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RESEARCH ARTICLE

Seed Priming with Plant Extracts Gives Enhanced Resistance Against *Alternaria Solani* in Tomato

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A B S T R A C T

Alternaria solani, the pathogen responsible for early blight disease, poses a severe threat to agricultural crops all over the world. In the quest for sustainable and environment friendly alternatives to chemical fungicides, this study investigates the antifungal potential of extracts derived from five plant species: *Azadirachta indica* (neem), *Citrullus colocynthis* (bitter apple), *Allium sativum* (garlic), *Mentha piperita* (peppermint), and *Moringa oleifera* (moringa) against *Alternaria solani in-vitro* and under natural conditions in a pot experiment. Our results demonstrated varying degrees of antifungal activity among the extracts, with neem and bitter apple displaying the most potent effects against *Alternaria solani* in an *in vitro* experiment using poisoned food technique. In another experiment, tomato seeds were primed with these plant extracts by dipping in the extract dilutions for 24 hours. Tomato plants grown with the primed seeds were screened for the development of early blight disease. Again, neem and bitter apple showed enhanced resistance with the least development of disease in tomato, as compared to the control and other extracts. These findings suggest that plant extracts, particularly neem and bitter apple, hold promise as eco-friendly alternatives to chemical fungicides for managing early blight disease. Further investigations are essential to elucidate the mechanisms underpinning the antifungal properties of these extracts and explore their practical applications in agriculture. This study contributes valuable insights into utilizing natural resources for sustainable disease management, aligning with the increasing emphasis on environmentally conscious agricultural practices.

Keywords: Antifungal activity, *Alternaria solani*, plant extracts, phytochemical analysis, sustainable agriculture.

INTRODUCTION

Tomato (*Solanum lycopersicum*) is the second most significant vegetable everywhere throughout the world (Nasir *et al.,* 2023). In Pakistan, significant tomato diseases include damping off, seed rot, early and late blight, leaf spots, anthracnose and root rot (Dellavalle *et al.,* 2011). Early blight disease in tomato is caused by the fungus *Alternaria solani* (phylum *Ascomycota*, family *Pleosporaceae*) (Usman *et al.,* 2023; Ahmad *et al.,* 2024). This disease is common in tomato growing areas. The disease has also caused huge losses in potato, chili, and eggplant. Early blight is common in the early season and in zones that receive heavy rainfall, high humidity and fairly high temperatures 24°-29°C, there are more chances of sever attack. Symptoms are more prominent in case of high humidity and favorable conditions. Symptoms begin with the spots formed from the tip of the leaf and the border of the petiole. In case of severe attack blight of leaves occurs when lesions extend and coalesce with each other. *Alternaria solani* causes the disease symptoms on stem, leaf and fruits. In case of

severe infection, all plants are damaged, and the plants may die at early stage (Abada *et al.,* 2008).

Among these type of diseases, early blight of tomato is one of the most significant and destructive diseases in the country. There are many types of diseases of tomato. Among them, the most common disease of tomato is early blight. The main reason of this disease is the necrotrophic fungus *Alternaria solani* Ellis and Martin is one of the very important foliar diseases of tomato. This disease is one of the most common tomato disease, which occurs nearly every season wherever tomatoes are grown, but it is quite eminent in the areas with bedew, and also depends on the rainfall and moisture ratio in the atmosphere (Roy *et al.,* 2019).

The disease symptoms appear on stems, leaves and fruits of tomato. Small and dark lesions appear on the leaves of tomato plant. Necrotic and concentric rings on leaves give a target like appearance on the surface of leaf. Lesions appeared on leaf surface are bound by yellow rings. Older leaves are firstly infected by *A. solani* followed by stems, petioles, twigs and fruits of tomato plant. Control of this disease is very difficult due to wide host range and active and prolonged life cycle of *A. solani* (Shoaib *et al.,* 2019). It affects leaves, fruits and stems and can be severely limiting the yield when susceptible cultivars are used and weather is favorable. Severe defoliation can occur and result in sunscald on the fruit (Chaerani and Voorrips, 2006).

Biological control is the technique in which pathogen is controlled with other living organisms and these organisms are known as biocontrol agents. Biocontrol agents have tendency to reduce the pathogen population. In many studies, it is observed that different types of biocontrol agents can suppress fungal growth. Bacterial bio-control agents belonging to the genera *Agrobacterium, Bacillus, Pseudomonas,* and *Streptomyces* have been found by observing zones of inhibition in petri plates (Larkin *et al.,* 1998). The data pertaining in-vitro evaluation of biocontrol agents against pathogen *A. solani* causing early blight of cherry tomato presented *Trichoderma harzianum* as the most effective biocontrol agent with statistically significant least mycelial growth (13.33mm) and highest mycelial growth inhibition of 85.13% against *Alternaria solani* followed by *T. viride* with mycelial growth inhibition of 80.67% (Naik *et al.,* 2020).

Biocontrol using plant extracts has emerged as promising alternatives to synthetic pesticides in managing plant diseases. Natural compounds, derived from various plant parts, contain bioactive substances that can inhibit or suppress pathogenic microorganisms. The use of plant extracts in plant pathology has gained attention due to their potential effectiveness, eco-friendly nature, and reduced risk of pathogen resistance. Researchers have identified numerous plant species with antimicrobial properties, including neem, garlic, and eucalyptus. As the demand for sustainable agricultural practices grows, plant extracts offer a promising avenue for developing novel disease management strategies in crop protection. Different plant extracts also have ability to inhibit the sporulation and fungal growth. Concentration of solution, plant extraction method, plant part, age of plant and storage conditions may be accountable factors. These all factors can disturb the activity of secondary metabolites. Studies of plant extracts show that working activity of disease suppression by using plant extract may be due to secondary metabolite found in them (Ayoola *et al.,* 2008).

Effect of five plant extracts *Azadirachta indica*, *Allium sativum*, *Parthenium hysterophorus*, *Datura stramonium* and *Eucalyptus camaldulensis* at three concentrations (5, 10 and 15%) was assessed through the poisoned food method in-vitro for their suppressive effect on the development of mycelial growth of *Alternaria solani.* Efficacy of different aqueous plant extracts from numerous plant species and families was also tested against pathogen growth (Zaker *et a1*., 2010). In particular, the plant species *Azadirachta indica* (neem), *Citrullus colocynthis* (bitter apple), *Allium sativum* (garlic), *Mentha piperita* (peppermint), and *Moringa oleifera* (moringa) have gained attention for their diverse phytochemical compositions and documented antimicrobial properties (Nascimento *et al.,* 2000; Cowan, 1999; Fahey *et al.,* 2001).

Azadirachta indica, commonly found in tropical and subtropical regions, is renowned for its broad spectrum of biological activities, including antifungal properties attributed to compounds such as *azadirachtin* and nimbin (Isman, 2006; Isman and Grieneisen, 2014). *Citrullus colocynthis*, a desert plant, is known for its bitter fruits and has been used traditionally in medicine (Cheng *et al.,* 2023). *Allium sativum*, or garlic is recognized worldwide for its numerous health benefits,

including potent antifungal properties linked to its sulfur-containing compounds like allicin (Ankri and Mirelman, 1999). *Mentha piperita*, or peppermint, is a widely cultivated herb known for its aromatic and medicinal qualities, with essential oils that exhibit antimicrobial activities (Nascimento *et al.,* 2000). *Moringa oleifera*, also known as the drumstick tree, is valued for its nutritional and medicinal properties, and it contains bioactive compounds such as flavonoids and glucosinolates, which have shown antifungal potential (Fahey *et al.,* 2001).

Pre-sowing treatment of seeds for their better germination and vigor is known as seed priming. It involves the soaking of seeds to hydrate them to a point where germination processes start but emergence of radical remains inhibited. Then, followed by re-drying the seeds for storage until their plantation. Priming not only enhances speed of germination but ensures the uniformity of the process as well. Increased seed tolerance to adverse environmental conditions is an added advantage (Harris *et al.,* 1999). It stimulates various biochemical changes in the seed anatomy, contributing towards breaking dormancy, mobilization of seed reserves, enzymatic activity, and the emergence of embryonic tissues (Asgedom and Becker, 2001; Çatav *et al.,* 2012). Root rot infection of *Fusarium spp*., *Rhizoctonia solani* and *Macrophomina phaseolina* in peanut, okra, sunflower and chickpea was significantly reduced when seeds were primed with *Acacia nilotica* and *Sapindus mukorossi* parts. It has various uses such as improvement of seedling vigor, enhanced germination, biotic and abiotic stress tolerance, early maturity, and yield improvement.

A few crucial agronomic techniques are used in seed priming to guarantee that seeds are ready for planting, which improves germination, seedling vigor, and crop performance in general. Firstly, the process starts with selecting the high-quality, disease-free seeds and cleaning them to remove debris. A variety of techniques, including hydro-priming, osmo-priming, halo-priming, hormonal priming, bio-priming, and matrix priming are used to soak seeds for varying lengths of time at the appropriate temperature (usually between 15 and 25°C) and with sufficient aeration (Rahman *et al.,* 2020; Koushal *et al.,* 2024). Primed seeds are sown in the field at the suggested time, and because of their increased germination rates, the seeding rate can be adjusted as needed. Proper planting depth and spacing are also followed. Proper crop establishment and growth is fostered by ongoing monitoring, which includes germination tests and field observations to ensure priming's efficiency.

This study aims to explore the antifungal potential of extracts derived from these five plant species against *Alternaria solani in-vitro* and in pot experiment using seed priming method. By investigating these plant extracts, we aspire to contribute to the development of sustainable and eco-friendly strategies for managing early blight in agricultural settings. Furthermore, understanding the phytochemical basis of their antifungal activity will shed light on the mechanisms underlying their efficacy. This research is not only relevant for agricultural and horticultural industries but also holds promise for promoting safer and more sustainable practices in the fight against *Alternaria solani*, ultimately benefiting food security and the environment. In the subsequent sections of this paper, we will delve into the methodologies employed for extract preparation, dilution and inoculation protocol, the results of our antifungal assays, and discussion of implications of our findings for the control of *Alternaria solani*.

MATERIALS AND METHODS

Extract Preparation: Five plant extracts were used in this study, which are neem, moringa, bitter apple, garlic bulb and mint. Plant parts were collected from UAF Sub-Campus Burewala and the city Burewala and geographical coordinates (latitude 30° 12' 53.00" N and longitude 72° 42' 42.37" E) were recorded using GPS map location. Plant parts were dried in shady place for a week. After drying, all material was ground into fine powder with the help of grinder. Extracts were prepared by the weight/volume method in flasks. One-gram powder was dissolved in 10 ml of distilled water. Flasks were kept shaking with vibratory shaker for 24 hours. After shaking incubation, the liquid was filtered by filter paper and stored in sterile plastic bottles. In this way, 10 % stock solution was prepared for each extract. Extracts were further diluted up to dilutions given in table 1.

Media Amendment with Plant Extract: Sterilized PDA media of 90 ml was taken in conical flask and added in it the 10 ml (v/v) of each plant extract. Separate flasks were prepared for each plant extract and were sterilized. All flasks were placed in laminar flow hood after autoclaving and media was poured in autoclaved petri

plates. All plates were labelled according to plant extracts. This technique of media amendment with plant extract is known as poisoned food technique.

Pathogen Inoculation *in vitro:* After cooling and solidification of media, took the 9 mm disk with sterile borer from 7-day old pathogen culture. Disks of 7-day old fungal culture were placed in the center of petri plates having different plant extracts and covered the petri plates with para-film. Three replications of each plant extract were prepared. For the control treatment, plant extract was not added in a PDA plate. All these activities were carried out in aseptic conditions by using the laminar flow hood. All the petri plates were placed in incubator at ± 28°C for 2 to 5 days. Fungal growth was observed by calculating its diameter on petri plates, after 24 hours, 48 hours, 72 hours, and last data was collected after 7 days.

Disease Inhibition: To record the growth inhibition % of *Alternaria solani* against various plant extracts, it was cultured on a potato dextrose agar media (PDA) along with plant extracts using *in vitro* poisoned food technique in petri plates of 9 cm. Total six treatments were applied including neem, bitter apple, garlic, moringa, mint, and distilled water as a control. 10 ml of each plant extract was mixed with PDA except control treatment. Disc of 9mm of *Alternaria solani* was shifted from pure culture to each treated media. Cultures were incubated at 26±2 ^{0}C for seven days in incubator. Radial growth was noted after seven days of incubation using length measuring scale (cm). Growth inhibition %age was calculated by using the following formula given by (Taskeen *et al.,* 2011).

$$\text{Growth inhibition \%} = \frac{(C-T)}{C} \times 100$$

C= Control radial growth *in vitro*

T= Treatment radial growth *in vitro*

Seed Priming with Plant Extracts: Tomato seeds of a susceptible line were received from National Agricultural Research Centre (NARC), Islamabad. All the seeds were soaked in plant extracts for 24 hours with required concentration. Seeds were kept in separate beaker. 10 to 15 seeds were taken and soaked in required concentration of plant extracts given in table 1.

Nursery Raising and Transplanting: After soaking the seeds in plant extracts with specific concentration, nursery was established in Horticulture Research Farm, UAF Sub-Campus Burewala. The dimension of each plot was 1 x 1 sq. foot. Six plots were prepared; five plots for seeds soaked in plant extracts (neem, bitter apple, garlic, moringa and mint) and one plot for seeds soaked in distilled water as a control. A thin layer of compost and wood powder was spread over the nursery bed. All the plots were watered with shower and plots were covered with polythene sheet for 3 weeks. After 3 weeks, polythene sheet was removed. Plants were transplanted into pots (30 cm height and 30 cm diameter). These pots were filled with mixture of soil, sand and compost by the ratio1:1:1. After transplanting, pots were placed in shade.

Pathogen Inoculation: Inoculum of the early blight pathogen *Alternaria solani* was cultured on PDA media for 10-12 days at room temperature. Conidia were collected by flooding the petri dish with distilled water (10 to 20 ml). Conidia concentration was known using hemocytometer (1x10^5 spores/ml). The fungal inoculum was further diluted with 300 ml distilled water for inoculation on the potted plants. Fungal suspension was collected in sterile bottle with plastic shower. Inoculum was sprayed on all 20-30 days old plants twice a day, early in the morning and in evening time when temperature is low, and humidity is high to assure the sporulation of pathogen. Data was recorded according to the disease rating scale published by Rahmatzai *et al.,* 2017.

Table 1. Plants used for extracts preparation

Sr. No.	English Name	Scientific Name	Local Name	Family	Used Part	Used dilution %age	Reference
1	Bitter apple	*Citrullus colocynthis*	Kour Tuma	Cucurbitaceae	fruit	5%	Ponsanker *et al.,* 2023
2	Neem	*Azadirachta indica*	Neem	*Meliaceae*	leaf	4%	Subhani *et al.,* 2014
3	Garlic	*Allium sativum*	Lahsan	Alliaceae	bulb	3%	Subhani *et al.,* 2014
4	Moringa	*Moringa oleifera*	Sohanjna	Moringaceae	leaf	3%	-
5	Mint	*Mentha spicata*	Podeena	Lamiaceae	leaf	5%	Al-Araji *et al.,* 2017

RESULTS

Disease Inhibition *In-vitro:* There are lot of methods to examine the antifungal activity of medicinal plants *in-vitro* such as spore suspension, dual culture technique, mycelia plug method and food poisoning technique. Here, we have used poisoned food technique in which five plant extracts with variable dilutions as given in table 1 were added in PDA media. All the treatments showed significant inhibition in the mycelial growth of target pathogen as compared to control. In this study, maximum inhibition was recorded from the neem leaf extract and minimum fungal growth (2.1cm) was observed, followed by bitter apple and garlic. Mint and moringa also showed significant reduction in mycelial growth, while in control the growth was maximum as shown in figure 1. Disease inhibition occurred due to plant extract application is represented by graph in figure 2.

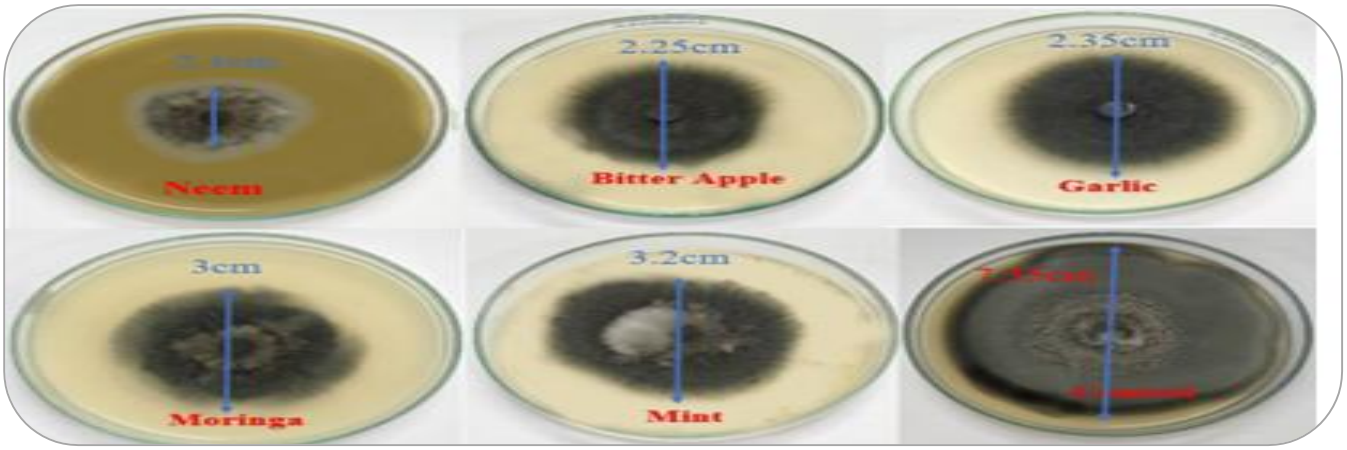

Figure 1. Poisoned food technique to show the reduction of mycelial growth of *A. solani* on PDA plates amended with plant extracts. Control plate shows the growth of *A. solani* on PDA without any amendment.

The reduction of mycelial growth of *A. solani* on PDA plates amended with plant extracts indicates the variations in mycelial growth. Petri plate containing neem extract indicating the minimum growth of about 2.1 cm. Following neem extracts petri plates treated with other plants extracts showing mycelial growth in an increasing order, neem < bitter apple < garlic < moringa < mint < control. In contrast, control showing the maximum growth as it was kept untreated. The plates that were treated with mint is not showing any significant control in mycelial growth. Hence, neem extract was found more effective in controlling *Alternaria solani* growth on PDA which is an important causal agent for early blight disease.

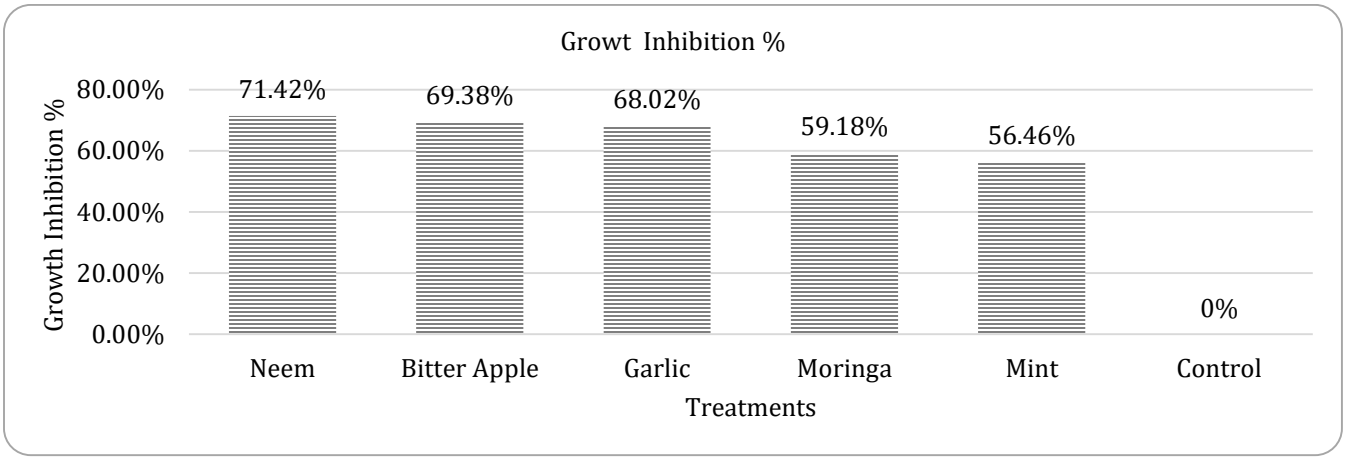

Figure 2. Graphical representation of radial growth inhibition percentage of *Alternaria solani* against various plant extracts in-vitro using poisoned food technique.

Growth inhibition indicated by a graph showing maximum rate of inhibition of 71.42% by neem extract. Inhibition rate was observed in a decreasing manner due to the treatment of plant extracts as neem > bitter apple > garlic > moringa > mint > control. There was no significant control found in control treatment as it was kept untreated. Mint extract showed the minimum inhibition than all other plant extracts by 56.46%.

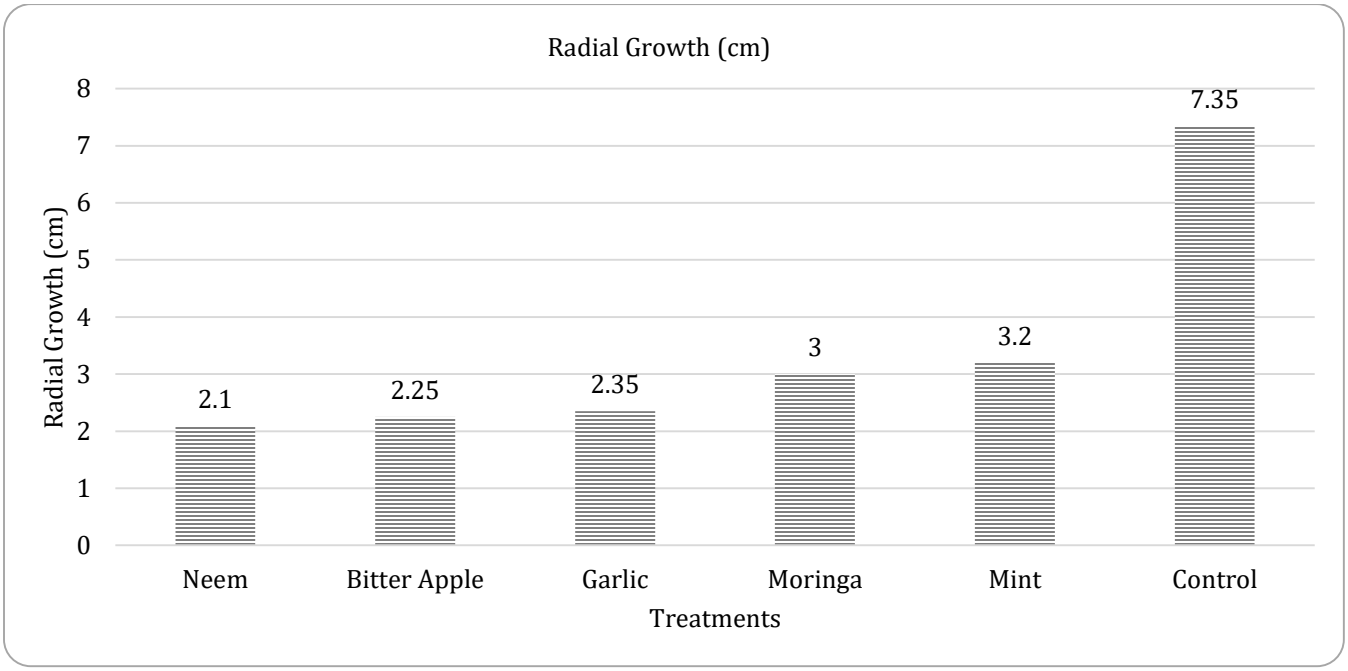

Figure 3. Graphical representation of radial growth of fungus on nutrient media plates amended with various plant extracts, showing a comparison of efficacy of plant extracts with the control.

Neem showing the highest growth reduction 2.1 cm, while bitter apple 2.25 cm and garlic showing 2.35 cm. Moringa and mint showing 3 cm and 3.2 cm respectively, over the control 7.35 cm.

Control of Early Blight of Tomato Using Seeds Primed with Plant Extracts**:** Plant extracts at the rate of 10ml from stock solution were used for seed priming against early blight of tomato. Neem extract showed the minimum disease severity of 18% after 3rd week of the inoculation of *Alternaria solani.* While the plants that were treated with mint extract showed minimum disease severity of 34%. In addition, control treatment showed maximum disease severity of 70%.

Table 2. Effect of Plant Extracts on Disease Severity of Early Blight

Treatment	Maximum Disease Severity %		
	1st Week Disease Severity %	2nd Week Disease Severity %	3rd Week Disease Severity %
Neem	5	12	18
Biter Apple	10	18	22
Garlic	17	26	35
Moringa	32	40	52
Mint	34	45	60
Control	40	55	70

Maximum disease severity was recorded after 1st, 2nd, and 3rd week of inoculation of *Alternaria solani* to the plants treated with plant extracts and as to the control treatment. In control treatment, the seeds were primed with simple water. A similar pattern of the effect of plant extracts was noted after each week. Minimum disease severity (18%) was noted for neem, followed by bitter apple, garlic, moringa, and mint, respectively. Maximum disease severity was noted in case of control treatment (70%), which were untreated plants.

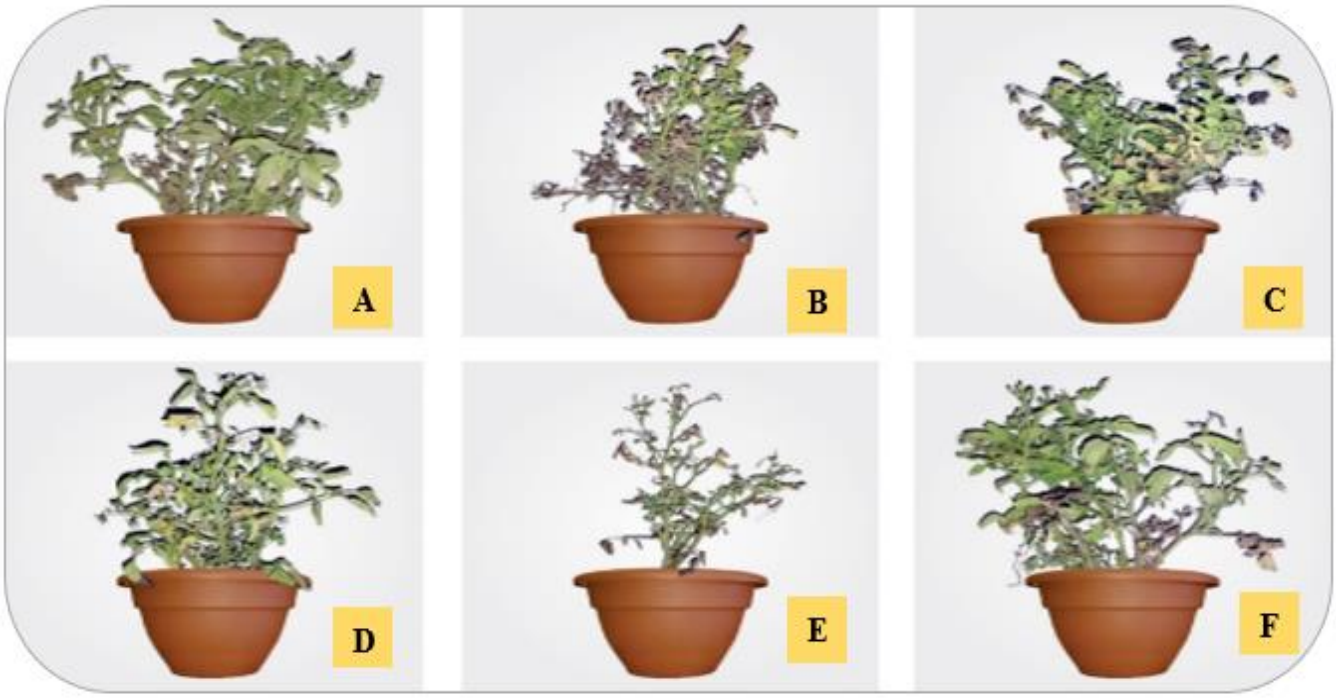

Figure 4. Disease severity after inoculation with *Alternaria solani* in tomato plants grown out of seeds primed with different plant extracts; (A) Neem extract, (B) Control, (C) Moringa, (D) Bitter apple, (E) Mint, and (F) Garlic. Pictures were taken 25 days' post inoculation (dpi). Pots were placed in the nursery area of UAF Sub-Campus Burewala.

Statistical Analysis for Effects of Plant Extracts against Early Blight Disease*:* In order to analyze the efficacy of plant extracts against early blight disease, analysis of variance (ANOVA) was performed by using SPSS software. Statistical analysis is given below in tables.

Table 3. ANOVA table of plant extracts' efficacy against early blight of tomato (1st week data)

Source	DF	SS	MS	F	P
Replication	2	0.3333	0.16667		
Treatment	5	28.6667	5.73333	57.33	0.0000
Error	10	1.0000	0.10000		
Total	17	30.0000			

ANOVA results reveal a highly significant treatment effect according to the LSD (least significant difference) test at $P<0.05$ with an F-value of 57.33 and a P-value of 0.0000, indicating substantial differences among treatments.

As can be seen from the ANOVA table's high F-value of 57.33 and P-value of 0.0000, which show substantial differences between treatment means, the treatments appear to have a significant impact on the outcome variable. With a mean square of 0.16667 and a sum of squares of 0.3333, the replication variability is minimal. The sum of squares of 30.0000 represents the total variability in the data, suggesting that treatments account for a significant portion of the variation overall.

Table 4. ANOVA table of plant extracts efficacy against early blight of tomato (2nd week data)

Source	DF	SS	MS	F	P
Replication	2	0.1111	0.05556		
Treatment	5	13.7778	2.75556	14.59	0.0003
Error	10	1.8889	0.18889		
Total	17	15.7778			

ANOVA results indicate a significant treatment effect according to the LSD (least significant difference) test at *P*<0.05 with an F-value of 14.59 and a P-value of 0.0003, demonstrating substantial differences among treatments.

The sum of squares for the data reflects the total variability in the data, which suggests that treatments account for a significant amount of the variance in the data. Treatments have a considerable impact on the response variable, as shown by the ANOVA table. Treatment differences are statistically significant at the 0.05 level, according to the F-value of 14.59 and P-value of 0.0003. The total sum of squares (SS) for the model as a whole is 15.778, which represents the data's overall variability. This analysis does not examine the significance of the replication effect.

Table 5. ANOVA table of plant extracts efficacy against early blight of tomato (3rd week data)

Source	DF	SS	MS	F	P
Replication	2	0.7778	0.38889		
Treatment	5	22.2778	4.45556	23.59	0.0000
Error	10	1.8889	0.18889		
Total	17	24.9444			

ANOVA results show a significant treatment effect according to the LSD (least significant difference) test at *P*<0.05 with an F-value of 23.59 and a P-value of 0.0000, indicating that treatments have a highly significant impact on the response variable.

With a p-value of 0.0000 and an F-value of 23.59, the Treatment effect is highly significant, indicating that the differences between treatments are statistically significant. Error and replication parameters contribute significantly less to the overall variation. All in all, almost all of the variability in data can be explained by the treatment component.

Table 6. LSD All Pairwise Comparison Test

Treatment	First Week Data	Second Week Data	Thirds Week Data
Neem	0.0000 D	2.0000 D	1.6667 C
Bitter Apple	0.6667 C	2.0000 D	2.3333 C
Garlic	2.0000 B	2.3333 CD	2.0000 C
Moringa	3.0000 A	3.0000 BC	3.3333 B
Mint	3.0000 A	3.6667 AB	3.3333 B
Control	3.3333 A	4.3333 A	5.0000 A

The similar letter within a column are not significantly different according to the LSD (least significant difference) test at *P*<0.05. While garlic fluctuated between C and D. Moringa showed a slight decrease in effectiveness over time from B to C.

The LSD (Least Significant Difference) Pairwise Comparison Test shows that lower disease severity scores indicate greater effectiveness in controlling the disease. In this context, neem is the most effective treatment, as it consistently shows the lowest disease severity scores across all three weeks (0.0000 in the first week, 2.0000 in the second week, and 1.6667 in the third week). The control, on the other hand, has higher disease severity scores (3.3333 in the first week, 4.3333 in the second week, and 5.0000 in the third week), indicating it is less effective compared to neem. Overall, neem's consistently low disease severity scores highlight its more efficacy in disease control compared to the other treatments.

DISCUSSION

The findings revealed that neem leaf extract exhibited the most substantial inhibitory impact, recording a mycelial growth of merely 2.1cm. Different studies have been done for the control of early and late blight disease using numerous plant extracts. Hassanein *et al.,* (2010) described that the mycelial development of early and late blight disease in tomato is inhibited by neem leaf extracts *in-vitro*. We have demonstrated here the potential of neem against early blight fungus. Arzoo *et al.,* 2012 reported that disease suppression has been recorded by neem leaf extract. Neem shows promising results against many plant pathogenic fungi attributable to its potential of being rich in phytochemicals (Sarkar *et al.,* 2021; Joshi *et al.,* 2011). These chemicals can

inhibit the fungal sporulation and suppress the disease, while some studies indicate that neem extract plays a role in yield increase as well (Kavishanker *et al.,* 2011). Some metabolites for example, terpenoids, quercetin, desactylimbin and sitosterol have been reported in extracts of neem, which are attributable to antimicrobial property (Siddiqui *et al.,* 2000).

Bitter apple (*Citrullus colocynthis*) ranks second in its ability to control the growth of early blight fungus allowing mycelial growth up to 2.25cm. Joshi *et al.,* 2011 mentioned that bitter apple has rich amount of metabolites and these metabolites have antifungal activity. *Ameh et al., 2013 reported that antifungal activity of garlic bulb extract is due to allicin compound that is found in the garlic extract, allicin having strong antifungal and antimicrobial potential.* The solid smell of new and squashed garlic is credited to oxygenated sulfur compound allicin which has antimicrobial properties (Davis, 2005). Rough concentrates of six plants were tested against root spoil, organic product decay and gummosis of citrus brought about by *Phytophthora* spp. The best outcome was accomplished with garlic which confined mycelial development at 5, 10 and 15% (Jagtap *et al.,* 2012). Here, we have optimized 10% concentration of the garlic bulb extract against *Alternaria solani*, however, further dilutions can be tested to screen out the best performing dilution.

Mint extract also demonstrated noteworthy inhibitory potential with reduced mycelial growth. Essential oil of mint has antimicrobial effects and is used as the biological control of pathogenic fungi. It is confirmed that the essential oil obtained from mint can prevent the growth of common fungi such as *Botrytis cinerea*, *Phytophthora infestans, Sclerotinia sclerotiorum and Verticillium dahliae, and it has a high fungicidal effect (Soylu et al., 2010).* Many other studies reported that plant extracts obtained from medicinal plants including mint show inhibitory effects to many plant pathogenic fungi. These extracts inhibit the sporulation and germination of mycelium (Dissanayake *et al.,* 2014).

The moringa extract exhibited a relatively lower inhibition effect, yet it still managed to significantly curtail mycelial growth, registering at (3.0cm). In comparison, the control treatment, devoid of any plant extract, displayed a markedly higher mycelial growth of (7.35cm). In many other literature studies *(Moringa oleifera)* show very effective results against *Alternaria solani* and *Fusarium oxysporum* (Fawzi *et al.,* 2009; Dwivedi and Enespa, 2012).

Significant treatment effects were found using ANOVA analyses carried out over a three-week period to assess the effectiveness of plant extracts against early blight disease in tomatoes. The differences between the treatments were statistically significant for each of the three weeks, as shown by the high F-values and extremely low p-values for each ANOVA result according to the LSD (least significant difference) test at $P<0.05$ (week 1: $F = 57.33$, $p = 0.0000$; week 2: $F = 14.59$, $p = 0.0003$; week 3: $F = 23.59$, $p = 0.0000$). The findings demonstrate that the plant extracts are highly successful in reducing Early Blight disease, and the main factor driving the observed variability is the treatment effects.

Concluding, the effectiveness of neem in suppressing mycelial growth underscores its potential as an eco-friendly alternative to synthetic fungicides, thus presenting a promising avenue for sustainable pest management strategies in agriculture. While the garlic bulb extract displayed a slightly lower inhibitory effect compared to the neem leaf extract. Its significant suppression of mycelial growth accentuates the potential of its bioactive constituents, particularly allicin, in combating fungal pathogens. Similarly, the observed inhibitory effects of bitter apple, mint, and moringa extracts suggest the presence of bioactive compounds that impede the growth of fungal pathogens, thus advocating for further exploration of their potential application in integrated pest management strategies. However, it is essential to note that the varying degrees of inhibition among the plant extracts might be attributed to differences in the composition and concentration of their bioactive constituents. Further investigations into the specific bioactive compounds responsible for the observed inhibitory effects are imperative for understanding their mechanisms of action and optimizing their application in sustainable agriculture practices. Additionally, assessing the long-term effects of these plant extracts on non-target organisms and the environment is crucial to ensure their safety and ecological sustainability. Three plant extracts out of five show effective results, while remaining two show moderate results against the disease. In current study, the effectiveness was in this order: *Azadirachta indica > Citrullus colocynthis > Allium sativum > Moringa oleifera > Mentha spicata.* Overall, the findings

underscore the promising potential of these plant extracts as natural alternatives for controlling fungal pathogens, thereby reducing the reliance on synthetic fungicides and promoting sustainable agricultural practices. Further research exploring their efficacy under diverse environmental conditions and their impact on crop productivity will contribute significantly to the development of eco-friendly and sustainable pest management strategies.

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