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Exploiting Phytopathogenic *Alternaria alternata* and *Alternaria macrospora* for Management of *Parthenium hysterophorus*

Komal Sahu[1], Kuldeep Kumar[2], Bikram Jit Singh[3], Anil K Sharma[4], Rippin Sehgal[5], Chhaya Singh[6], Raj Singh[1]

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ABSTRACT

Background: *Parthenium hysterophorus* is a fast-spreading invasive weed that poses serious challenges to agriculture and the environment due to its aggressive growth and allelopathic effects. The conventional use of mechanical or chemical methods for its control, often result in environmental hazards and some times limited effectiveness of these methods. As a sustainable alternative, biological management using pathogenic fungi is gaining attention due to its specificity and ecological safety.

Methods: The study aimed to isolate and evaluate pathogenic fungi from infected *Parthenium* plants to assess their potential as biological control agents. Four fungal isolates were collected from naturally infected *Parthenium* plants and tested for pathogenicity on *Parthenium*, using the disc plate technique under controlled laboratory conditions.

Result: Results revealed that *Alternaria macrospora* strain PHMMU1 exhibited the highest disease incidence (67.27%) and most severe defoliation, followed by *Alternaria alternata* strain PHMMU2 (50.06%), *A. macrospora* strain PHMMU3 (40.70%) and *A. macrospora* strain PHMMU4 (35.18%). All isolates fulfilled Koch's postulates, confirming their pathogenicity. The findings highlight the potential of *Alternaria* spp., particularly *A. macrospora*, as effective mycoherbicides for the sustainable management of *Parthenium hysterophorus*, supporting integrated weed management strategies and environmental conservation efforts.

Key words: Aggressive, Allelopathic, *Alternaria*, Conventional, Mycoherbicides, *Parthenium*.

INTRODUCTION

Invasive alien plant species pose critical threats to global biodiversity, agricultural productivity and ecosystem stability (Rai, 2022). These species often outcompete native flora and crops for growth factors like light, nutrients, space, soil moisture *etc*. due to their rapid growth, adaptability to changing environmental conditions and prolific seed production (Kashyap *et al*., 2022; Bhattacharya *et al*., 2025). Among them, *Parthenium hysterophorus* L., known as Congress grass, carrot weed or bitter weed, is one of the most aggressive (Singh *et al*., 2023). It is an annual herbaceous weed known for its toxicity and rapid growth. Belonging to the Asteraceae family, *Parthenium* is native to tropical and subtropical Americas, Mexico and the southern USA (Rana, 2022), but has spread widely across Asia, Africa and Australia, owing to its prolific reproduction, adaptability and human-mediated dispersal (Kaur *et al*., 2021; Strathie *et al*., 2021; Adhikari *et al*., 2023). This weed severely impacts native flora, ecosystems and human and animal health (Sarita *et al*., 2024). It causes up to 80% yield loss in agriculture, triggers allergic reactions such as dermatitis and asthma and adversely affects livestock, leading to reduced milk production and health issues (Mao *et al*., 2021; Sharma and Kumawat, 2023). Its allelopathic properties suppress the growth of nearby vegetation, further diminishing agricultural productivity (Dheer *et al*., 2023). Infestation in croplands, pastures, roadsides and wastelands leads to arable land degradation and economic

[1]Department of Bio-Sciences and Technology, Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala-133 207, Haryana, India.
[2]Department of Botany, Vardhaman College, Bijnor-246 701, Uttar Pradesh, India.
[3]Department of Mechanical Engineering, Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala-133 207, Haryana, India.
[4]Department of Biotechnology, Amity University, Mohali-140 306, Punjab, India.
[5]Department of Biotechnology, Ambala College of Engineering and Applied Research, Devsthali, Ambala-133 001, Haryana, India.
[6]Department of Botany, Government PG College, Thalisain, Pauri Garhwal-246 285, Uttarakhand, India.

Corresponding Author: Raj Singh, Department of Bio-Sciences and Technology, Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala-133 207, Haryana, India.
Email: dr.rajsingh09@gmail.com

ORCIDs: https://orcid.org/0009-0002-7716-4081, https://orcid.org/0000-0001-5378-6818, https://orcid.org/0000-0002-7250-0165, https://orcid.org/0000-0002-9768-1644, https://orcid.org/0009-0009-4026-5728, https://orcid.org/0000-000-9372-2349, https://orcid.org/0000-0002-6331-9196

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losses, estimated at AUD 109 million annually in rangelands (Chhogyel *et al.*, 2021; Shabbir *et al.*, 2020). Traditional control strategies-mechanical, chemical and cultural-have shown limited long-term success. Mechanical removal, like hand weeding is common but this method is labour-intensive and often ineffective due to rapid regrowth during peak times (Meena *et al.*, 2022), while chemical herbicides may harm non-target organisms, promote resistance and cause environmental contamination (Bashar *et al.*, 2021; Bogale and Tolossa, 2021; Madrewar *et al.*, 2024). These drawbacks underscore the urgent need for environmentally sound alternatives. Biological control, employing living organisms to suppress invasive weeds, presents a promising long-term solution (Bekeko, 2021; Petraki *et al.*, 2024). Biocontrol agents such as insects, nematodes and microbes have been explored globally (Rani *et al.*, 2021; Nagar *et al.*, 2022; Singh *et al.*, 2022; Sahu *et al.*, 2024). Notable insect biocontrol agents include *Bucculatrix parthenica*, *Carmenta ithacae*, *Conotrachelus albocinereus*, *Epiblema strenuana*, *Listronotus setosipennis*, *Platphalonidia mystica*, *Semicronyx lutulentus* and *Zygogramma bicolorata* (Khan and Fahad, 2020; Samanta *et al.*, 2021; Madrewar *et al.*, 2024; Sarita *et al.*, 2024).

Among microbial agents, phytopathogenic fungi offer advantages such as high infectivity, host specificity, mass producibility and compatibility with integrated weed management (Singh and Pandey 2019; Peng *et al.*, 2021; Sharma, 2021). Fungi like *Puccinia abrupta* var. *partheniicola*, *P. melampodi*, *Entyloma compositarum*, *Alternaria alternata*, *Fusarium oxysporum*, *Colletotrichum* spp., *Aspergillus* spp., *Drechslera* spp. and *Valsa mali* have shown varying levels of efficacy against *Parthenium* (Dukpa *et al.*, 2020; Ahmad *et al.*, 2020; Maharjan *et al.*, 2020; Ocán *et al.*, 2024; Sheikh *et al.*, 2020; Tsehaye and Semere, 2023). Mycoherbicides-fungal agents used similarly to chemical herbicides-can effectively manage invasive weeds in both agricultural and non-agricultural contexts (Sırrı and Özaslan, 2023; Aneja, 2024). This study aims to isolate and characterize fungal pathogens, particularly *Alternaria alternata* and *A. macrospora*, from infected *Parthenium* plants and evaluate their pathogenicity. The findings may support the integration of biological control into weed management strategies, contributing to agricultural sustainability, biodiversity conservation and ecosystem restoration.

MATERIALS AND METHODS

Sample collection and preparation

Fresh infected *Parthenium hysterophorus* leaves were aseptically collected from Maharishi Markandeshwar (Deemed to be University) (Mullana), Ambala and nearby areas, by using clean sterilized tools and sterile collection bags to minimize contamination. After surface sterilization with ethanol and sodium hypochlorite and rinsing with sterile water, samples were dried in a laminar airflow chamber and used for fungal isolation (Ahmad *et al.*, 2020). The experimental work was conducted from January 2023 to December 2024.

Isolation of fungi from *Parthenium* plants

Fungal pathogens were isolated from sterilized *Parthenium* leaf segments on PDA with streptomycin at a concentration of 100 mg/L to suppress bacterial contamination. After 5-7 days of incubation at 25°C, emerging colonies were subcultured for purity by placing 6 mm mycelial discs onto fresh PDA plates with the help of sterile corkborer and placed for incubation at 25°C. Pure cultures were stored on PDA slants at 4°C and in glycerol stocks at -20°C in the Department of Bio-Sciences and Technology for future use (Karim *et al.*, 2018).

Identification and molecular characterization of pathogenic fungi

Fungal identification was based on colony morphology and microscopic examination using lactophenol cotton blue staining (Patyal *et al.*, 2024). For molecular characterization, DNA was extracted and 18S rRNA along with the ITS region were amplified using ITS1 and ITS4 primers. PCR products were sequenced, followed by BLAST analysis and phylogenetic tree construction to confirm species identity through sequence similarity with known fungal taxa (Sahu *et al.*, 2025).

Pathogenicity of fungal strains

The disc plate technique assessed fungal pathogenicity by placing 8 mm discs from week-old cultures on petri dish lids. Six-week-old sterilized *Parthenium* leaves on moist filter paper were incubated at 28-30°C for 72 hours. Spore dispersal led to disease symptoms, which were observed after three days (Singh, 2020). The disease incidence percentage was evaluated through the following method.

$$\text{Disease incidence (\%)} = \frac{\text{Infected area of the leaf}}{\text{Total area of the leaf}} \times 100$$

Statistical analysis

Each experiment was conducted in triplicates. The statistical analysis was carried out using the SPSS software, version 21. Experimental results were analysed following appropriate methods such as Analysis of Variance (ANOVA) and multiple regression analysis. Critical differences were computed at the p = 0.05 level after single-way ANOVA.

RESULTS AND DISCUSSION

In the MMU region of Ambala, *Parthenium hysterophorus* was affected by leaf spot diseases caused by four fungal isolates cultured on PDA. Morphological and microscopic analysis identified them as *Alternaria* species with distinct variations (Pavicich *et al.*, 2022). Molecular characterization *via* 18S rRNA gene sequencing and ITS region PCR confirmed isolates as *Alternaria alternata* strain PHMMU2 and three *Alternaria macrospora* strains (PHMMU1,

PHMMU3, PHMMU4) (Table 1). Sequences were submitted to NCBI GenBank (PQ483096, PQ433119, PQ483101, PQ483102). Phylogenetic analysis validated these identifications (Fig 1). Pathogenicity was tested using the Parthenium disc plate technique (Fig 2). Spore germination occurred within 24 hours on healthy leaves, with maximum leaf defoliation at 72 hours (25±5°C). *Alternaria macrospora* PHMMU1 caused the highest disease incidence (67.27%),

Table 1: List of isolated fungi used in the research with their accession number.

Isolate no.	Fungus name	GenBank submission name	Accession number
LC	*Alternaria alternata*	*Alternaria alternata*strain PHMMU2	PQ483096
LR	*Alternaria macrospora*	*Alternaria macrospora*strain PHMMU1	PQ433119
L3b	*Alternaria macrospora*	*Alternaria macrospora*strain PHMMU3	PQ483101
L1b	*Alternaria macrospora*	*Alternaria macrospora*strain PHMMU4	PQ483102

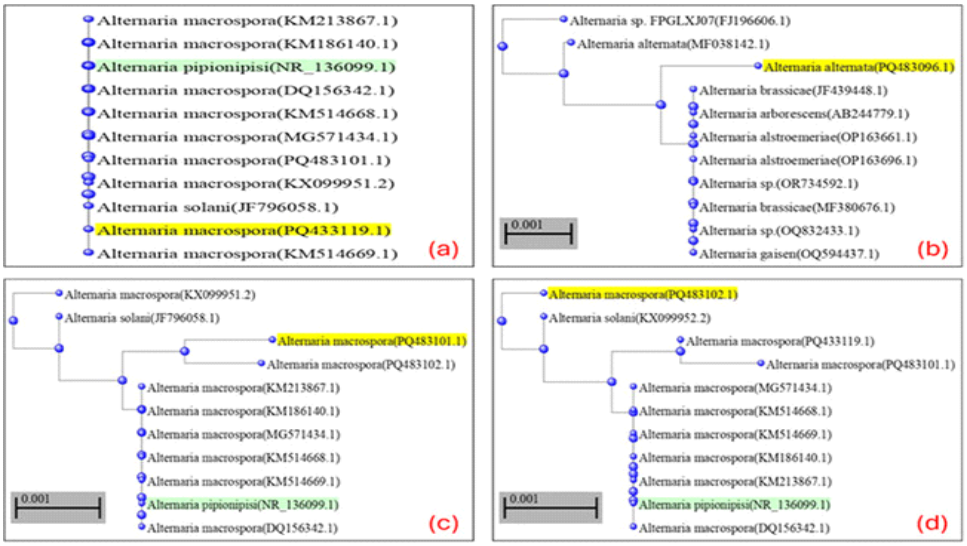


Fig 1: Phylogenetic trees of (a) PHMMU1 (b) PHMMU2 (c) PHMMU3 and (d) PHMMU4.

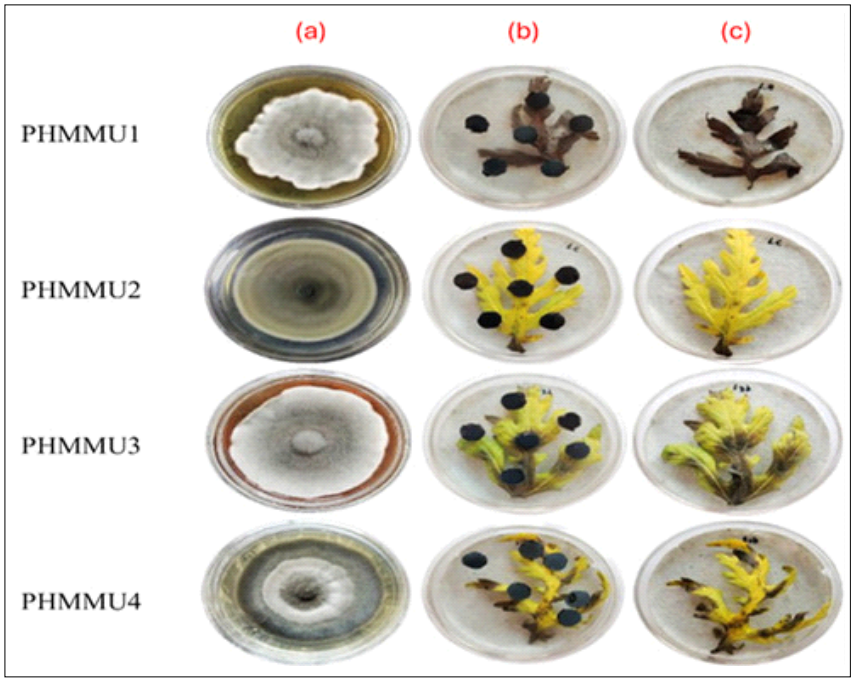


Fig 2: (a) Fungal strains isolated from infected *Parthenium* leaves (b) Release of spores from fungal culture discs and invasion into leaves and (c) Disease incidence on *Parthenium* leaves after 72 hours of incubation.

followed by *A. alternata* PHMMU2 (50.06%), *A. macrospora* PHMMU3 (40.70%) and PHMMU4 (35.18%). Re-isolation confirmed their identity, fulfilling Koch's postulates and establishing pathogenicity to *Parthenium hysterophorus*.

Statistical analysis

Four fungal isolates (PHMMU1-PHMMU4) were tested for pathogenicity against *Parthenium hysterophorus* using the disc plate method. Disease progression, including necrotic lesions and leaf degradation, varied among strains over 72 hours. Multiple regression analysed the effects of incubation time and fungal strain on disease incidence, while ANOVA confirmed significant differences in virulence, validating the observed infection patterns.

Evaluation of disease incidence through multiple regression

Multiple regression analysis assessed the impact of incubation time and fungal isolates on *Parthenium hysterophorus* disease incidence. The model showed a strong fit (high R^2) and significant predictors ($p<0.05$). Disease incidence increased with time, especially for isolate PHMMU1, which had the highest infection rates-from 33.21% at 24 hours to 67.27% at 72 hours-demonstrating its strong virulence. These results confirm incubation time's significant role in disease progression and validate PHMMU1 as the most pathogenic isolate (Fig 3).

Multiple regression model building report

The multiple regression model predicting *Parthenium hysterophorus* disease incidence was developed using incubation time (X1) and fungal isolate (X2) as predictors (Fig 4). The fungus variable (X2) contributed most, increasing adjusted R^2 to 50.06%. Adding incubation time (X1) improved the model accuracy to 99.84%. Including the interaction term (X1*X2) further raised adjusted R^2 to 99.99%, indicating a strong synergistic effect. All predictors were statistically significant ($p<0.05$). The incremental impact chart showed fungus had the highest contribution, followed by incubation time, with their interaction enhancing model performance. Regression models for each isolate revealed quadratic disease progression over time, characterized by a negative squared time coefficient (-0.002951), showing an initial increase then decline in severity. *Alternaria macrospora* PHMMU1 had the highest linear coefficient (1.5569), indicating the fastest, most severe infection, followed by PHMMU2 (1.3604), PHMMU3 (1.1569) and PHMMU4 (1.0951). The control group exhibited minimal disease incidence (0.2833), confirming the isolates' pathogenic effectiveness (Table 2).

Analysis of pathogenicity using ANOVA

The significance of one-way ANOVA lies in its ability to detect and validate statistical differences in disease incidence among fungal isolates, enabling a reliable interpretation of their pathogenic potential. This statistical method was chosen to evaluate whether the observed variation in pathogenic effects among the treatments was statistically significant. The results revealed notable differences in the degree of infection produced by each isolate, indicating that the effectiveness of the fungal strains in inducing disease symptoms varied considerably. This analysis provides clear evidence supporting the differential virulence of the tested isolates.

Effect of fungal treatments on disease incidence

One-way ANOVA showed a highly significant difference in disease incidence among fungal treatments ($f = 15.10$; $p = 0.001$).

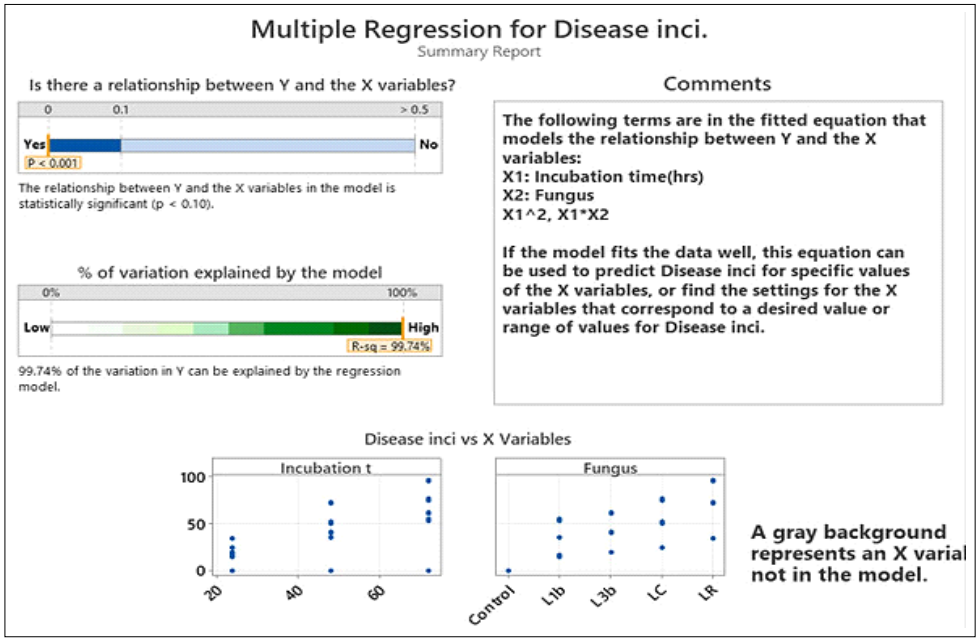


Fig 3: Multiple regression analysis of disease incidence.

The control had no symptoms (0.00%), while all fungal treatments significantly increased disease. *A. macrospora* PHMMU1 caused the highest incidence (67.27%), followed by *A. alternata* PHMMU2 (50.06%), PHMMU3 (40.70%) and PHMMU4 (35.18%). The results highlight PHMMU12's strong pathogenicity, with moderate variability within treatments (SD = 19.14) but consistent differences across groups (Fig 5).

Dunnett multiple comparisons with control

Dunnett's multiple comparisons test further substantiated the significance of these findings. Each fungal treatment group (PHMMU4, PHMMU3, PHMMU2 and PHMMU1) was found to be significantly different from the control group at the 95% confidence level ($p<0.05$). Notably, the control group was distinctly separated in grouping from all fungal

Table 2: Multiple regression equations for disease incidence (%).

Fungus (Type)		Final equation
Control	Disease incidence (%)	= -5.67+0.2833 Time (hrs) - 0.002951 [Time (hrs)]^2
PHMMU1	Disease incidence (%)	= 0.47+1.5569 Time (hrs) - 0.002951 [Time (hrs)]^2
PHMMU2	Disease incidence (%)	= -7.31+1.3604 Time (hrs) - 0.002951 [Time (hrs)]^2
PHMMU3	Disease incidence (%)	= -6.90+1.1569 Time (hrs) - 0.002951 [Time (hrs)]^2
PHMMU4	Disease incidence (%)	= -9.46+1.0951 Time (hrs) - 0.002951 [Time (hrs)]^2

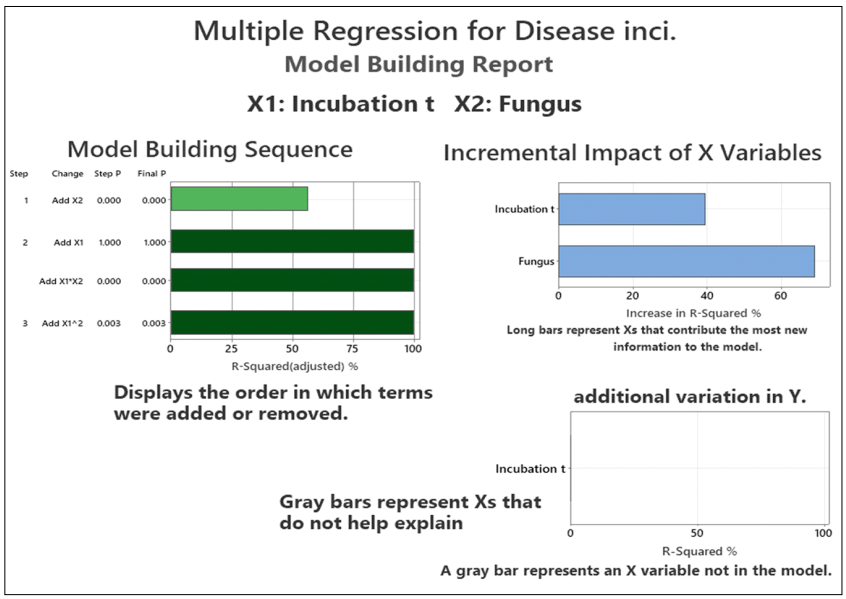


Fig 4: Model building report for multiple regression analysis of disease incidence.

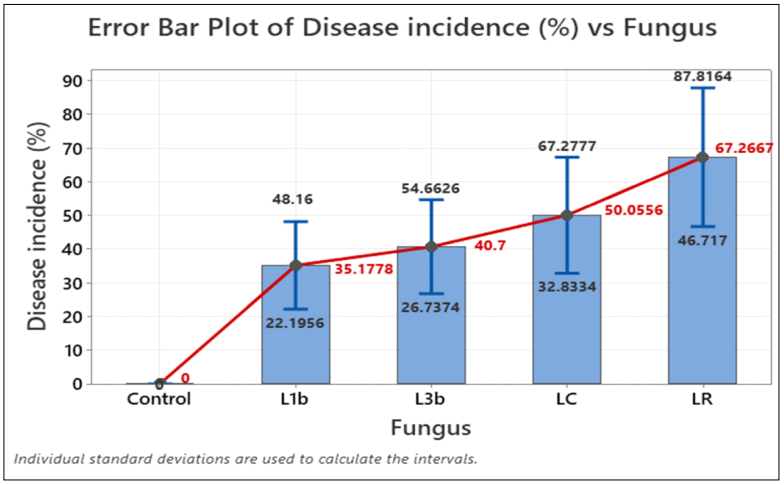


Fig 5: Error bar plot showing the effect of different fungal treatments on disease incidence.

treatments, confirming that disease incidence following fungal inoculation was not due to random variation but was attributable to treatment effects (Fig 6). The data indicate a dose-response-like trend where certain fungal isolates, particularly *A. macrospora* strain PHMMU1, induced more aggressive disease symptoms compared to others.

Effect of incubation time on disease incidence

The effect of incubation duration on disease incidence was also evaluated through a separate one-way ANOVA. Analysis revealed a statistically significant difference among the three incubation periods (f = 9.30; p = 0.001), indicating that the length of incubation played a critical role in disease progression. Mean disease incidence was observed to increase substantially with longer incubation times (Fig 7). At 24 hours post-inoculation, the mean disease incidence was relatively low (18.70%). However, after 48 hours, the mean incidence nearly doubled to 39.77% and further increased to 57.45% at 72 hours. This stepwise increase suggests a time-dependent enhancement in disease severity, with the most substantial disease symptoms manifesting at the longest incubation period tested. The pooled standard deviation for the incubation time analysis was 24.64, indicating a moderate spread of disease incidence values within each incubation group. However, the distinct upward trend across 24, 48 and 72 hours indicates that prolonged exposure significantly facilitates fungal pathogen establishment and symptoms development.

Taken together, the results clearly demonstrate that both fungal treatment and incubation time are critical determinants of disease incidence. The highest disease severity was observed when the most aggressive fungus (*A. macrospora* strain PHMMU1) was combined with the longest incubation time (72 hours), highlighting a synergistic effect between pathogen virulence and environmental conditions favouring disease progression.

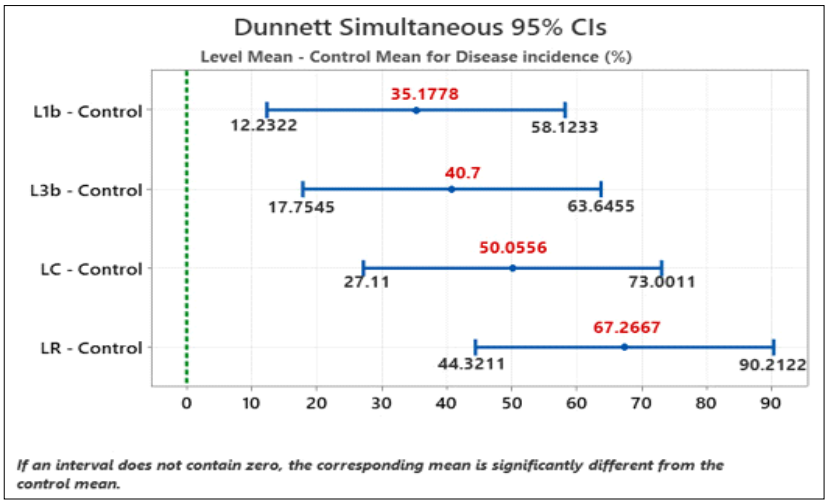


Fig 6: Dunnett's multiple comparison plot showing the 95% confidence intervals for mean differences in disease incidence between each fungal treatment and the control group.

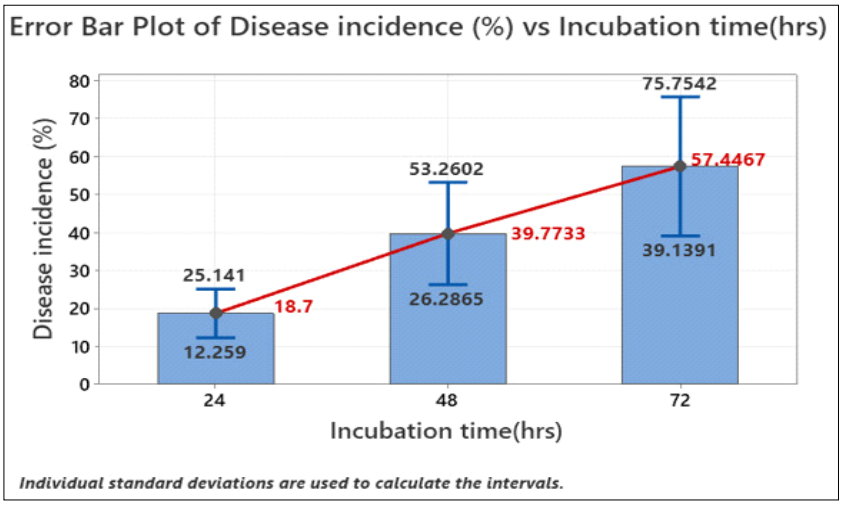


Fig 7: Error bar plot showing the effect of incubation time on disease incidence.

This study identified four fungal isolates as pathogenic to *Parthenium hysterophorus* under *in-vitro* conditions. Among them, *Alternaria macrospora* strain PHMMU1 exhibited the highest virulence, with a 67.27% disease incidence, rapid spore germination within 24 hours and maximum leaf defoliation by 72 hours, suggesting a highly efficient infection cycle. These findings indicate its strong potential as a promising candidate for mycoherbicide development. The results align with earlier studies, such as those by Kaur and Kumar (2019); Kumar *et al.* (2021); Kausar *et al.* (2022) which demonstrated the effectiveness of *A. macrospora* MKP1 against Parthenium. Singh (2020) also reported *Fusarium*, *Alternaria*, *Cephalosporium* and *Cladosporium* species as pathogens, with *F. equiseti* showing the highest defoliation. Similarly, Naseem *et al.* (2016) found *Nigrospora oryzae* to induce severe symptoms in Parthenium. Overall, these findings support the potential of *Alternaria* and other fungal pathogens as biocontrol agents in integrated weed management.

CONCLUSION

This study focused on isolating and identifying four fungal pathogens (*Alternaria alternata* and three strains of *Alternaria macrospora*) from naturally infected *Parthenium hysterophorus* plants and evaluating their pathogenic potential under laboratory conditions. Among these, *Alternaria macrospora* strain PHMMU1 showed the highest virulence, with significant leaf defoliation, establishing it as a promising bioherbicidal agent. The isolates fulfilled Koch's postulates, confirming their pathogenicity and potential for development as mycoherbicides. These results highlight the effectiveness of *Alternaria alternata* and *Alternaria macrospora*, particularly *Alternaria macrospora* (PHMMU1), as environmentally friendly alternatives to chemical or mechanical methods of controlling *Parthenium* weed. The adoption of such biocontrol agents aligns with sustainable agricultural practices and integrated weed management strategies. Further research should include large-scale field trials, development of suitable formulations and host-specificity testing to ensure safety and effectiveness. Successful implementation of these fungi as biocontrol agents can reduce the ecological and economic impacts of *Parthenium hysterophorus*, while promoting biodiversity, environmental sustainability and long-term resilience of agro-ecosystems.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

No any approval required by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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