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Research Article

Integrated Management of Tikka Disease in Groundnut Using Chemical, Botanical, and Biocontrol Approaches

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ABSTRACT

Groundnut (*Arachis hypogaea*) in Pakistan faces a major challenge from tikka disease, caused by *Cercospora arachidicola* and *Cercosporidium personatum*. The present study was undertaken to isolate the causal agents and evaluate the efficacy of fungicides and Bio-botanical protectants. Infected leaf samples were collected from the University Research Farm, Koont (Chakwal), and pathogens were isolated based upon cultural and morphological characteristics (e.g., colony color, texture, spore size and shape). Pathogenicity was confirmed by fulfilling Koch's postulates proving *Cercospora arachidicola* and *Cercosporidium personatum* to be the actual cause for tikka disease of ground nut. Following isolation, pot experiment was conducted under Completely Randomized Design (CRD) comprising nine treatments and three replications. Means were analysed statistically by applying ANOVA using Statstix 8.1 software. Varying concentrations of Mancozeb (0.1% and 0.2%), neem extract (5% and 10%), garlic extract (5% and 10%), and *Trichoderma* spp. (5 mL and 10 mL), with combined treatments of Mancozeb 0.2%, neem 10%, garlic 10%, and *Trichoderma* 10 mL. Key parameters viz., disease incidence, severity, plant height, number of leaves, fresh and dry biomass were recorded. The results revealed combined treatments effectively reducing disease incidence by 48.66% and severity by 50.59% and enhancing plant height (30.32 cm), leaf number (15), and biomass. The disease was suppressed highly using 0.2% Mancozeb, followed by moderate *Trichoderma* treatments while neem and garlic extracts showed minimum potential. The study highlighted combined biogenic inputs to manage tikka disease and improve plant growth with effectivity, sustainability, and eco-friendly strategies. Keywords: Groundnut, *Cercospora arachidicola*, *Cercosporidium personatum*, Mancozeb, *Trichoderma*, Botanicals, Biocontrol.

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INTRODUCTION

Groundnut, also known as peanut (*Arachis hypogaea* L.), is one of the most important oilseed and leguminous crops grown widely across the world. It plays a crucial role in the agricultural systems of regions like Asia and Africa, where countless smallholder farmers depend on its cultivation as a major source of income (Nigam, 2014). Due to its rich nutritional profile groundnut contributes



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significantly to human diets and also serves as a valuable ingredient in livestock feed, thereby strengthening food and economic security in developing regions (Balasubramanian et al., 2024).

Groundnut is a key cash crop in Pakistan, predominantly cultivated in the rain-fed regions of Punjab, especially in districts such as Attock, Chakwal, Mianwali, and Rawalpindi, which together account for nearly 84% of the country's groundnut production (Zamurrad et al., 2013), providing 43-55% oil content and 25-32% protein (Hussain et al., 2022). The total area under groundnut cultivation is 145.1 thousand hectares, resulting in a production of 133.3 thousand tonnes (Crop Report, 2023). Despite its cultivation and production, Pakistan is importing raw peanuts worth US\$2.65 million from Nigeria (Trading Economics, 2023). This insufficiency is largely driven by various abiotic stresses and biotic factors that act as major constraints, significantly hampering crop productivity and profitability. Among various biotic and abiotic stresses, diseases viz., tikka leaf spot, *Fusarium* wilt, *Alternaria* leaf spot, stem rot, rust and crown rot have been reported as key constraint in groundnut cultivation. Out of all these diseases, tikka (Ascomycota: Dothideomycetes, Capnodiales, *Mycosphaerellaceae*) disease of groundnut is the most destructive disease attacking 1-2 months old plants causing a yield loss (up to 30-50%) by spots and reduction in pod yield by 29% (Faisal and Tiwari, 2015).

Under field condition, disease is diagnosed as early leaf spot (*Cercospora arachidicola*) with subcircular-irregular, 1-10 mm diam. reddish, dark brown to black coloured lesion with yellow halo on upper and lower surface of leaves. Whereas, late leaf spot (*Phaeoisariopsis personata*) is diagnosed as smaller, 1-6 mm diameter, dark grey/black spots on lower leaf surface (Dutta et al., 2021). Several, conventional management strategies, including cultural practices such as removal of crop debris, elimination of volunteer plants, crop rotation, and early planting, have been commonly used to control tikka disease (Niwas et al., 2021). Meanwhile, these methods are time consuming and slow in providing effective outcomes. However, there is urgent need to find and embrace more specific and better solutions to control the disease and ensure the proper production of groundnut.

MATERIALS AND METHODS

Potato Dextrose Agar (PDA) was used for the isolation of fungal pathogen (Moosa et al., 2020; Syamsia et al., 2021). Infected leaf samples showing characteristic symptoms were used for the isolation of the pathogen. The samples were washed under running tap water to remove debris and then air-dried. Infected portions, along with some healthy portion, were carefully cut and surface-sterilized by dipping in sodium hypochlorite (NaOCl), followed by washing twice with sterile distilled water to remove any residual disinfectant and debris. The disinfected tissue pieces were placed on the PDA plates. The plates were then sealed and incubated at $27 \pm 2^\circ\text{C}$ for 5–7 days (Srinivas et al., 2024).

Morphological Characterization

Morphological identification of the pathogens was carried out using pure cultures grown on PDA after 7-10 days of incubation. For microscopic examination, a small amount of fungal spores was mixed in sterile distilled water on a clean glass slide, gently covered with a cover slip, and observed under a compound microscope at magnifications ranging from 10X to 100X. Microscopic characteristics such as size, conidial shape, conidiophore structure, and mycelial nature were examined to help in the identification. *Cercosporidium personatum* were smaller and cylindrical conidia with heavy sporulation whereas *Cercospora arachidicola* developed elongated and septate conidia. The morphological observations identified the isolates as the causal organism of tikka leaf spot in ground nut (Sundaramoorthy and Ivin., 2023).

Pathogenicity Test

For the confirmation of the pathogenicity of the fungus, healthy groundnut plants at the stage of 4–6 leaf, were inoculated with spore suspensions of *Cercospora arachidicola* and *Cercosporidium personatum*, prepared from 7–10 days old cultures grown on potato dextrose agar (PDA). Fungal spores were scraped from the culture surface using a sterile cork borer, added in sterile distilled water, and filtered through muslin cloth to remove any mycelial debris. The spore concentration was then adjusted to $1 \times 10^4 - 1 \times 10^5$ spores/mL using a hemocytometer, by adding 0.01% Tween-20 to enhance spore adhesion and uniform distribution.

The prepared spore suspension was uniformly sprayed onto healthy groundnut plants using a hand sprayer until runoff, ensuring complete coverage of the foliage. Control plants were treated with sterile distilled water under the same conditions. To facilitate infection, inoculated plants were enclosed in transparent plastic bags for 48 hours to maintain high humidity. Thereafter, the plants were transferred to a greenhouse maintained at $25-28^\circ\text{C}$ with relative humidity exceeding 80%. Typical disease symptoms began to appear within 10–14 days of inoculation. To confirm the involvement of the inoculated fungi, the pathogens were successfully re-isolated from the symptomatic tissues, thereby

fulfilling Koch's postulates and confirming their pathogenicity to be the actual cause of tikka disease (Chen et al., 2024).

Application and Evaluation of Pot Treatments

The experiment was carried out at Plant Pathology Department in PMAS-Arid Agriculture University during the groundnut growing season. A pot experiment was performed under controlled environmental conditions to examine the efficacy of different disease management approaches against tikka disease. Naturally involved plant extracts, biocontrol agents, and synthetic chemical treatments, were tested to determine their ability to decrease the disease incidence and severity under uniform conditions (Akinbode, 2010). The experiment was designed under Completely Randomized Design (CRD) which involved 27 pots with eight treatments together with three replications of each treatment to provide statistical validity against experimental basis. The treatments were randomly assigned to each pot to enhance uniformity and enable the assessment of the treatment effectiveness in managing the disease. The spore suspensions were prepared and sprayed gently on all the experimental plants with a hand-held atomizer until runoff, to cover the surfaces of the leaves well. After inoculation, the plants were placed under high humid environment (>80%) by covering them with transparent polyethylene bags to ensure infection after 48h. Following the appearance of characteristic symptoms of tikka disease (typically within 7–10 days), respective treatments were applied as per the experimental layout.

Table 1. Treatments for management of tikka leaf spot disease.

Treatment	Description
T ₁	Fungicide (Mancozeb 0.1%)
T ₂	Fungicide (Mancozeb 0.2%)
T ₃	Neem Extract (5%)
T ₄	Neem Extract (10%)
T ₅	Garlic Extract (5%)
T ₆	Garlic Extract (10%)
T ₇	<i>Trichoderma</i> (5 mL)
T ₈	<i>Trichoderma</i> (10 mL)
T ₉	Combination of Mancozeb (0.2%) + Garlic Extract (10%) + Neem Extract (10%) + <i>Trichoderma</i> 10ml)
T _c	Control Only

A susceptible groundnut variety was selected for the study to accurately evaluate the effectiveness of different disease management strategies. Healthy, certified seeds were sourced from the National Agricultural Research Centre (NARC) to ensure genetic uniformity and high germination potential. In each pot, three seeds were sown at a depth of 3–5 cm in sterilized soil to promote uniform germination and minimize external variability.

Extracts of neem and garlic plants were also tested during the trial to determine their effectiveness against tikka disease in groundnut.

Fresh leaves of neem (*Azadirachta indica*) were collected from field and washed with tap water to remove surface impurities and then crushed in mortar and pestle to make a paste. The resulting paste was diluted using distilled water and filtered with muslin cloth in 1:10 (w/v) ratio in order to obtain a clear extract. The neem extract was later used as a foliar spray on groundnut plants at a concentration of 5% and 10% to assess its effectiveness against tikka disease.

Garlic (*Allium sativum*) was washed and crushed and mixed with distilled water at a ratio of 1:10 (w/v). Then the mixture was filtered through muslin cloth to get clear extract. To evaluate the efficacy of this garlic extract (Gr Ext) in the control of tikka disease, it was sprayed as a foliar spray of 5% and 10% percent on experimental groundnut plants.

A pure culture of *Trichoderma* spp. was obtained from the Plant Pathology Department, PMAS-Arid Agriculture University. The fungus was mass-produced on Potato Dextrose Agar (PDA) medium under sterile conditions. After sufficient growth, a spore suspension (liquid culture in distilled water) of *Trichoderma* (Trich) was prepared and applied as a foliar spray on groundnut plants at concentrations of 5 mL and 10 mL to evaluate its efficacy against tikka disease.

In the chemical treatment, the fungicide Mancozeb (Mnz) was selected for evaluation due to its broad-spectrum antifungal activity. It was prepared at concentrations of 0.1% and 0.2% according to the manufacturer's guidelines and applied as a foliar spray using a hand sprayer to the groundnut plants.

The first application of treatments was carried out 24 hours prior to pathogen inoculation to allow for protective action against infection. Two additional foliar sprays were applied at 10-day intervals to enhance and maintain disease suppression throughout the experimental period.

The percentage of plants exhibiting tikka disease symptoms was recorded and the incidence calculated by formula:

Disease Incidence (%) = Number of infected plants/ total Number of observed plants x100

Disease severity was assessed using a 0–9 rating scale based on the percentage of leaf area infected. This scale was adopted as described by Azmat et al., (2012), commonly used for visually estimating plant disease severity.

To assess the impact of disease and treatments on plant growth, the Plant height (cm), Number of leaves per plant and Fresh and dry biomass (g) parameters were recorded:

The treatment efficacy in disease suppression was calculated using the following formula:

Efficacy (%) = Disease in control-Disease in treatment /Disease in Control x100

The data collected from each treatment were subjected to Analysis of Variance (ANOVA) using Statistix 8.1 software.

Treatment means were compared using the Least Significant Difference (LSD) test at a 5% probability level ($p \leq 0.05$) to determine statistically significant differences among the treatments.

Statistical Analysis

The data was analysed in STATISTIX 8.1 using ANOVA followed by LSD to compare means of the treatments. Significance of the data was determined by $p \leq 0.05$ and the result was presented as mean standard error with letter groups indicating statistical differences.

RESULTS AND DISCUSSION

Symptomatic groundnut (*Arachis hypogaea*) leaves showing typical tikka disease lesions were collected from the University Research Farm, Koont. Infected tissues were surface sterilized and cultured on Potato Dextrose Agar (PDA). After 5–7 days of incubation at room temperature, slow-growing, circular colonies with dark brown to black pigmentation developed. Pure cultures were obtained using the hyphal tip method.

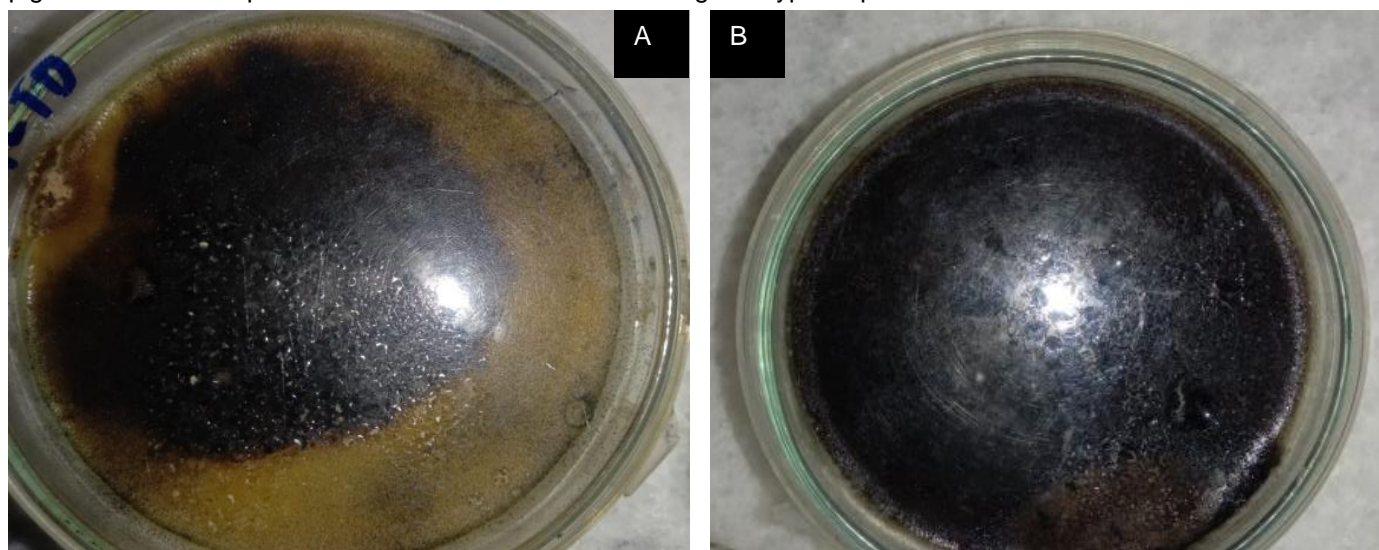


Figure 1. Pure cultures of tikka disease pathogens on PDA: (A) colony growth after 4 days showing initial development, and (B) full plate coverage with dark pigmentation after 10 days of incubation.

Colony characteristics and microscopic features were assessed to identify the pathogens. Fungal spores were scraped from PDA cultures, suspended in sterile distilled water, and examined under a compound microscope (10x to 100x) using temporary slides. The conidiophores were brown to dark brown, septate, and arose singly or in fascicles, measuring 30–100 μm in length and 3–5 μm in width. Conidia were slender, hyaline, cylindrical to slightly curved, 35–90 μm long, 2.5–4.5 μm wide, possessed four to eight septa. According to goirdano et al., (2021) “Tikka” disease, greatly caused by *Passalora arachidicola* (Hori) U. Braun (syn. *Cercospora arachidicola* Hori) and *Nothopassalora personata* (Berk. & M.A. Curtis) U. Braun, C. Nakash, Videira & Crous (syn. *Cercosporidium personatum* (Berk. & M.A. Curtis) Deighton), was distinguished by its lighter colonies and larger, fewer-septate conidia, while *N. personata*, the late leaf spot pathogen, exhibited darker colonies and more septate conidia. These identifications were consistent with reports by Sundaramoorthy, and Ivin, (2023).

The pot experiment was conducted under controlled conditions to evaluate the efficacy of various treatments against tikka disease in groundnut. All plants were uniformly inoculated with the pathogen, and respective treatments were applied according to the experimental design. The visual response of plants to each treatment is shown in Figure 2.



Figure 2. Experimental layout of groundnut plants inoculated with tikka pathogens under treatments (A) Mancozeb 0.1% (B) Mancozeb 0.2% (C) Neem Extract 5% (D): Neem Extract 10% (E) Garlic Extract 5% (F) Garlic Extract 10% (G) Trichoderma 5 mL (H) Trichoderma 10 mL (I) Mancozeb 0.2% +Garlic 10%+Neem 10%+Trichoderma 10 mL and (J) Inoculated control without treatment.

Treatment effects on tikka disease and plant growth parameters in groundnut

Various treatment effects on tikka disease and plant growth parameters in groundnut were assessed systematically shown in table 2.

Table 2. Summary of treatment effects on disease and growth parameters in groundnut.

Treatments	DI (%)	DS (%)	Plant Height (cm)	No. of Leaves	Fresh Biomass (g)	Dry Biomass (g)
Mancozeb 0.1% (T1)	57.23	53.29	28.70	11.00	15.95	09.67
Mancozeb 0.2% (T2)	54.49	56.44	26.53	12.00	15.83	08.68
Neem 5% (T3)	72.84	63.47	16.67	07.00	10.43	05.50
Neem 10% (T4)	66.41	63.83	16.17	08.00	11.36	06.85
Garlic 5% (T5)	65.90	65.34	17.00	07.00	09.23	05.45
Garlic 10% (T6)	67.48	68.55	17.65	07.00	09.41	05.30
Trichoderma 5 mL (T7)	64.96	60.49	21.67	10.00	14.90	08.37
Trichoderma 10 mL (T8)	65.13	60.84	20.33	10.00	14.23	07.98
Mnz 0.2% +Gr 10%+Neem 10%+ Trich 10% (T9)	48.66	50.59	30.32	15.00	19.03	10.46
Control Tc	83.49	80.23	10.17	05.00	06.40	04.22

The detailed findings are presented as under.

Comparative analysis of disease incidence and severity among treatments

Treatment effects on tikka disease and plant growth parameters in groundnut were observed showing variations in plant response to chemical, botanical, and biological treatments. The data was compared with the control values presenting the highest DI (83.49%) and DS (80.23%) values as described in figure 3. The combined application of *Mancozeb 0.2% + Garlic 10% + Neem 10% + Trichoderma 10%* showed the highest efficacy, resulting in the lowest disease incidence (48.66%) and disease severity (50.59%), indicating superior protection against the pathogen. These findings align with those of Mapari and Zacharia (2017), who also reported a notable reduction in disease severity when combining chemical and biological agents against leaf spot pathogens.

While, among individual treatments, *Mnz 0.2%* was the most effective with DI of 54.49% and DS of 56.44%, followed closely by *Mnz 0.1% (T1)* with DI of 57.23% and DS of 53.29%. The superior performance of *Mnz* corroborates earlier studies that recognized its potent fungicidal properties for managing foliar diseases (Mapari and Zacharia, 2017). Similarly, *Trich 5 mL* and *10 mL* also provided considerable disease reduction, with DI ranging from 64.96% to 65.13% and DS between 60.49% and 60.84%, showing moderate efficacy. This is consistent with previous research by Lee et al. (2019) and Kim (2021), which highlighted *Trichoderma's* capacity to suppress fungal pathogens and enhance plant immunity. Garlic and Neem ext at both 5% and 10% concentrations exhibited comparatively higher disease levels, with DI values ranging from 65.90% to 72.84% and DS values from 63.47% to 68.55%, indicating lower effectiveness and support the findings of Kumar (2017) and Zeng et al. (2019), who reported moderate antifungal activity of Garlic and Neem extracts.

In contrast, the control treatment recorded the highest disease incidence (83.49%) and severity (80.23%), confirming the significant impact of biocontrol and chemical treatments in suppressing disease. Analysis of variance (ANOVA) also revealed significant differences ($P < 0.05$) among treatments in reducing disease incidence (DI) and disease severity (DS).

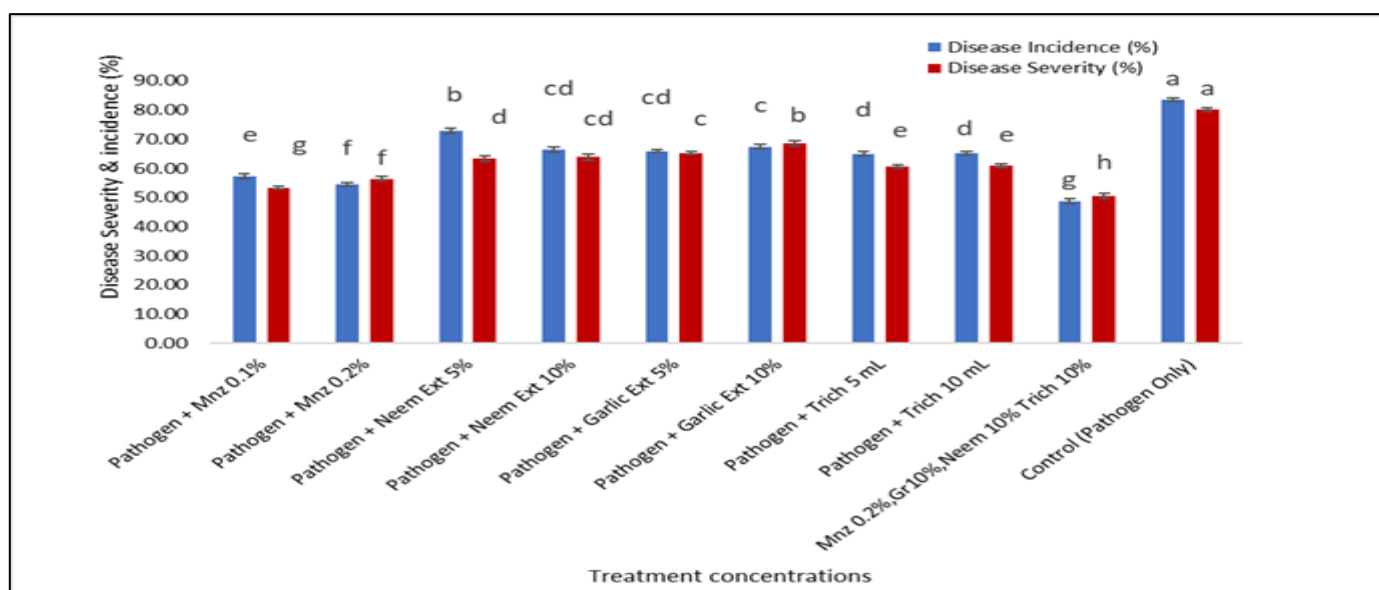


Figure 3. Disease incidence and severity across treatments.

Differential plant height response to treatments

Treatment effects on tikka disease and plant growth parameters in groundnut were observed showing variations in plant response to chemical, botanical, and biological treatments. As shown in Figure 4, significant differences in plant height were observed among treatments applied to pathogen-inoculated groundnut plants. The tallest plants (30.32 cm) were observed in the combined treatment group (*Mnz 0.2% + Gr 10% + Neem 10% + Trich 10%*), indicating a strong correlation between disease suppression and enhanced growth. These findings are similar to the work done by Mapari and Zacharia (2017), where the combined application of fungicides and biocontrol showed a significant increase in plant height, highlighting the advantages of integrated disease management strategies. *Mnz 0.1%* also supported relatively good plant height (28.70 cm), followed by *Mancozeb 0.2%* at 26.53 cm. These results are

supported by the findings of Devi, (2015). Furthermore, the moderate increase in plant height (21.67cm) with *Trichoderma* treatments aligns with the earlier findings of Chen et al., (2025), who reported that *Trichoderma* enhances plant growth through mechanisms such as auxin production and improved nutrient uptake. while Neem and Gr treatments recorded comparatively shorter plants (16.17–17.65 cm). The control group exhibited the least plant height (10.17 cm), likely due to severe disease stress. These findings align with the results of Kumar (2017) indicating effectiveness of neem and the findings of Zeng et al., (2019) indicating the efficacy of garlic.

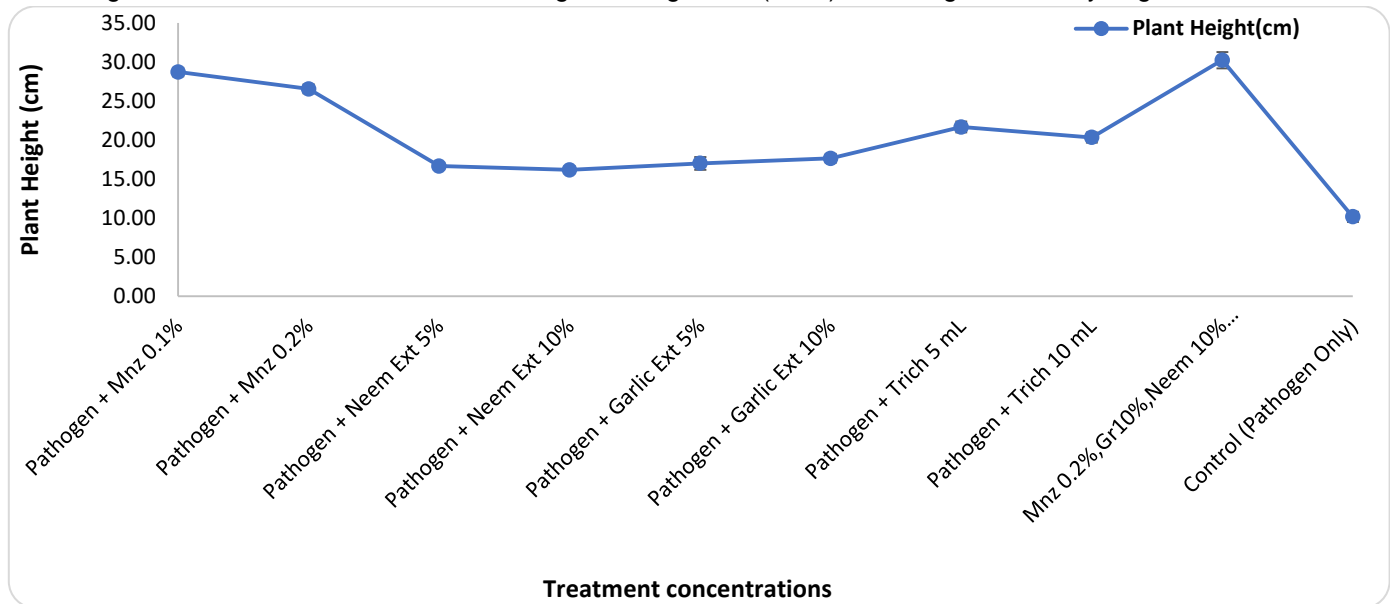


Figure 4. Plant height across treatments.

Comparative analysis of leaf count across treatments

Treatment effects on tikka disease and plant growth parameters in groundnut were observed showing variations in plant response to chemical, botanical, and biological treatments. As depicted in Figure 5, the number of leaves varied notably across treatments, indicating the influence of disease management strategies on plant vigor and foliage development. The maximum number of leaves (15) was produced under the combined treatment, reflecting overall plant health and vigor. These findings were also reported by Kumar et al., (2017). Similarly, *Mnz* 0.2% (T2) and *Mnz* (0.1%) also enhanced foliage with 12 and 11 leaves, respectively. *Trich* treatments (T7 & T8) followed with 10 leaves each. Lower leaf counts (7–8) were observed under *Neem* and *Garlic* treatments, while the control had the lowest leaf count (5). These findings are consistent with those of Smith et al. (2020), who reported that effective disease control, particularly with chemical and integrated treatments. The growth-promoting effects of *Trichoderma* observed in the present study also align with the results of Lee et al. (2019) and Kim (2021), who highlighted its role in inducing systemic resistance and improving nutrient uptake.

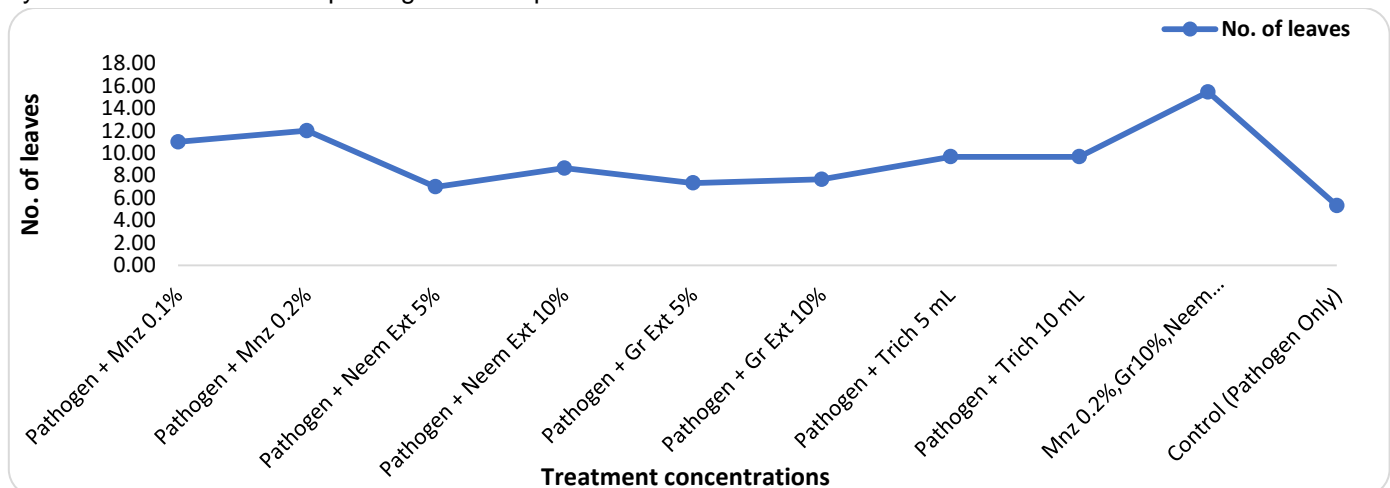


Figure 5. Number of leaves across treatments.

Effect of experimental treatments on plant biomass

Treatment effects on tikka disease and plant growth parameters in groundnut were observed showing variations in plant response to chemical, botanical, and biological treatments. As illustrated in Figure 6, fresh and dry biomass accumulation in groundnut plants varied significantly among treatments, reflecting the impact of disease management strategies on overall plant development.

Fresh biomass was significantly higher in the combined treatment (19.03 g), indicating better plant development. This was followed by *Mnz* 0.1% (15.95 g) and *Mnz* 0.2% (15.83 g). *Trichoderma* treatments also performed well (14.90–14.23 g), while Garlic and Neem treatments produced lower biomass values (9.23–11.36 g). The control again had the least fresh biomass (6.40 g).

The highest dry biomass (10.46 g) was recorded in the combined treatment, confirming improved plant health and resource allocation. *Mnz* 0.1% and *Trich* 5 mL also resulted in relatively high dry biomass (9.67 g and 8.37 g, respectively). *Mnz* 0.2%, and other botanicals showed moderate to low dry matter accumulation, with the control registering the lowest dry biomass (4.22 g).

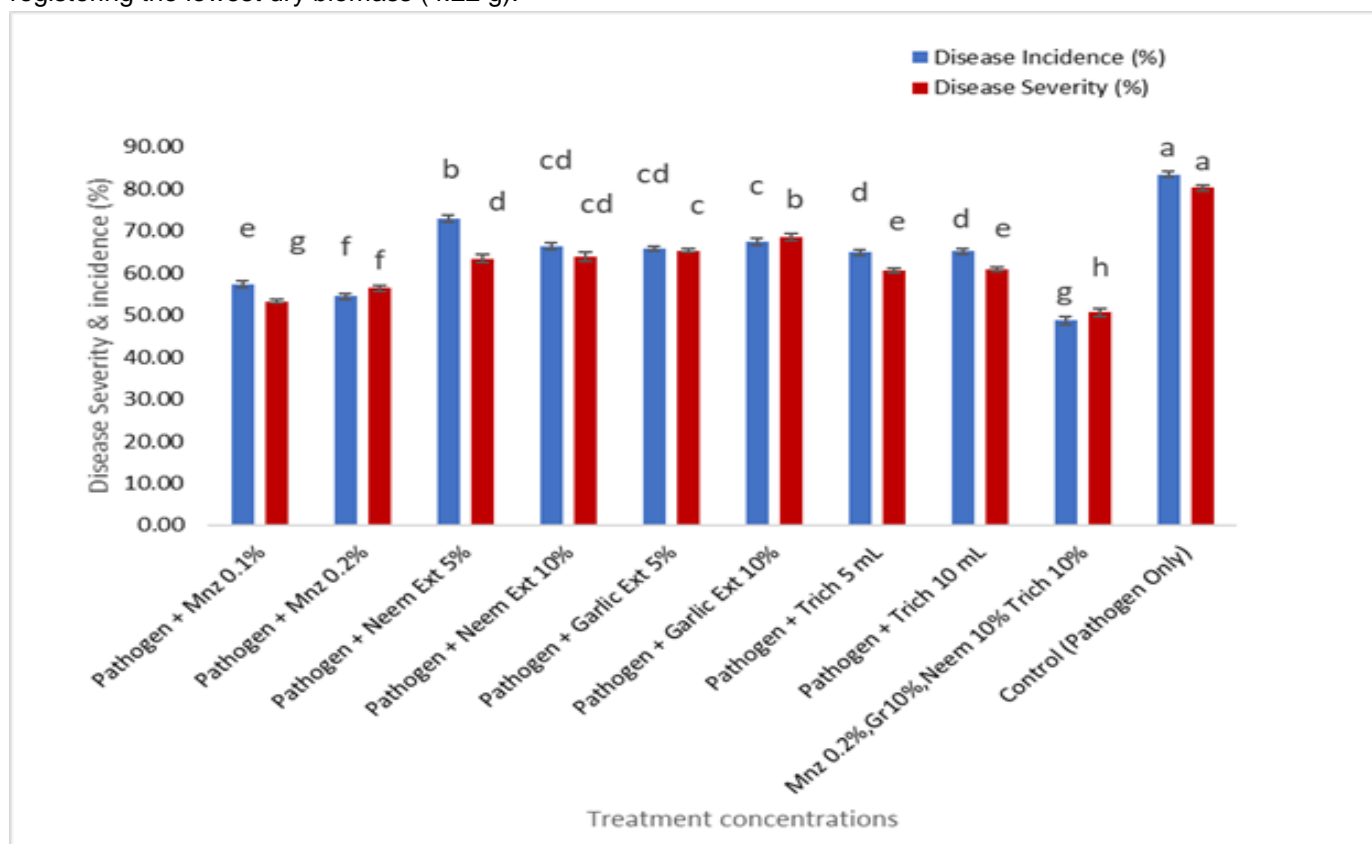


Figure 6. Plant biomass across treatments.

CONCLUSION

Groundnut as an important cash crop in rainfed regions, Khyber Pakhtunkhwa and irrigated regions of Sindh in Pakistan plays vital role in food security and rural livelihood. Groundnut remains significant despite challenges like tikka leafspot disease climate variability and limited access to improved varieties. This study highlights the effectiveness of various treatments in managing tikka disease of groundnut and improving plant growth. The combined application of Mancozeb 0.2%, Neem extract, Garlic extract, and *Trichoderma* emerged as the most effective strategy, significantly reducing disease incidence and severity while enhancing plant height, number of leaves, and biomass. This study highlights that integrating chemical, botanical, and biological agents, particularly the combination of Mancozeb with neem, garlic, and *Trichoderma*, provides an effective, sustainable, and eco-friendly strategy for managing tikka disease in groundnuts while promoting plant growth. Among individual treatments, Mancozeb 0.2% performed the best, showing strong fungicidal activity. *Trichoderma* also gave promising results by moderately reducing disease and supporting plant growth. Although Neem and Garlic extracts were less effective alone, they still played a role in disease reduction, indicating their potential as natural disease management tools.

AUTHOR CONTRIBUTIONS

The study, led by Muhammad Nawaz, a postgraduate scholar, benefited from the scholarly expertise of Prof. Dr. M. Inam-ul-Haq, renowned for his significant contributions. The experiments were conducted, and the data were analysed by Syed Zulfiqar, Dr. Gulshan Irshad, Dr. Muhammad Naveed Tahir. The manuscript underwent a rigorous review process by esteemed mentors Prof. Dr. M. Inam-ul-Haq and Dr. Gulshan Irshad, whose intellectual input greatly enhanced its overall quality. Mehak Zahra, Atta ul Rehman, Hurmain Aslam and Laiba Zarar Noor collaborated in the experimental setup and actively assisted with all activities during the experiments.

COMPETING OF INTEREST

The authors declare no competing interests.

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