isolates against both the 4th instar nymphs and adults of B. tabaci. For nymphal assays, fifteen 4th instar nymphs per leaf were selected, and upper surface of washed leaves. Leaves were sprayed with 1 mL of each spore concentration. Each treatment was replicated five times. Similarly, adults were transferred to clip cages on freshly cleaned leaves using an aspirator. were the same as those for nymphs. Control treatments included only distilled water with 0.05% Tween 20. All treatments were kept in an insectary at 30°C, 55% RH, and a 12 h light/dark photoperiod.

Mortality Assessment:

Mortality was recorded at 24-hour intervals for 6 consecutive days post-inoculation. Dead insects were identified by visual observation under a stereomicroscope and confirmed by checking for fungal sporulation. Insects were considered dead when unresponsive to gentle probing and showing signs of desiccation or fungal growth.

Data Analysis:

Mortality data were corrected using Abbott's formula [18] to account for natural mortality in the control. Chi-square (χ^2) tests were used to analyze fungal occurrence across agroecological zones. Pathogenicity data were subjected to Fisher's analysis of variance (ANOVA) using Statistix 8.1 software at a 5% probability level. Means were compared using at $\alpha = 0.05$. Standard deviations were calculated using MS Excel [19].

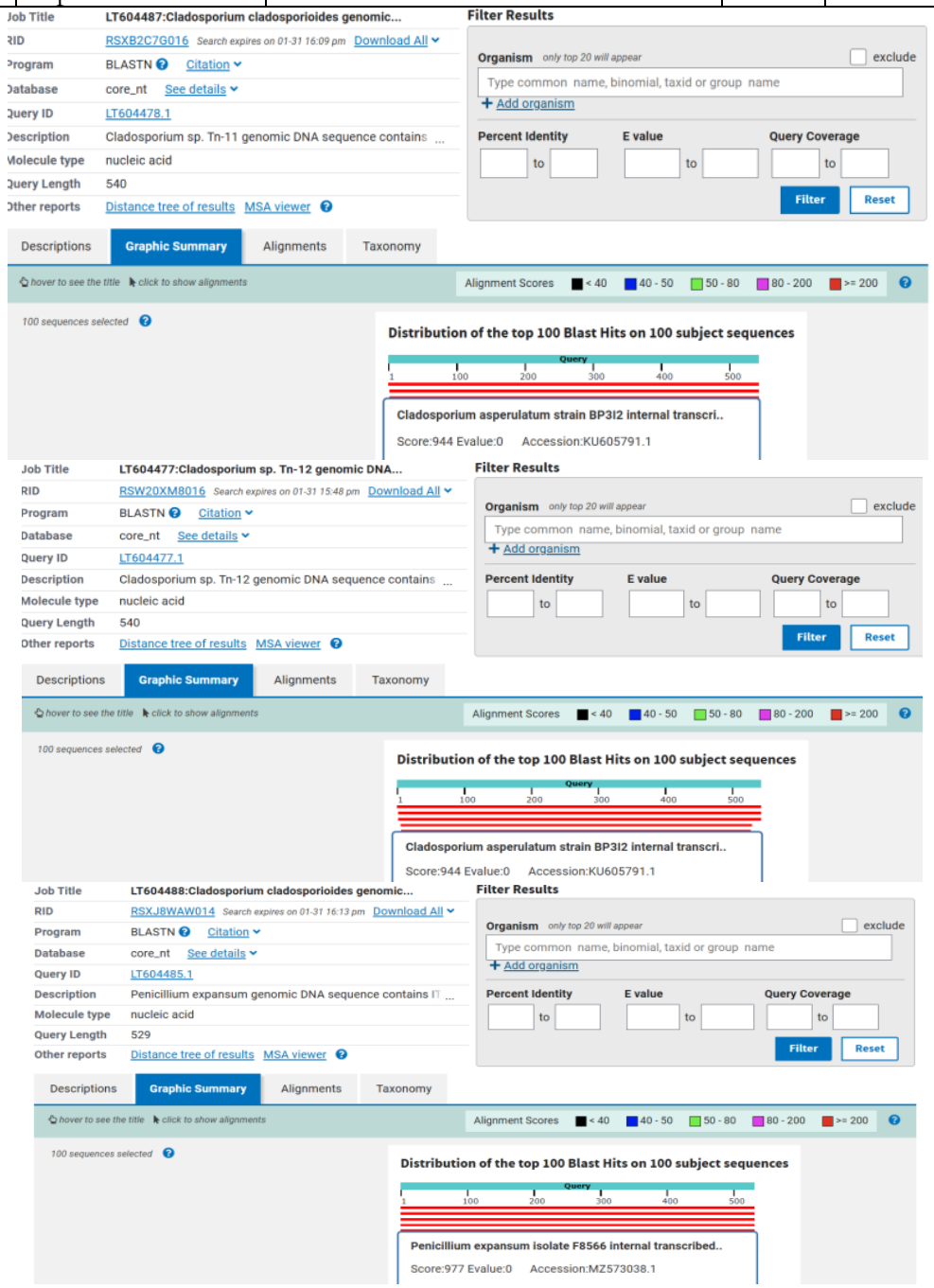
Results:

A total of 900 naturally dead insect samples were collected from three agro-ecological zones of Punjab, Pakistan (Cotton, Central Mixed, and Hot Arid zones). Fungal isolates associated with these samples were purified and identified as Cladosporium sp., Cladosporium cladosporioides, Penicillium expansum, and Penicillium polonicum (Table 1).

Table 1. Details of Cladosporium and Penicillium with their isolation Source.

Sr. No	Name of fungi	Source of isolation	Code	Gen Bank No.
01	Cladosporium sp.	Bemisia tabaci, cotton field, Kasur	Tn-12	LT604477
02	Cladosporium sp.	Aphid from the cotton field, Layyah	Tn-11	LT604478
03	Cladosporium sp.	Mealy bug of cotton field, Multan	Tn-20	LT604479
04	Cladosporium cladosporioides	Aphid from the cotton field, Layyah	W08	LT604487
05	Cladosporium cladosporioides	Bemisia tabaci, cotton field, Sahiwal	W09	LT604488
06	Penicillium expansum	Aphid from the cotton field, Kasur	Tn-14	LT604485

07	Penicillium expansum	Bemisia tabaci, cotton field, Muzafargarh	W01	.LT604486
08	Penicillium expansum	Mealy bug of cotton field, Multan	Tn-18	.LT604493



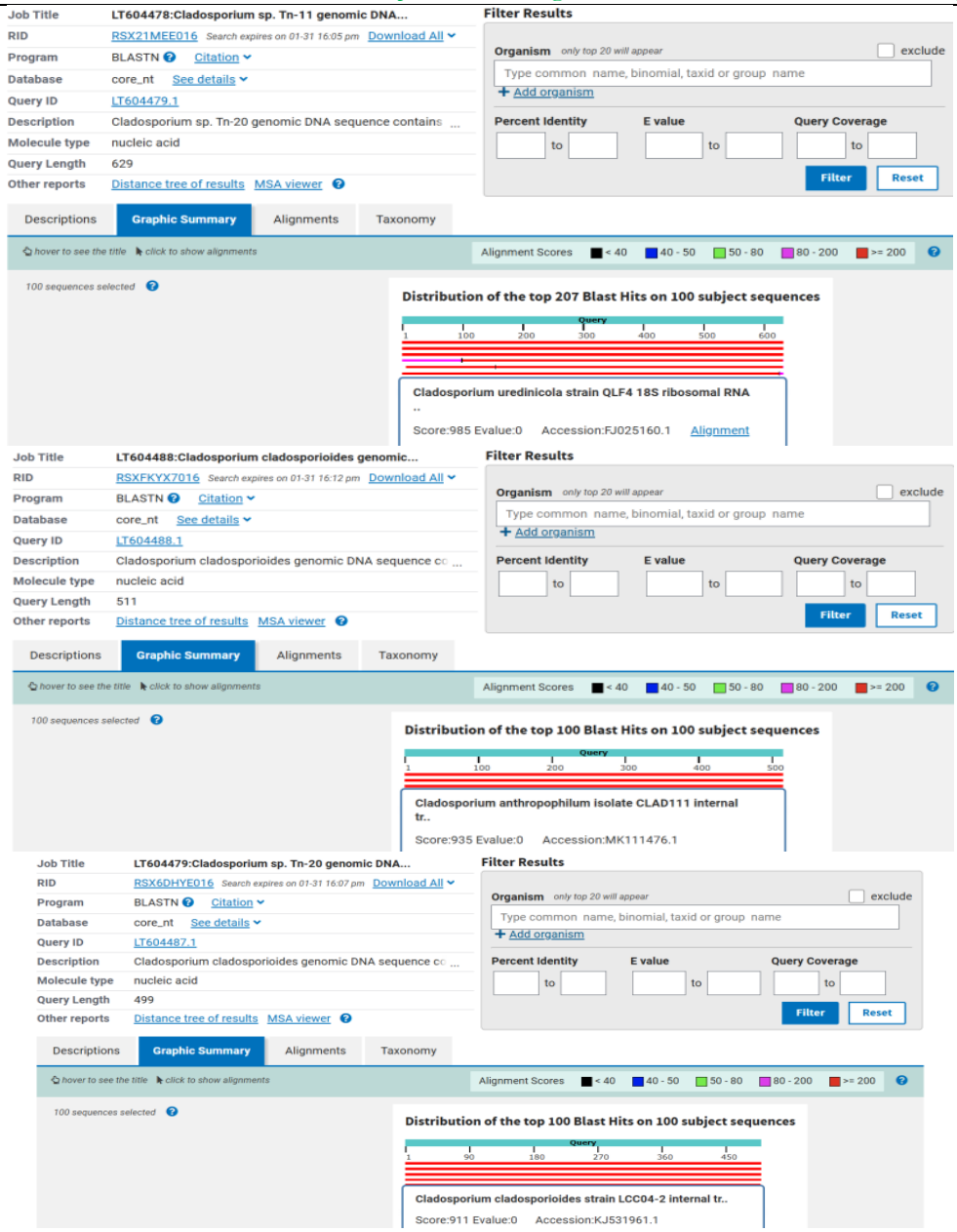


Figure 2. NCBI blast homology analysis of all 6 isolates.

Morphological characterization of Penicillium and Cladosporium

Morphological analysis of the Penicillium and Cladosporium isolates revealed distinct colony textures, spore shapes, and conidiophore sizes (Table 2; Fig. 3), aiding in preliminary identification before molecular confirmation.

Pathogenicity Bioassay:

To evaluate entomopathogenic potential, bioassays were conducted against 4th instar nymphs and adults of Bemisia tabaci using conidial suspensions at two concentrations: 4×10^{8} and 4×10^{4} spores/mL. These concentrations were selected based on standard ranges commonly used in entomopathogenic fungal bioassays.

Overall, mortality patterns across all treatments and fungal isolates clearly demonstrated that 4th instar nymphs were consistently more susceptible to fungal infection than adults, indicating higher vulnerability of the nymphal stage to entomopathogenic fungi.

Table 2. Morphological characterization of Penicillium and Cladosporium

Fungal species	Colony color	Colony reverse	Colony texture	Conidia/ Spore diameter	Conidia/ Spore shape	Conidiophores	Fig.
Penicillium polonicum	dull green	light brown	Floccose	4-5µm	Globose	Conidiophore= 300 µm	Fig. 2. (A, D, G)
Penicillium expansum	grayish turquoise	yellow brown	Velutinous	3.7-4.5 µm	Subglobose	Conidiophore= 400 µm	Fig. 2. (B, E, H)
Cladosporium cladosporioides	olivaceous green	olivaceous black	Velutinous	2-3 µm	ellipsoidal to lemon	Conidiophore= 360 µm	Fig.2 (C, F, I)

Table 3. Mortality range and percentage mortality of different species of Penicillium against the 4th instar nymphal stage of B. tabaci.

Sr. No	Fungal taxa	Conidial Conc. mL^{-1}	MORTALITY					
				AFTER				
				48 hrs	72 hrs	96 hrs	120 hrs	144 hrs
1	Penicillium polonicum	4×10^{8}	% ± SD	--- d	6.72±2.93 cd	14.65±7.22 C	28.48±10.03 B	44.06±9.64 a
		4×10^{4}	% ± SD	--- d	5.61±2.91 cd	13.17±6.05 C	22.39±1.5 b	42.31±8.29 a
2	Penicillium Expansum	4×10^{8}	% ± SD	--- d	8.78±6.37 cd	14.28±5.80 C	29.91±10.10 b	44.36±10.97 a
		4×10^{4}	% ± SD	--- d	7.94±6.04 cd	12.84±5.62 Cd	25.69±9.37 b	45.16±10.95 a

Table 4. Mortality range and percentage mortality of different species of Penicillium against the adult stage of B. tabaci.

Sr. No.	Fungal taxa	Conidial Conc. mL^{-1}	MORTALITY					
				AFTER				
				48 hrs	72 hrs	96 hrs	120 hrs	144 hrs
1	Penicillium polonicum	4×10^{8}	% ± SD	--- d	---d	---d	10.6±4.62 c	22.40±7.30 Ab
		4×10^{4}	% ± SD	--- d	--- d	--- d	9.8±4.00 c	23.22±6.27 Ab
2	Penicillium Expansum	4×10^{8}	% ± SD	--- d	--- d	--- d	15.69±7.44 bc	27.59±8.64 A
		4×10^{4}	% ± SD	--- d	--- d	--- d	11.83±7.56 c	25.49±8.63 A

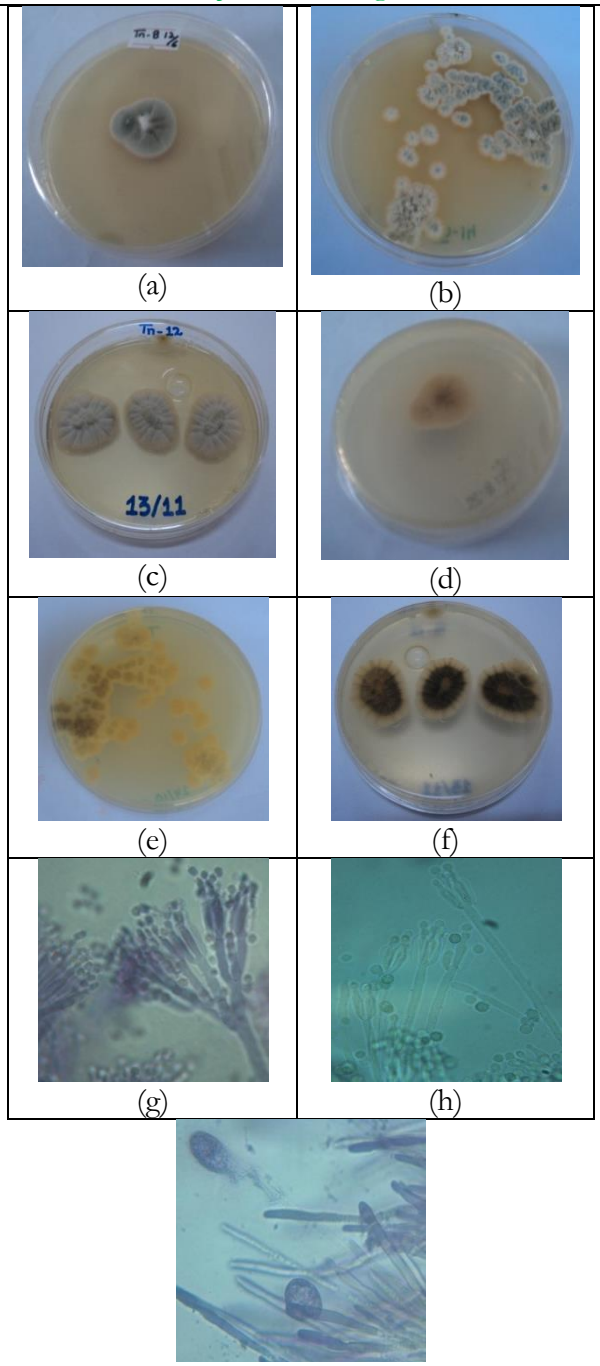


Figure 3. Microscopic characteristics of Penicillium and Cladosporium**. A.** Colony front of Penicillium polonicum. **B**. Colony front of Penicillium expansum. **C**. Colony front of Cladosporium cladosporioides. **D**. Colony reverse of Penicillium polonicum. **E**. Colony reverse of Penicillium expansum **F**. Colony front of Cladosporium cladosporioides **G**. Microscopic view of Penicillium polonicum **H**. Microscopic view of Penicillium expansum **I**. Microscopic view of Cladosporium cladosporioides

Pathogenicity of Penicillium spp:

The activity of the Penicillium species towards the developmental stages of B. tabaci was significantly higher, with P. expansum always having a higher virulence compared to P. polonicum. The higher conidial concentration resulted in a higher level of mortality, resulting in a clear dose-related response. In general, nymphal stages were more vulnerable than adults (Tab. 3 and Tab. 4).

LSD= (Similar small alphabetic showed no significant difference; P> 0.05)

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The pathogenicity of P. polonicum and P. expansum was compared on 4th instar nymphal and adult stages of B. tabaci. P. expansum was virulent against 4th instar nymphal and adult stages after 6 days, as compared to P. polonicum, which showed less pathogenicity against adults (Figure 3).

Pathogenicity of Cladosporium spp:

Cladosporium species exhibited strong entomopathogenic potential, whereby efficacy was highest at increased conidial concentrations and more effective against nymphal stages than adults. Cladosporium sp. and C. cladosporioides showed differences in virulence, with both species showing dose-dependent pathogenic responses (Tables 5 and 6).

Table 5. Mortality range and percentage mortality of different species of Cladosporium against the 4th instar nymphal stage of B. tabaci

Sr. No	Fungal taxa	Conidial Conc. mL^{-1}		MORTALITY AFTER				
				48 hrs	72 hrs	96 hrs	120 hrs	144 hrs
1	Cladosporium sp.	4 x 10^{8}	% ± SD	--- I	8.43±2.38 h	20.74±3.06 f	45.62±3.38 c	60.05±2.89 A
		4 x 10^{4}	% ± SD	--- I	--- i	12.52±3.02 gh	30.9 ± 4.96 e	48.71±5.86 Bc
2	Cladosporium cladosporioides	4 x 10^{8}	% ± SD	--- I	--- i	15.19±4.55 g	42.78±3.55 d	54.14±3.73 B
		4 x 10^{4}	% ± SD	--- I	--- i	13.27±4.31 g	28.65±4.41 e	44.16±3.06 C

LSD= (Similar small alphabetic showed no significant difference; P> 0.05).

Table 6. Mortality range and percentage mortality of different species of Cladosporium against the adult stage of B. tabaci.

Sr. No.	Fungal taxa	Conidial Conc. mL^{-1}		MORTALITY AFTER				
				48 hrs	72 hrs	96 hrs	120 hrs	144 hrs
1	Cladosporium sp.	4 x 10^{8}	% ± SD	--- F	--- f	7.05±2.69 de	15.48±4.70 c	35.57±7.19 a
		4 x 10^{4}	% ± SD	--- F	--- f	--- f	8.43±3.92 d	18.22±4.60 c
2	Cladosporium cladosporioides	4 x 10^{8}	% ± SD	--- F	--- f	4.5 ± 3.4 ef	20.57±4.24 c	40.25±4.27 a
		4 x 10^{4}	% ± SD	--- F	--- f	--- f	13.45±3.14 c	23.15±4.52 b

LSD= (Similar small alphabetic showed no significant difference; P> 0.05).

The pathogenicity of Cladosporium and C. cladosporioides was compared on 4th instar nymphal and adult stages of B. tabaci. Cladosporium was virulentwas virulent against both 4th instar nymphal and adult stages after 6 days, as compared to C. cladosporioides, which showed less pathogenicity against adults (Figure 4).

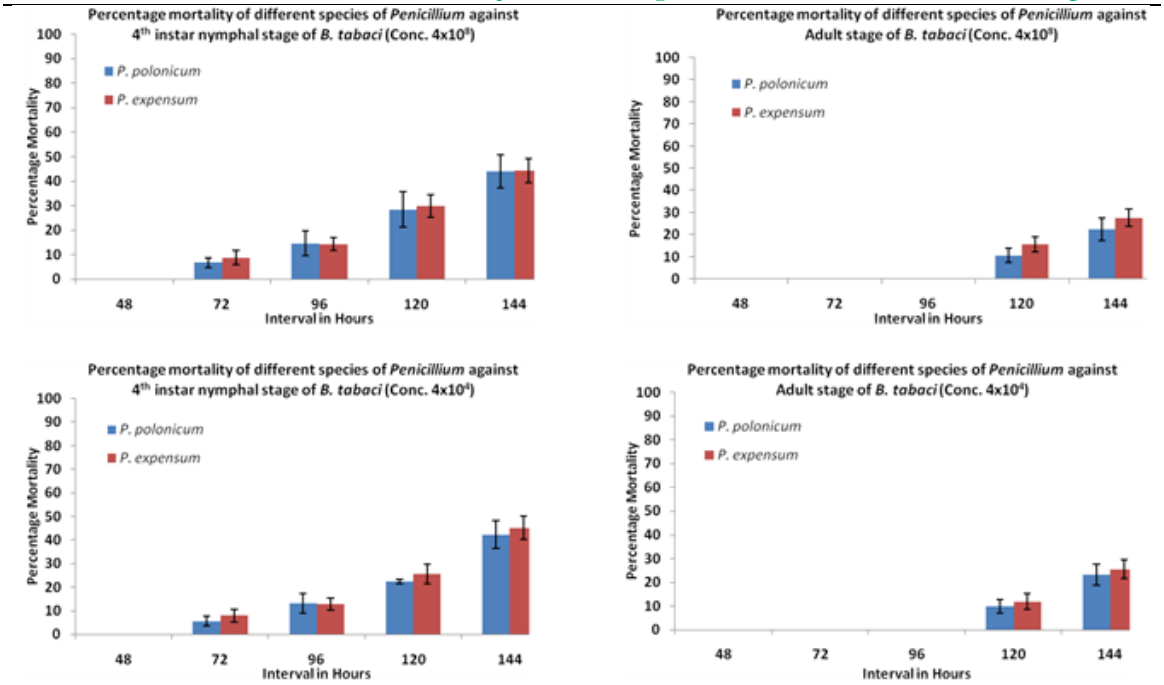

Figure 4. Comparison in percentage mortality of 4th instar nymphs and adults of B. tabaci by different concentrations of Penicillium

Discussion:

The present study contributes to the growing body of knowledge on the insecticidal potential of less-studied entomopathogenic fungi, particularly Penicillium and Cladosporium species, against Bemisia tabaci. Despite increasing interest in microbial control agents, ecological and pathogenic investigations of these genera remain limited, particularly in the context of whitefly management.

Our findings showed that Cladosporium sp. (4×10^8 spores/mL) caused higher mortality in nymphs (60.05%) compared to adults (35.57%) after 6 days, while C. cladosporioides showed slightly less efficacy in nymphs (54.14%) but higher adult mortality (40.25%). These results align with [20], who observed a 63.98% mortality of aphids after 7 days of treatment with C. cladosporioides. Moreover, [21] reported that different entomopathogenic fungi induced a range of mortality (up to 87.8%) in whitefly nymphs, suggesting that life stage significantly influences fungal pathogenicity. This trend was also confirmed in our study, with nymphs consistently more susceptible than adults, possibly due to differences in cuticle structure, behavior, and habitat exposure. The recent research also affirms the high susceptibility of the nymphal stages of B. tabaci to different EPFs, with a mortality of 45% to 82% with respect to the fungal strain, formulation, and environmental conditions [22].

In contrast, Penicillium species demonstrated comparatively lower mortality against B. tabaci, which is consistent with earlier findings. For example, P. citrinum caused high larval mortality (98.67%) in Spodoptera litura [23], but the extrapolation of such results to B. tabaci may not be entirely appropriate due to fundamental differences in biology, ecology, and immune responses between lepidopteran larvae and hemipteran pests. Our study recorded a gradual increase in whitefly mortality over time, peaking after 7 days, which is in line with [24], who observed delayed but significant mortality of Tribolium castaneum treated with Penicillium spp. Recent studies indicate that combining Penicillium spp. with adjuvants or plant-based compounds could be one of the new trends to increase the field efficacy of non-conventional EPFs.

One important observation is that fungal efficacy varies considerably not only by fungal species but also by host insect species, environmental conditions, and conidial concentrations. For instance, Penicillium sp. combined with nanosilica enhanced Myzus persicae mortality [25], highlighting the potential of synergistic formulations in improving fungal efficacy. Such approaches were not tested in the present study but could be highly relevant for enhancing Penicillium performance against B. tabaci. Notably, Beauveria and Metarhizium often require specific environmental conditions, such as high humidity, for optimal infection. In contrast, some isolates of Cladosporium and Penicillium have demonstrated tolerance to a broader range of environmental stresses, which could provide practical advantages under variable field conditions. Although the Penicillium species showed entomopathogenic potential against B. tabaci, they should be taken into consideration regarding their ecological effects. Penicillium spp. have been known to produce mycotoxins, including patulin, penicillic acid, and citrinin, which may impact non-target organisms when the spores or the mycotoxin accumulate in the environment [26]. Their emission into the field may cause a potential disruption in the ecological balance by affecting pollinators, natural predators of pests, or microbial communities with a positive influence on the health of the soil. Hence, although Penicillium spp. are promising biocontrol agents, the research on the fate, persistence, and non-target effects of their mycotoxins in agricultural systems should be scrutinized. This involves the assessment of risks to useful insects, the soil microbiome diversities, and plant safety in a realistic field environment. The measures are important in the establishment of safe and sustainable biocontrol methods with Penicillium species.

The integration of fungal entomopathogens into IPM programs could reduce dependence on chemical insecticides, which are often associated with pest resistance, environmental contamination, and non-target effects. Our findings contribute to the foundation for developing biocontrol strategies that are environmentally sound and compatible with organic farming systems. Given the specificity and delayed action of fungal pathogens, their application could be synchronized with other biological agents or cultural practices to maximize efficacy. For instance, timing fungal applications during the early nymphal stages, when susceptibility is highest, may improve control outcomes while reducing the need for repeated applications. The emerging entomopathogenic fungi tested in this paper (Penicillium and Cladosporium species) possess a good potential in the Integrated Pest Management (IPM) programs of the sustainable control of Bemisia tabaci. The integration of the IPM strategies can facilitate a multi-tactic approach to keep pests suppressed using a combination of biological, cultural, and chemical control strategies to ensure the long-term reduction in the environmental impact of the management.

Despite these promising outcomes, several limitations exist. The present study focused on laboratory assays under controlled conditions. Thus, extrapolating results to field environments requires caution. Field trials are necessary to assess fungal persistence, formulation stability, environmental tolerance, and compatibility with Integrated Pest Management (IPM) practices. Furthermore, the long-term safety of using Penicillium spp. must be carefully evaluated, as certain strains are known to produce mycotoxins with potential health risks for humans and animals [27].

Conclusion:

The paper gives a novel overall assessment of the entomopathogenic potential of Penicillium and Cladosporium species with regard to various stages of development of Bemisia tabaci. The results show that these non-conventional genera of fungi are highly pathogenic on nymphal and adult stages, showing evident disparity in virulence between isolates as well as in different conidial concentrations. The fact that there is a difference between the susceptibility of two developmental stages brings about the significance of using a stage-specific targeting when using biological control strategies. On the whole, it is possible to state that the obtained

results provide evidence that Penicillium and Cladosporium species will be promising alternative biocontrol agents to diversify the range of entomopathogenic fungi that can be used in sustainable whitefly management. These new entomopathogens have a promising potential in terms of bio-safety (no residues), resistance reduction, and bio-safety (eco-friendliness, and should be considered as good components of integrated pest management (IPM) to reduce reliance on chemical insecticides.

Real-World Applicability and Practical Implications:

This research paper has shown the possibility of the non-conventional entomopathogenic fungi as a sustainable biocontrol agent in the control of Bemisia tabaci. It can be demonstrated that Penicillium and Cladosporium species are pathogenic in laboratory conditions, which implies that they could be applied in integrated pest management (IPM) programs to limit the use of chemical insecticides. These fungi can be engineered into mycoinsecticide preparations, which greenhouse and open field settings especially when dealing with the more vulnerable nymphal stages. Also, they may be incorporated into the current IPM strategies to supplement cultural practices, biological control agents, and selective insecticides, which would help in the management of resistance and environmental safety. Nevertheless, the field experimentation, optimization of the formulation, and evaluation of environmental persistence, non-target action, and regulatory safety must be carried out to conduct practical implementation. This will require formal development pathways, such as formulation science, large-scale production, etc., that will be required to translate laboratory efficacy to field-level applications.

Prospects and Suggestions in the Field of Validation:

Even though the current investigation proves good laboratory effectiveness of Penicillium and Cladosporium species in B. tabaci, additional confirmation under semi-field and field environments is required prior to being used practically. The research of the future must be conducted with large-scale greenhouse and outdoor trials aimed at assessing their performance in the conditions of a variable environment, such as temperature, humidity, UV exposure, and pressure of natural pests. The development of the formulations, shelf-life stability, and compatibility with popular agrochemicals and IPM components should also be evaluated. Moreover, the research on the non-target effects, environmental safety, persistence in agro-ecosystem, and possible effects on beneficial insects would be needed to ensure ecological sustainability. As an additional step in the development of standardized mycoinsecticide preparations, molecular characterization of virulence factors and secondary metabolites associated with pathogenicity can be used. This field-based validation and the optimization of the formulation will be essential in the process of commercialization and the practical application of such non-conventional entomopathogenic fungi in the sustainable management of Bemisia tabaci.

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