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### ASSESSMENT OF PHYTOEXTRACTS AND SYNTHETIC CHEMICALS FOR CONTROLLING LEAF BLIGHT OF *SYZYGIUM CUMINI*

<sup>a</sup>Rabeet Ahsan Khan, <sup>a</sup>Muhammad Atiq, <sup>a</sup>Nasir Ahmed Rajput, <sup>b</sup>Irfan Ahmad, <sup>c</sup>Abdul Jabbar, <sup>d</sup>Muhammad Jalal Arif, <sup>e</sup>Abuzar Hasnain, <sup>a</sup>Ahmad Nawaz, <sup>a</sup>Muhammad Mehtab, <sup>a</sup>Waqas Ahmad

<sup>a</sup> Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan.

<sup>b</sup> Department of Forestry and Range Management, University of Agriculture, Faisalabad, Pakistan.

<sup>c</sup> Department of Agronomy, Community College PARS Campus, University of Agriculture, Faisalabad, Pakistan.

<sup>d</sup> Department of Entomology, University of Agriculture, Faisalabad, Pakistan.

<sup>e</sup> Department of Botany, Faculty of Sciences, University of Agriculture, Faisalabad, Pakistan.

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#### ABSTRACT

Jamun (*Syzygium cumini*) is a distinguished source of protein, fat, minerals (Iron, Calcium, and Potassium), fiber, carbohydrates, phosphorous, and vitamin C (ascorbic acid). Leaf blight of *S. cumini*, caused by *Pestalotiopsis* spp., poses a potential threat to the successful production of Jamun fruit. The appearance of small, round, dark, and sunken spots on the fruit diminishes its quality and results in yield losses of 10 to 20%. Fungicides and plant extracts were employed to address this fungal problem under laboratory and greenhouse conditions using a Completely Randomized Design whereas field trials were performed using a Randomized Complete Block Design. *In vitro* experiments revealed that among the fungicides, minimum fungal growth was observed with Score (12.5 mm), followed by Topsin M (16.89 mm), Fossil (18.37 mm), Excel (23.17 mm), Evito (27.56 mm), and Bloom (32.32 mm), as compared to the control (55.56 mm). Among the phytoextracts, Moringa extracts showed the least fungal growth (15.7 mm), followed by Neem (18.76 mm), Eucalyptus (19 mm), Garlic (22.72 mm), Ginger (27.57 mm), and *Cinnamomum verum* (Dar Cheni) (31 mm), in comparison to the control (53.17 mm). The most effective fungicides and plant extracts determined in the laboratory experiments were further evaluated in greenhouse and field conditions, both alone and in combinations. In the greenhouse evaluation, the combination of Moringa + Score exhibited the lowest disease incidence (23.63%), followed by Score (28.12%) and Moringa (29.56%), in contrast to the control (52.9%). Under field conditions, among all treatments, Moringa + Score exhibited the least disease incidence of 17.44%. These findings confirm that leaf blight of *S. cumini* can be managed by using Score fungicide and *Moringa oleifera* extract.

Corresponding Author: Muhammad Atiq

Email: dratiqpp@gmail.com

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#### INTRODUCTION

*Syzygium cumini*, commonly known as Jamun, Jaman, Black plum, Java plum or Jambul is a widely cultivated fruit tree with an annual production of 13.5 million tons

(Verma et al., 2019). The Punjab and Sindh provinces of Pakistan have conducive climates for the production of *S. cumini* (Patel et al., 2010). It is grown on an area of 870 hectares in Pakistan with total production of 4811 tons

(GOP, 2019). Its fruit is consumed in raw as well as in processed form like jam, wine and fermented beverages and various other value-added products (Chhikara et al., 2018). It is rich source of moisture, crude fat, protein, carbohydrates and fiber (Joshi et al., 2019). Jamun is well famous for its various health benefits as it is loaded with various bioactive compounds like flavonoids, carotenoids, polyphenols that are effective in curing various diseases such as treatment of stomach pain, carminative, anti-scorbutic, diuretic and urine problems (Joshi et al., 2019).

Trunk disease (Panahandeh et al., 2019), leaf spot of jamun, and bacterial leaf blight are the major diseases of this multipurpose tree but leaf blight caused by *Pestalotiopsis* spp is the most significant disease. Pathogen has a wide distribution and found on a variety of hosts. Its conidia have 3-5 septa, their center cell is large where apical cells are hyaline and appendages are present which looks like as branched form. The basal cell is hyaline with a stipe, causing various diseases including stem canker, necrotic lesions on leaf, seed and root rots in different hosts (Vujanovic et al., 2000; Dhingra et al., 2002, 2003). *Pestalotiopsis* spp. primarily proliferates at 25°C temperature (Bhuiyan et al., 2021) during dry and rainy season in March. It has been reported to cause 17% losses in production in southern India (Joshi et al., 2009) and 10 to 20% yield loss in Japan (Chen et al., 2018).

*Pestalotiopsis* spp. is seed as well as soil borne pathogen, being spread through rain splashes and physical contact. Water present on surface of leaves, carry the spores and then splash on healthy plants, effectively transmitting the pathogen (Mordue, 1984). A number of strategies have been adopted to manage plant diseases such as application of fungicides, use of plant defense activator, implementation of antagonistic organisms (Iqbal and Mukhtar, 2020a, b; Ahmed et al., 2021; Azeem et al., 2021; Haq et al., 2021; Mukhtar et al., 2021, 2023; Saeed et al., 2021; Shakoor et al., 2015; Bibi et al., 2017) but the most economical, eco-friendly and cost-effective approach is the use of resistant varieties. However, when resistant varieties are not available and disease appears in epidemic form and farming community is bound to use synthetic chemicals due to their quick action and easily availability. By knowing the beneficial importance of these chemicals, in the current study, disease was managed through application of chemicals.

Keeping in view the residual effect of chemicals on the environment, it is the need of time to manage plant diseases through eco-friendly approaches (Shahbaz et al., 2023). One of the eco-friendly methods to combat plant diseases is use of plant extracts. Plant extracts have been used in plant disease management as they accommodate antimicrobial compounds like alkaloids, polyphenols, tannins, flavonoids, glycosylates, and indoles which trigger the plant defense system through activation of defense related genes (Cowan, 1999; Mandiriza et al., 2018). Some plant components such as eugenol and thymol possess high anti-microbial potential and proved to be highly effective against pathogens and boost up plant defense system (Vaseeharan and Thaya, 2014). By observing the beneficial aspects of plant extracts, these were evaluated against leaf blight of *S. cumini*.

## MATERIALS AND METHODS

### Isolation, purification and identification of pathogen

Disease samples of *Syzygium cumini* were collected from botanical garden, University of Agriculture, Faisalabad and brought to the laboratory for isolation of causative agent. Potato Dextrose Agar (PDA) media was prepared for cultivation of *Pestalotiopsis* spp. Regarding this, 250 g of potatoes were washed, peeled, cut into small cubes and boiled in 500 ml distilled water for 15 min. Potato starch was obtained by the filtration of boiled potato through doubled layered muslin cloth. One liter of PDA media was prepared by dissolving 20 g of extracted potato starch and 20 g of Agar in distilled water. Further distilled water was added to make 1000 ml volume then closed the bottle lid. The suspension was shaken well till all ingredients were dissolved completely. The media was then autoclaved (RTA 85 Robus technologies) at 121°C at 15 psi pressure for 15 min. When PDA media cooled down to 55°C, 0.5 ml of an antibacterial (Streptomycin) was added to avoid bacterial contamination. The collected diseased samples were washed with tap water to remove debris. The samples were cut into small pieces by using sterilized scissors. These pieces were surface sterilized in 1% solution of Sodium hypochlorite and rinsed through distilled water for the removal of toxic effect of Sodium hypochlorite. PDA media was poured in glass Petri plates and diseased samples were placed after solidification. After wrapping, the Petri plates were

placed for incubation at 25±5°C and fungal growth was observed after 3-4 days. All work was performed in laminar flow chamber (Robus technology, UK) to avoid contamination.

Hyphal tip method was used to purify pathogen culture. For this purpose, hypha was picked with the help of sterilized needle from the margins of colony and inoculated to new Petri plates containing PDA media. The Petri plates were wrapped with the help of wrapping tape and placed inside incubator at 25±5°C and fungal growth was noticed after 3-4 days.

Causative agent was identified on morphological basis (growth pattern, colony color etc.). For microscopic identification of pathogen, glass slides were prepared. 1-2 drops of distilled water were placed on glass slide and fungal spores from 5 days old purified culture of pathogen was shifted on this slide by using sterilized needle. The glass slide was covered by cover slip and the spores were observed under light microscope.

#### **Pathogenicity test**

Koch's postulates were assessed for the confirmation of pathogen. One year old plants of jamun were obtained from the nursery of the Department of Forestry and Range Management, University of Agriculture Faisalabad. Plants were grown in pots (30 cm diameter) with sterilized soil. Spore suspension ( $3 \times 10^7$  spores/ml of water) of pathogen was prepared from 7 days old culture and applied to the plants by using spray method. After 5-7 days of inoculation, disease symptoms were appeared. Pathogen was reisolated and compared with primary pathogen culture.

#### ***In vitro* evaluation of phytoextracts and chemicals against leaf blight of *S. cumini***

Experiment was designed to assess the antifungal efficacy of synthetic chemicals and phytoextracts against *Pestalotiopsis* spp. under *in vitro* condition by using poisoned food technique. For this purpose, fresh leaves of Neem (*Azadirachta indica*), Eucalyptus (*Eucalyptus camaldulensis*), bulbs of Garlic (*Allium sativum*), rhizome of Ginger (*Zingiber officinale*) and leaves of Moringa (*Moringa oleifera*) were collected, washed by using distilled water, dried under shade, and placed in hot air oven at 65°C for 4 hours. Dry leaves were ground and fine powder was obtained after sieving through double layer muslin cloth. Three concentrations of phytoextracts (10, 15, 20%) and chemicals (100, 200, and 300 ppm) were prepared by adding 10, 15, and 20 g of each plant extract and 10, 20 and 30 mg of each chemical separately in 100

ml PDA, respectively. The poisoned PDA media was poured into Petri plates. After solidification of PDA media, the bits (5 mm) of fungal mycelia from purified colony were placed by using the sterilized cork borer. Fungal growth was observed after 48, 72, and 96 hours.

#### **Assessment of combination of most effective chemicals and phytoextracts against leaf blight of *S. cumini* under greenhouse conditions**

The most effective chemicals and phytoextracts observed under lab condition were evaluated under greenhouse conditions alone and in combination against leaf blight of *S. cumini*. One year old seedlings were collected from nursery of the Department of Forestry and Range Management University of Agriculture, Faisalabad and transplanted in pots (11 inches) containing clay loam soil sterilized with 1% formalin solution. Seedlings were allowed for two months to establish properly under greenhouse condition. After establishment, seedlings were artificially inoculated by spray method. When disease symptoms appeared, treatments were applied with already prepared three concentrations through foliar spray method. The control plants were treated with distilled water. Experiment was designed under Completely Randomized Design and disease incidences were recorded after 5, 10 and 15 days.

#### ***In vivo* evaluation of phytoextracts and chemicals against leaf blight of *S. cumini***

Plant extracts and chemicals, alone and in combination form were evaluated *in vivo* with three concentrations against leaf blight of *S. cumini* under CRD. Host plants were collected from nursery of the Department of Forestry and Range Management, University of Agriculture, Faisalabad. Spore suspension ( $3 \times 10^7$  spores/ml of water) of pathogen was measured through haemocytometer and plants were inoculated by using foliar spray methods. Treatments were applied after development of disease symptoms and disease incidence was recorded after 5, 10 and 15 days.

## **RESULTS**

#### ***In vitro* assessment of chemicals against *Pestalotiopsis* spp. causing leaf blight of *S. cumini***

Among synthetic chemicals, Score exhibited the minimum fungal growth (12.500 mm) followed by Topsin M (16.889 mm), Fossil (18.370 mm), Excel (23.167 mm), Evito (27.56 mm), Bloom (32.315 mm) as compared to control plates (Table 1, Figure 1). Impact of

treatments and concentrations (T×C) revealed that Score expressed minimum fungal growth at concentration of 300 ppm (5.33 mm), followed by Topsin M at 300 ppm (9.0 mm), Fossil at 300 ppm (10.0 mm), Excel at 300 ppm (12.833 mm), Evito at 300 ppm (19.722 mm), Bloom at 300 ppm (25.833 mm), as compared to control

(Figure 2). Impact of treatments and time (T×t) expressed that, after 96 hours the minimum fungal growth was expressed by Score (15.22 mm) followed by Topsin M (19.444 mm), Fossil (20.889 mm), Excel (26.667 mm), Evito (30.22 mm), Bloom (34.50 mm) as compared to control (Figure 3).

Table 1. Impact of fungicides on fungal growth under laboratory conditions.

Treatments	Active Ingredients	Fungal growth (mm)
Score	Difenoconazole (100%)	12.500 g
Topsin M	Thiophanate Methyl (70%)	16.889 f
Fossil	Azoytrobin + Difenocoazole (60%)	18.370 e
Excel	Difenocoazole (80%)	23.167 d
Evito	1,2-benzisothiazolin-3- one (0.0193%)	27.556 c
Bloom	Myuobutonyl (45%)	32.315 b
Control	Distilled water	55.556 a

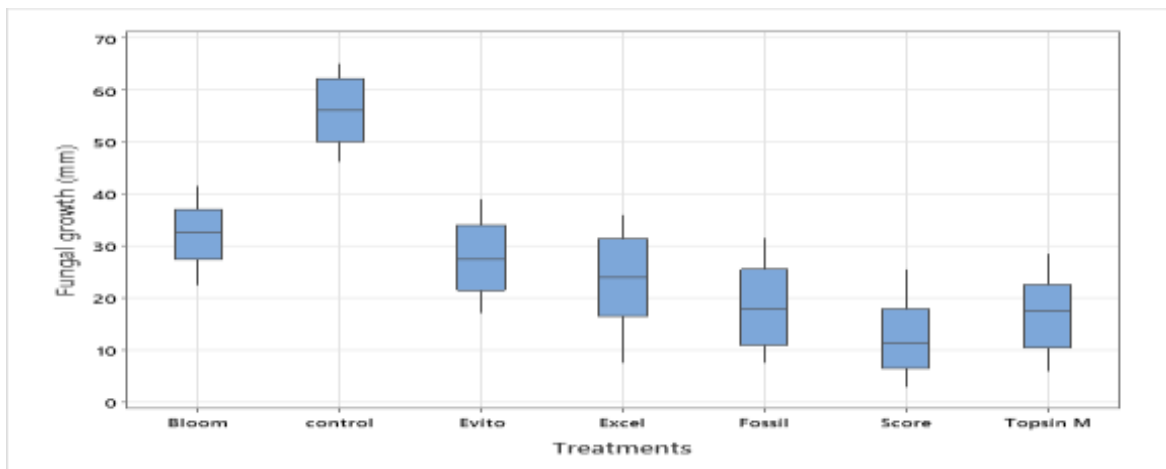


Figure 1. Impact of different chemicals on fungal growth under laboratory conditions.

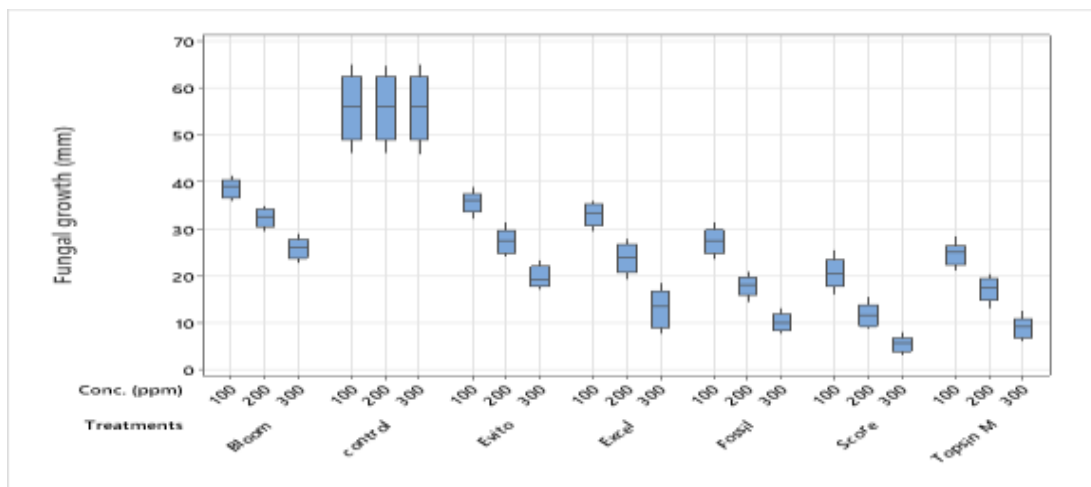


Figure 2. Impact of interaction between chemicals and their concentrations on fungal growth under laboratory conditions.

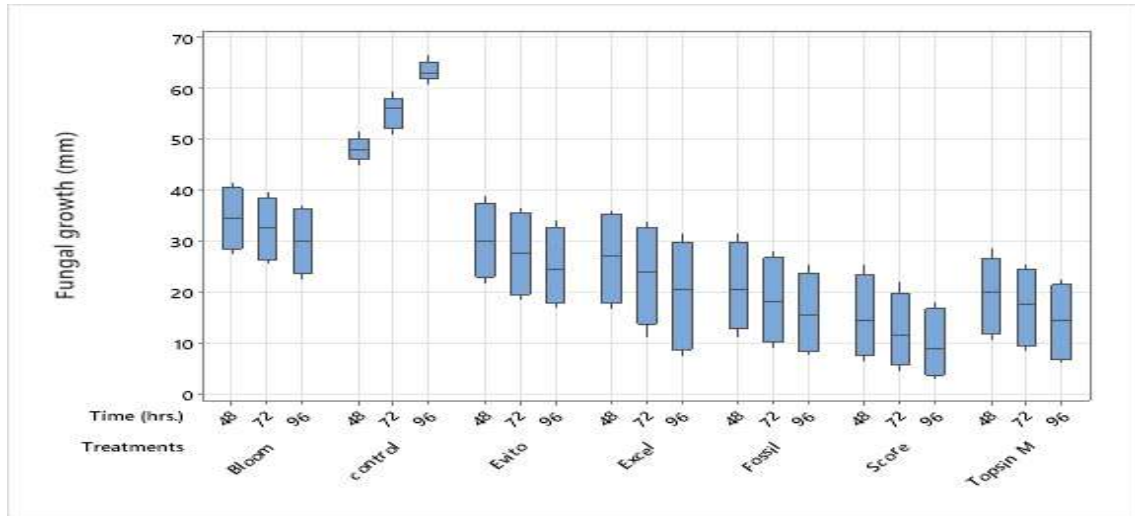


Figure 3. Impact of interaction between chemicals and time against fungal growth under lab. conditions.

**In vitro evaluation of plant extracts against Pestalotiopsis spp. causing leaf blight of S. cumini**

Among plant extracts, Moringa showed the minimum fungal growth (15.704 mm) followed by Neem (18.759 mm), Eucalyptus (19.000 mm), Garlic (22.722 mm),

Ginger (27.574 mm), Dar Cheni (31.000 mm) as compared to control plates (Table 2, Figure 4). Similarly, results of interaction between treatments and their concentrations (T×C), treatments and time (T×t) are expressed in figure 5 and figure 6 respectively.

Table 2. Impact of plant extracts on fungal growth under laboratory conditions.

Treatments	Scientific name	Fungal Growth (mm)
Moringa	<i>Moringa oleifera</i>	15.704 f
Neem	<i>Azadirachta indica</i>	18.759 e
Eucalyptus	<i>Eucalyptus globulus</i>	19.000 e
Garlic	<i>Allium sativum</i>	22.722 d
Ginger	<i>Zingiber officinale</i>	27.574 c
Dar Cheni	<i>Cinnamomum verum</i>	31.000 b
Control		53.167 a

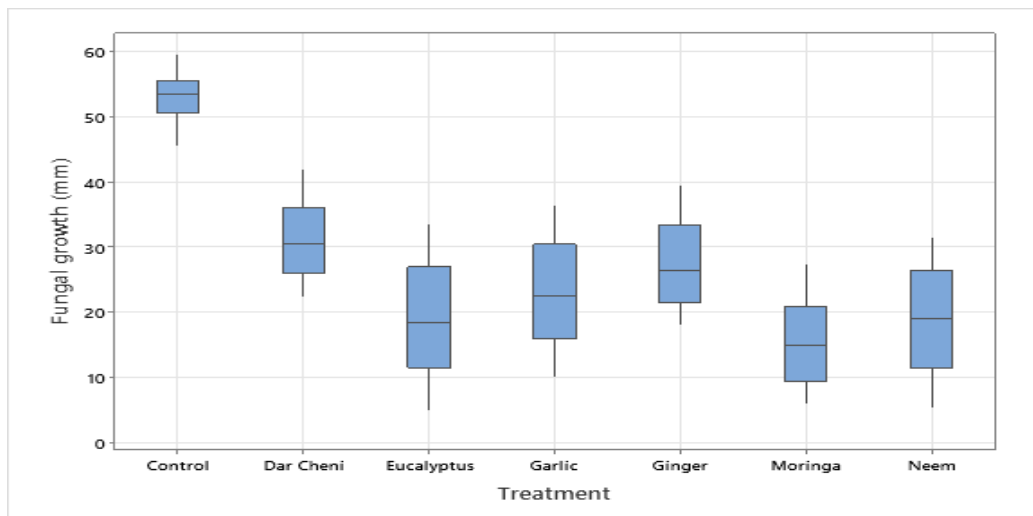


Figure 4. Impact of different plant extracts on fungal growth under laboratory conditions.

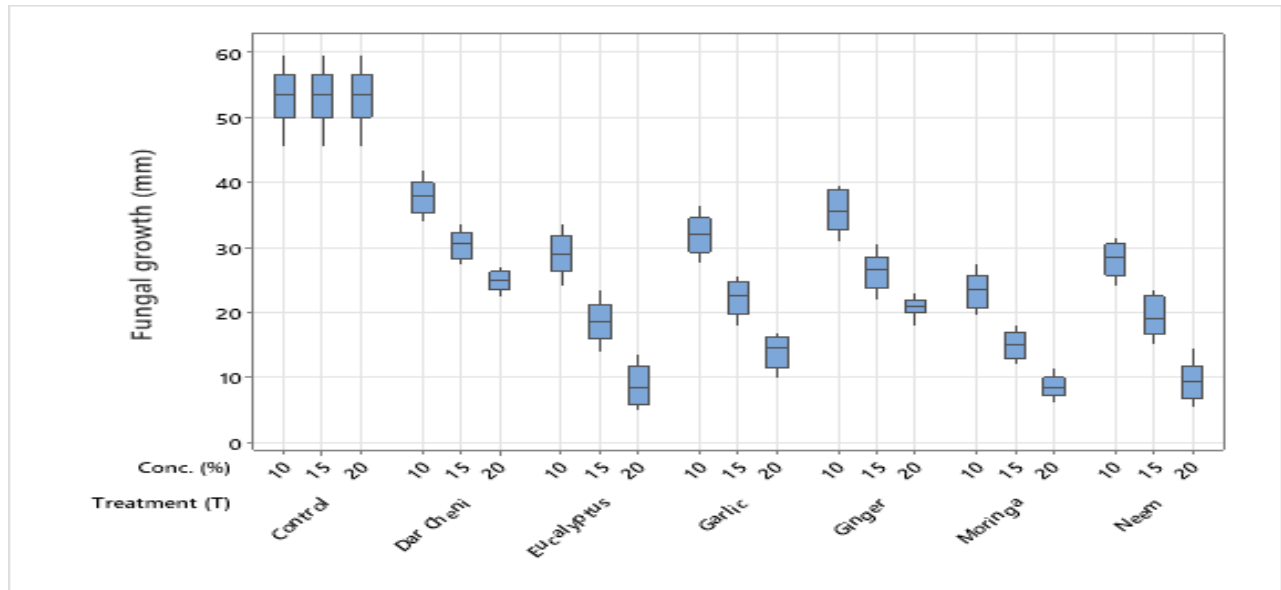


Figure 5. Impact of interaction between plant extracts and their concentrations on fungal growth under lab. conditions.

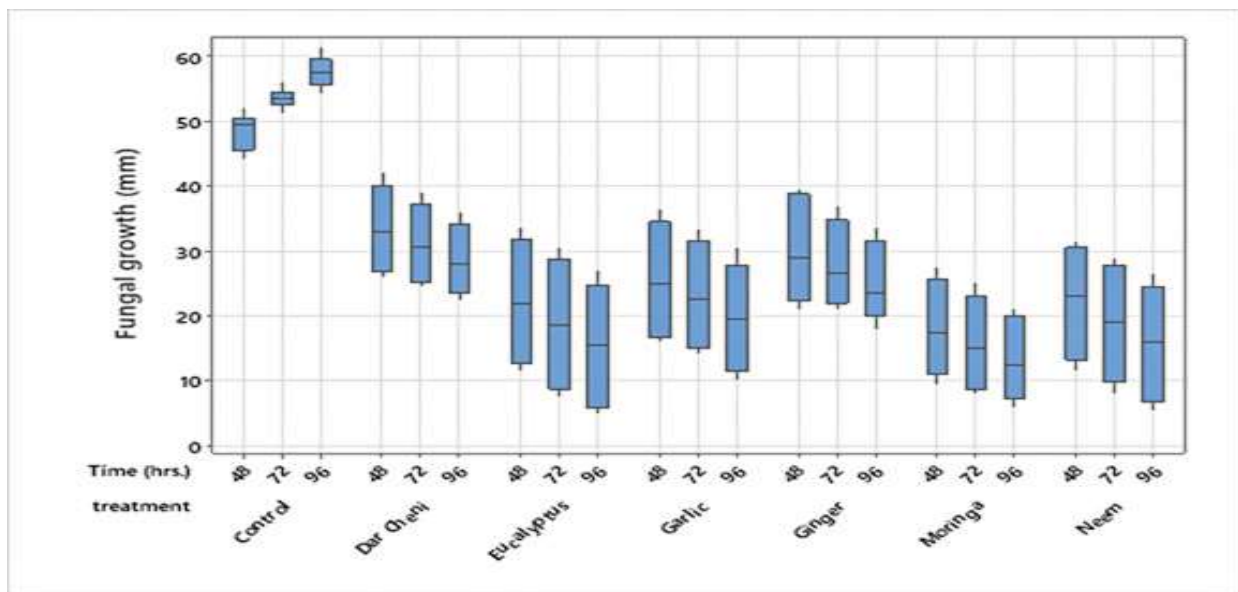


Figure 6. Impact of interaction between plant extracts and time against fungal growth under lab. conditions.

**Assessment of chemicals and phytoextracts against leaf blight of *S. cumini* under greenhouse conditions**

Among all treatments under greenhouse conditions, Score + Moringa expressed minimum disease incidence (23.631%) followed by Score (28.115%) and Moringa (29.559%) as compared to control (Table 3, Figure 7). The interaction between treatments and concentrations (T×C) indicated that minimum disease incidence was expressed by Score + Moringa at concentration of 20% (18.711%), 15% (23.724) and 10% (28.457%) followed

by Score (24.432, 28.370 and 31.543 %) and Moringa (26.068, 29.433 and 33.174%) at concentrations of 20, 15 and 10 %, respectively as compared to control (Figure 8). The interaction between treatments and days (T×D) exhibited that minimum disease incidence with gradual decrease was expressed by Score + Moringa after 5 days (26.379%), 10 days (23.382%) and 15 days (21.131%) followed by Score (30.849, 27.960, 25.537%) and Moringa (32.177, 29.553 and 26.946%) respectively as compared to control (Figure 9).

Table 3. Impact of treatments on disease incidence under greenhouse conditions.

Sr. No.	Treatments	Disease incidence (%)
1	<i>Moringa oleifera</i> + Score	23.631d
2	Score	28.115c
3	<i>Moringa oleifera</i>	29.559b
4	Control	52.895a
	LSD	0.8548

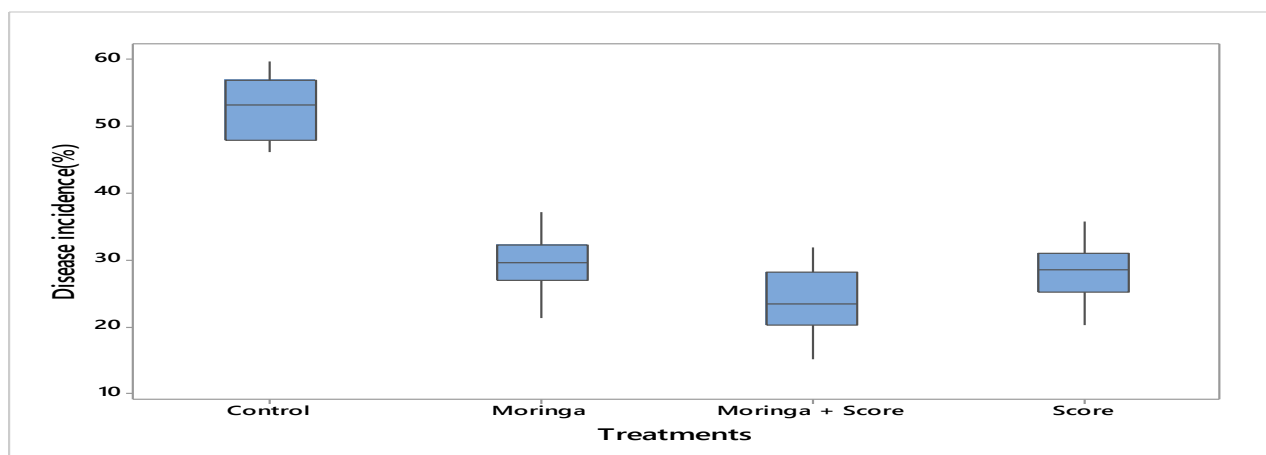


Figure 7. Impact of chemical and plant extract against leaf blight of *S. cumini* under greenhouse conditions.

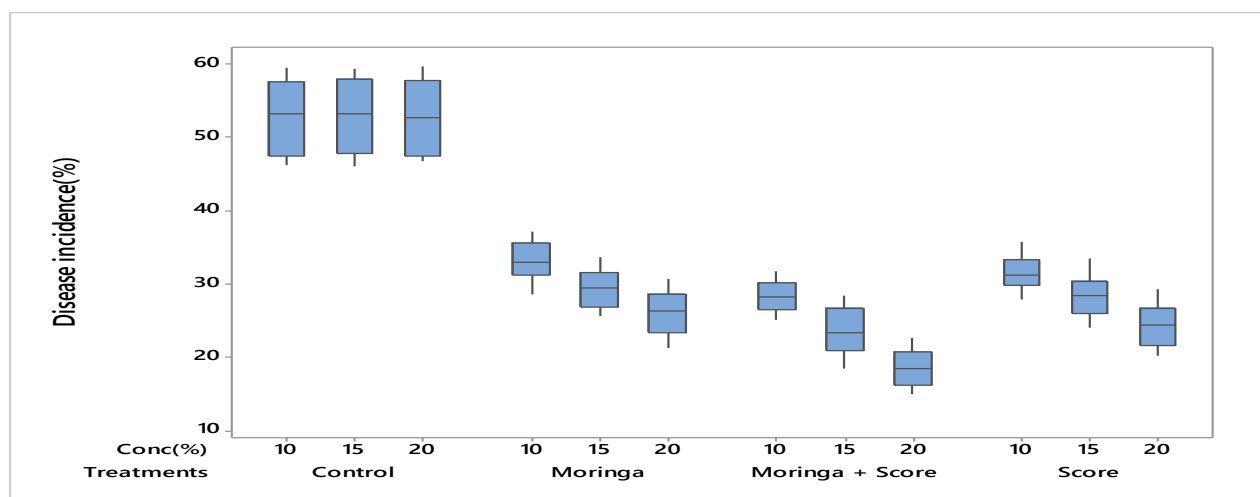


Figure 8. Impact of interaction between treatments and concentrations against leaf blight of *S. cumini* under greenhouse conditions.

**In vivo assessment of chemical and phytoextracts against leaf blight of *S. cumini***

Among all treatments under field conditions, Score + Moringa exhibited the minimum disease incidence (17.444%) followed by Score (16.88%) and Moringa (21.741%) as compared to control (Table 4, Figure 10). The interaction between treatments and concentrations

(T×C) revealed that minimum disease incidence was expressed by Score + Moringa at concentration of 20% (9.833%), 15% (17.056%) and 10% (25.444) followed by Score (10.389, 20.556, 29.056%) and Moringa (11.333, 21.500, 32.389%) at 20, 15 and 10% concentrations, respectively as compared to control (Figure 11). The interaction between treatments and

days (T×D) concluded that minimum disease incidence with gradual decrease was observed by Score + Moringa after 5 days (19.944%), 10 days (17.444%) and 15 days

(14.944%) followed by Score (23.111, 20.0, 16.889%) and Moringa (25.556, 21.61, 18.056%) respectively as compared to control (Figure 12).

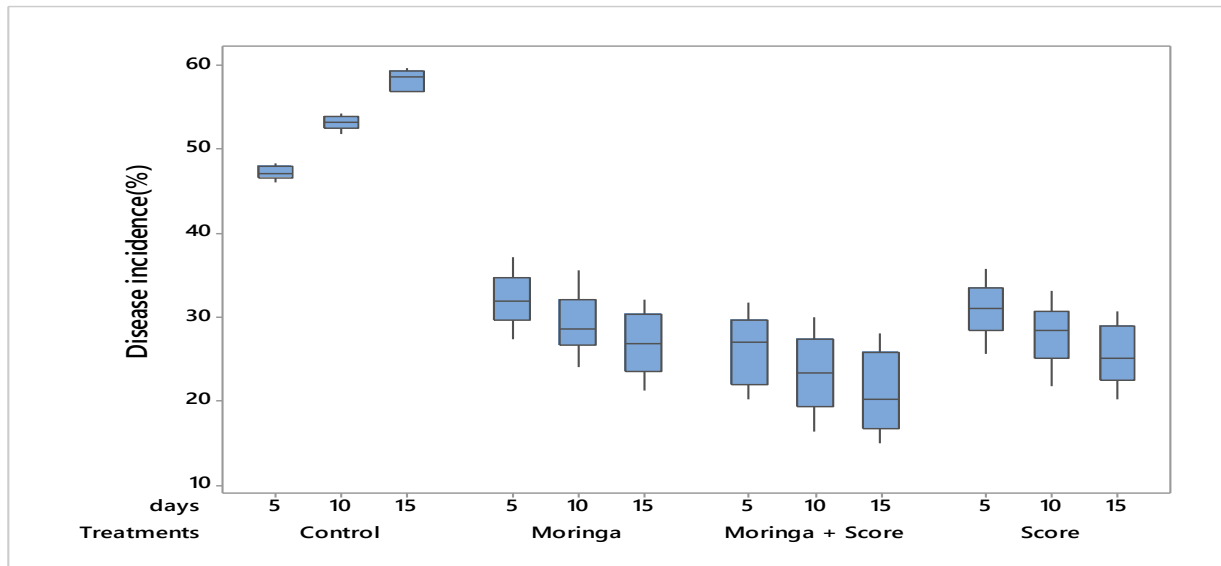


Figure 9. Impact of interaction between treatments and days against leaf blight of *S. cumini* under greenhouse condition

Table 4. Impact of treatments on disease incidence under field conditions.

Treatments	Description	Disease Incidence (%)
Moringa+Score	<i>Moringaolerifas</i> +Difenoconazole (100%)	17.444d
Score	Difenocoazole (60%)	20.00c
Moringa	<i>Moringa olerifas</i>	21.741b
Control		53.167a

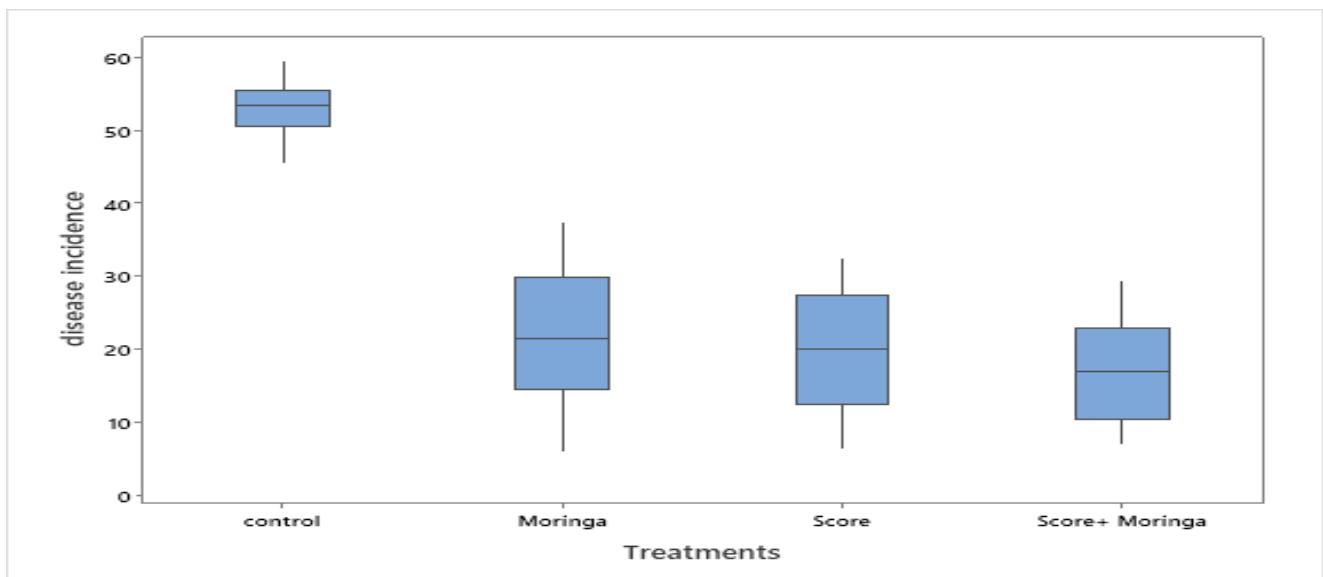


Figure 10. Impact of different treatments against leaf blight of *S. cumini* under field conditions.



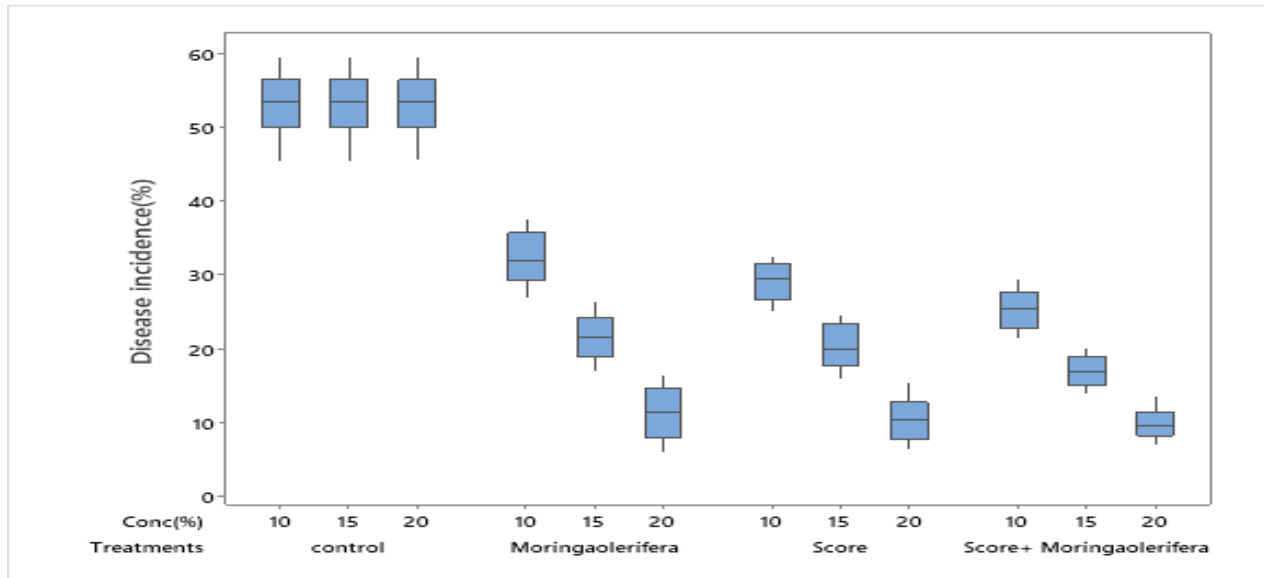


Figure 11. Impact of interaction between treatments and concentrations against leaf blight of *S. cumini* under field conditions.

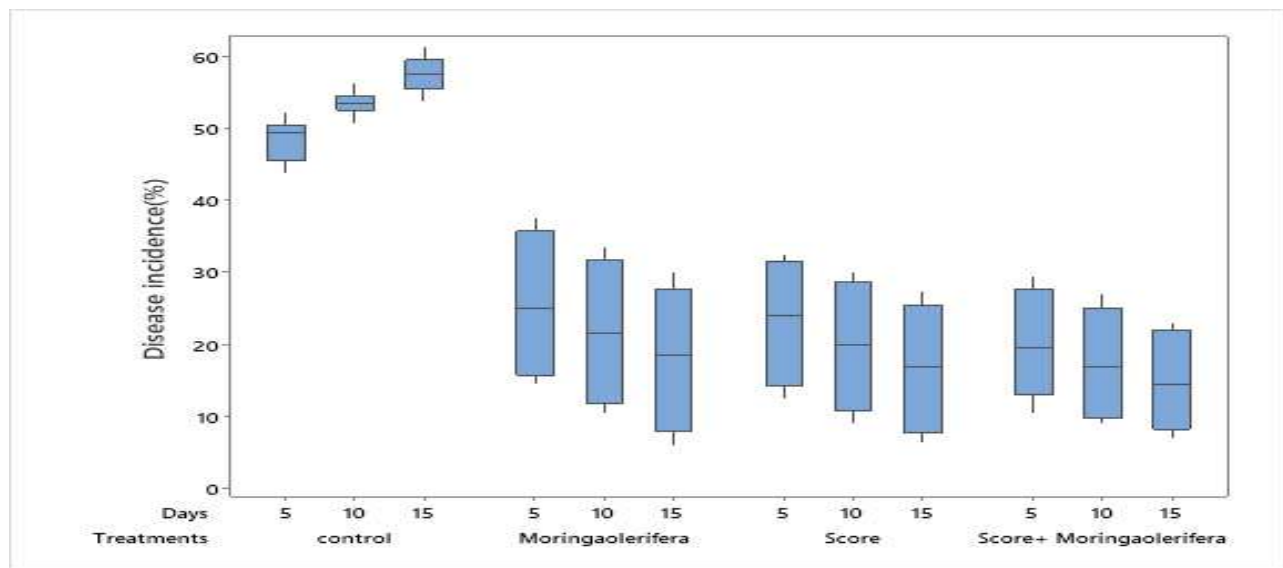


Figure 12: Impact of interaction between treatments and days against leaf blight of *S. cumini* under field conditions.

**DISCUSSIONS**

Fungi play a significant role in prevalence of various diseases posing substantial damage and economic losses to Jaman tree. Leaf blight of *Syzygium cumini* caused by *Pestalotiopsis* spp. is one such prevalent disease which causes critical postharvest issues leading to production and quality losses of fruit in different Asian countries, including Pakistan, India and Bangladesh. Present study was designed to evaluate different fungicides and plant extracts against *Pestalotiopsis* spp. Six chemical treatments consisting of

Score, Topsin M, Fossil, Excel, Evito and Bloom with three concentrations were evaluated under laboratory conditions with three replications. Result revealed that, Score fungicide was the most effective against *Pestalotiopsis* spp. Topsin M also showed better result as compared to other fungicides. According to Bhanumathi and Ravishankar (2007), Bavistin and Roko against *Pestalotiopsis* spp. were found effective on mycelial growth at different concentrations such as 50, 100, 150 mg/L. Similarly, Moshayedi et al. (2017) revealed that maximum growth was inhibited by using

different fungicides against *Pestalotiopsis* spp. Bavistin 50WP, Dithane M-45, Aliette, Benlate 50WP and Topsin M 70WP exhibited significant results to inhibit the mycelial growth at different concentrations such as 1000, 2000, and 3000 ppm. Another eco-friendly approach for the management of fungal diseases is the use of phytoextracts. Plant extracts such as Moringa, Neem, Eucalyptus, Garlic, Ginger, Dar Cheni were used in laboratory conditions to manage the leaf blight of *S. cumini*. Moringa and Neem extracts were the most significant among the phytoextracts. Our results were in line with experiment conducted by Haider et al. (2020) who described that extracts obtained from *Azadirachta indica*, *Allium sativum* and many others extracts were effective against the mycelial growth of *Pestalotiopsis* spp. Barman et al. (2015) studied that eucalyptus oil and neem oil expressed 98.1 and 94.3% inhibition of mycelial growth over the control. Tripathy et al., (2018) also evaluated the extracts of six plant species against alternative pathogen that potentially inhibited the pathogen mycelial growth. The finger extract of Turmeric having fungal colony diameter of 24.03 mm allowed minimum growth of the pathogen followed by clove extracts of Garlic (68.44 mm), finger extract of Ginger (78.13 mm) and leaf extract of Black Tulsi (82.00 mm). Similar experiment was designed by the Yousaf et al. (2015) who evaluated different plant extracts towards late blight disease of tomato and reported that maximum disease was reduced by *A. indica* (32.15%) followed by *M. oleifera* (41.09%), *Z. officinales*(48.88%), *Citrus calosynthis* (49.88%) and *Calotropis gigantea* (53.41%) as compared to control.

Difenoconazole is the main constituents of score fungicide that was extensively studied against various plant pathogens. A wide range of fungi, including those in the Ascomycetes, Basidiomycetes, and Deuteromycetes families, are controlled by the broad-spectrum fungicide difenoconazole which acts as a systemic fungicide. These are the inhibitor of the biosynthesis process that transforms lanosterol into ergosterol, the end product of fungal sterol production, known as the C-14 demethylation of lanosterol or 24-methylenedihydrolanosterol (Koller and Scheinpflug, 1987).

Small peptides found in the leaf extracts of *M. oleifera* may be crucial to the plant's antimicrobial defense mechanism (Dahot, 1998). According to Chuang et al. (2007), peptides are thought to have a role in the

defense mechanism against phytopathogenic fungi by preventing the development of microorganisms through a variety of molecular mechanisms, such as attaching to chitin or increasing the permeability of the fungal membranes or cell wall. Antimicrobial peptides likely engage in a two-stage interaction with membranes. Due to presence of negative charge on phospholipid head group, it engages with positive charge amino acids. After that, positively charged peptide and hydrophobic patches attaches the anionic and aliphatic components respectively (Zasloff, 2002; Koczulla and Bals, 2003). This results in membrane destabilization, and pathogens are killed by the leakage of cytoplasmic contents, loss of membrane potential, change in membrane permeability, distribution of lipids, entry of the peptide and blocking of anionic cell components, or induction of autolytic enzymes (Zasloff, 2002).

## CONCLUSION

Among the chemicals that were tested, Score showed the lowest incidence of leaf blight on *Syzygium cumini* and among phytoextracts, Moringa oleifera exhibited the minimum occurrence of leaf blight on *S. cumini*, both in greenhouse and field conditions. Therefore, these options are highly recommended for farmers to effectively manage leaf blight on *S. cumini*.

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## AUTHORS' CONTRIBUTIONS

RAK conducted research and wrote original manuscript; MA conceived the idea and supervised research experiments; NAR and IA helped in conducting the trials; AJ corrected the references and edited the manuscript; MJA thoroughly corrected and edited the manuscript; AH drew graphs of the manuscript; AN assisted in lab experiments; MM helped in data collection and rearrangement; WA performed statistical analysis.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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