# Antifungal Potential of Synthetic Chemicals and Phytoextracts towards Leaf Spot of *Pongamia pinnata*

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***Abstract-*** The "karum" tree, *Pongamia pinnata* (L.) Pierre is mainly cultivated in Punjab and Sindh, as well as in India, Burma, Malaya, Bangladesh, China, and Australia. It is indigenous to East and Tropical Asia. *P. pinnata* belongs to the Fabaceae family of plants, which can grow well in various weather conditions. It is one of the miscellaneous plants with medicinal properties, and its components are used as medicine in many regions to cure various diseases. It is vulnerable to various fungal diseases, including leaf spots, tar spots, blight rust, leaf rust, etc. The maximum disease was reported at maximum temperature (25-40°C) and relative humidity (50-80%). Hence, this study investigated the leaf spot disease and environmental factors involved in disease development. The main objective was the management of the disease through five chemicals, Mancozeb, Fostyl-aluminum, Thiophonate-methyl, Azoxystrobin, and Copper-oxychloride at three concentrations (50 ppm, 100 ppm, and 150 ppm), and five plant extracts *Azadirachta indica*, *Aleo barbadensis miller*, *Citrus reticulata*, *Moringa oleifera*, and *Eucalyptus camaldulensis* at three concentrations (3%, 5% and 7%) with three replications of each treatment under both in-vitro and in-vivo conditions. Among five chemicals and plant extracts Mancozeb (5.408 mm) and *Azadirachta indica* (0.3222 mm) showed minimum fungal growth. While the minimum disease incidence (%) was recorded by *Azadirachta indica* (8.168%) from plant extract and fungicides alone and in combination

***Index Terms***- Plant extract, environment, *Pongamia pinnata*, diseases, fungicides

## I. INTRODUCTION

*Pongamia pinnata* Pierre is a fast-growing leguminous tree (Huang et al., 2018). *P. pinnata* is a perennial legume tree with a short stem that may grow to 30-40 feet and provide moderate shade through its canopy (Huang et al., 2018). It is also known as Karanja and malapari tree as well as *Derris indica* and *Pongamia glabra* are the other names for *P. pinatata* (Daehler, 2018). Minerals, amino acids, and secondary metabolites are substantial in pongamia cake. Fatty acids are found in Pongamia oil, and nutrients are found in Pongamia leaves. The macro and micronutrients such as nitrogen, phosphorus, potassium, calcium, magnesium, zinc, copper, and iron in *P. pinnata* make it a good source of fertilizer for organic farming (Usharani et al., 2019). The entire plant has been used as a rudimentary medicine to cure tumors, piles, skin conditions, itches, abscesses, severe rheumatic joint wounds, ulcers, diarrhea, etc. (Anjana and Paulsamy, 2016). Additionally, it is well known for its use as a fish poison, green manure, timber, food, medicine, and animal fodder (Usharani et al., 2019).

It is mostly grown in Pakistan's two regions of Sindh and Punjab. The three primary areas for growth are Lahore, Karachi, and Islamabad. It is indigenous to Australia, Bangladesh, India, Myanmar, Nepal, Thailand, New Zealand, and Eastern and Tropical Asia. It is widely spread in China, Bangladesh, Australia, and India (Shaheen et al., 2020). Humid tropical and subtropical regions are the natural areas of *P. pinnata* (Wunderlin et al., 2020). Areas with an average annual rainfall of 500 to 2500 mm, a maximum temperature of 27 to 38°C, and a minimum temperature of 1 to 16°C are excellent for farming (Cooper et al., 2019). Probability spans from Subtropical Dry to Moist Forest Life Zones through Tropical Dry to Moist Forest Life Zones (Nadeem et al., 2016). There are abiotic factors that affect the growth of *P. pinnata* (temperature, light, water, humidity, and rainfall) as well as biotic factors (leaf spot, powdery mildew, leaf rust, and leaf blight) (Mohan, 2016).

The leaf spot of *P. pinnata* is one of the most crucial emerging threats to the successful growth of the Pongamia tree (Usharani et al., 2019). The size, shape, and color of leaf spots might vary based on the age and kind of cause of infection (Douglas, 2020). Spots on the leaves harm plants, shrubs, and trees by reducing the available foliar area for photosynthesis (Anembom et al., 2022). Leaf spot disease affects plants by causing brownish or black spots on plants' leaves (Douglas, 2020). The borders of the rings are often black and concentric.

Along with ovate to elliptical bi-celled conidia, measuring 15.64 -23.463.91 m and having a bright yellow color, black circular patches are also observed (6–10 mm diameter) on the ventral side and concurrent yellow dots on the dorsal surface (Rana and Brar, 2017). For the disease development, the maximum and minimum temperature ranges from 21.3 to 33.6 °C, relative humidity of 55-

94.3%, the average rainfall of 76 mm, and optimum pH of 7.3 or above are required (Fagodiya et al., 2022). During the growth season, the disease is dispersed throughout the canopy by wind and raindrops splashing against susceptible plant tissue (Giordano et al., 2021). Elongated acervuli conidiomata carried the conidia of *C. gloeosporioides*. The shape of the conidial varies, ranging from cylindrical to oblong to dumbbell, with an oil globule in the middle. The size of the obtained *Colletotrichum* isolates ranged from 9.6 mm to 12.2 mm in length and 3.8 mm to 4.7 mm in breadth (Nisha and Heera, 2016).

Several approaches, such as chemicals and plant extract, have been used to manage leaf spot disease. The use of chemicals to treat leaf spot disease is efficient since they are readily available and produce results quickly. (Watve et al. 2009), evaluated different fungicides against leaf spot disease, in which carbendazim, difeconazole, propiconazole, and copper oxychloride completely inhibit the growth of *Colletotrichum gleosporioides*. (Jayalakshmi et al. (2013) tested six different chemicals against *Colletotrichum gleosporioides*. Propiconazole and carbendazim showed the most effective antifungal activity against the growth of *Colletotrichum gleosporioides*. Mridha et al. (2007) investigated three fungicides against *Colletotrichum gleosporioides*. Cuprivate and Bavistin showed high and lowest mycelial growth at a concentration of 0.5. The results showed that Bavistin has the highest effectiveness compared to the other three fungicides, followed by Diathane M-45, while cupravite was unsuccessful due to its relatively poor mycelial growth inhibition.

Chemicals are the most effective technique for treating leaf spot disease. However, chemicals have some long-term negative consequences on human and animal health and the environment. As a result, it is necessary to implement new strategies for treating *Pongamia pinnata* leaf spot disease. A plant extract is a substance or active ingredient with the desired quality treated and extracted from plant tissue for a particular use. It is a complicated mixture of several phytochemicals, including phenolics, sugars, flavonoids, xanthones, and others. (Johnny et al., 2010) evaluated the antifungal activity of fifteen plant extracts on the pathogenic fungus *C. gleosporioides*. Five treatments were used in the antifungal experiment on potato dextrose media: distilled water as a negative control, crude leaf extract in methanol, chloroform, acetone, and benomyl as a positive control. The most effective and potent antifungal agents against *C. gloeosporioides* were *A. galanga* extracts (Nduagu et al., 2008) researched how certain plant extracts affect the development of various Colletotrichum species. To ascertain their impact on the colony diameter and sporulation of Colletotrichum species, crude extracts of the leaves, stems, and roots of *Annona senegalensis, Azadirachta indica, Chromolaena odorata, Citrus limon, Cochlospermum planchonii, Hymenocardia acids, Ocimum gratissimum, Psidium guajava* and *Ricin* were used. All leaf extracts had little effect on the Colletotrichum spp colony's diameter, while the stem bark and root bark extracts of *Azadirachta indica* and *Vernonia* amygdalina had significant antifungal activity. Kwodaga et al. (2019), investigated the aqueous and ethanol extracts of *Azadiratcha indica, Balanites aegyptiaca, Jatropha curcas, Khaya senegalensis* seeds, *Icacina oliviformis* leaves, and *Capsicum annuum* for their ability to inhibit *Colletotrichum gloeosporioides*. Each extract reduced the spore germination and mycelia growth of C. gloeosporioides (p .05).

## II. MATERIAL AND METHOD

***Collection and preservation of samples***

Leaf spot specimens were collected in polythene bags from the nursery of the Department of Forestry and Range Management. The samples were preserved at -4°C until they were processed in the Bacteriology Laboratory, Department of Plant Pathology, the University of Agriculture Faisalabad, to isolate the pathogen.

***Isolation, purification, and identification***

Leaf spot samples were collected in polythene bags from the nursery of the Department of Forestry and Range Management. The samples were preserved at -4°C until they were processed in the Bacteriology Laboratory, Department of Plant Pathology, the University of Agriculture Faisalabad, to isolate the pathogen.

Petri plates were filled with PDA medium. When the media become solidify, the sample pieces were placed in the media-containing Petri plates. For mycelium development, plates were incubated at 25-28°C for 24-48 hours. For fungal purification, the single spore technique was used. A single hyphae of mycelium was taken during sporulation and put on a petri dish with a PDA medium. The Petri plate was wrapped, labeled, and incubated. The fungal colonies that developed on inoculated tissue were white to grey mycelium encircled by white space. By using microscopy, it was possible to identify the pathogen by its morphological characteristics.

***Pathogenicity test***

*P. pinnata* leaves that were fresh and healthy after 30 days were collected and surface-sterilized for 2 minutes with 1% HgCl2 solution. The leaves were put in a sterile Petri dish and kept moist with wet blotting paper after two rounds of sterile distilled water washing. Then the conidial suspension was used to inoculate the leaves. The leaf was similarly inoculated with sterile distilled water to create a control set simultaneously. The Petri plates were kept at 25 °C for 5 days. The pathogen was re-isolated from the disease leaf and maintained in PDA.

***Evaluation of fungicides against Colletotrichum gloeosporioides in vitro***

Under a completely randomized design (CRD), five fungicides (Mancozeb, Fostyl-aluminum, Thiophonate-methyl, Azoxystrobin, and Copper-oxychloride) were tested at three concentrations (50, 100, and 150 ppm) with three replications of each treatment to check the antifungal activity of available chemicals on mycelial growth of isolated pathogen by using the poisoned food technique. The PDA was prepared. The stock solutions of each fungicide were prepared. An electronic weighing balance was used for weighing chemicals with the accurate amount, and added 100 mL distilled water. To achieve the desired concentration of fungicides, an accurate amount of stock solution was transferred into distilled water. To prepare 50, 100, and 150 ppm concentrations, 0.5 ml, 1 ml, and 1.5 ml of stock solution were added to 100 ml of distilled water. An amended PDA solution of 5 mL was added to a sterilized

petri plate (90 mm) and allowed to solidify. All plates were incubated in an incubator at 25±2°C. Data was collected using a vernier caliper and specified formulas (Pun et al., 2020).

Growth inhibition percentage (I) = $\frac{(C-T) \times 100}{C}$

Where

I = Percent growth inhibition

C = Mycelia fungal growth in control plate (mm)

T = Mycelia fungal growth in the treated plate (mm)

***Assessment of plant extracts against C. gloeosporioides by poisoned food technique under lab condition***

Under a completely randomized design (CRD), five plant extracts (i.e., *Azadirachta indica, Aleo barbadensis miller, Citrus reticulata, Moringa oleifera,* and *Eucalyptus camaldulensis*) were tested at specific concentrations (i.e., 3, 5 and 7%) with three replications of each treatment to check the antifungal activity of plant extracts on mycelial growth of isolated pathogen by using the poisoned food technique. After properly washing, cutting, and grinding each plant leaf, 50 g of powder from each plant was thoroughly mixed separately in a grinder with 250 ml of distilled water and 1 g of washing detergent. After that, the extracts were filtered and kept for 16 hours. Each plant extract was blended with 50 mL of distilled water. Before use, this was extracted as a 100 % basic stock solution and diluted in distilled water to the required concentrations. The final pure extracts that were obtained constituted the normal plant extracts with a concentration of 100%. The specific concentration of plant extracts added to distilled water was 100mL. Then with the help of a sterile cork borer, cut the 5mm circular disc of culture and transfer it into the new plate containing PDA.

Each treatment was replicated three-time and over the next seven days, the plates were incubated at 25± 2°C or until the untreated control plates were completely covered by the test pathogen's fungal growth. Data was collected using a vernier caliper and specified formula (Pun et al., 2020).

Growth inhibition percentage = (I) = $\frac{(C-T) \times 100}{C}$

Where

I = Inhibition of growth in percent

C = Mycelia fungal growth in control plate (mm)

T = Mycelia fungal growth in the treated plate (mm)

Table: Detail of each plant extract

| Botanical name | Plant tissues | Active ingredient |
|---|---|---|
| *Azadirachta indica* (Neem) | Leaf | Azadirachtin Nimbolin |
| *Aloe barbadensis miller* (Aleovera) | Leaf | Salicyclic acid Potassium silicate |
| *Moringa oleifera* (Moringa) | Leaf | Phenolic acid |
| *Citrus reticulate* (Citrus) | Leaf | Citric acid |
| *Eucalyptus camaldulensis* (Sufaida) | Leaf | Eucalyptol |

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## III. RESULTS

### *A. In-vitro assessment of plant extract against C. gloeosporioides*

Among all the 6 treatments, Neem showed minimum fungal growth (0.3222 mm) as compared to the control treatment (Fig. 1a). Among the interaction between concentration and treatments, the Neem showed minimum fungal growth (0.1444 mm, 0.3111 mm, and 0.5111 mm) followed by Sufaida (0.1533 mm, 0.36781 mm, and 0.66889 mm), Citrus (0.1900 mm, 0.2378 mm, and 1.021 mm), Aleo vera (1.1433 mm, 1.1967 mm, and 1.3222 mm), Moringa (1.3556 mm, 1.5556 mm, and 1.7444 mm) at 3%, 5%, and 7% concentrations respectively as compared to control treatment (Fig. 1b). The interaction between treatments and duration expressed that Neem showed the minimum fungal growth (0.2778 mm, 0.3222 mm, and 0.3667 mm) followed by Sufaida (0.3300 mm, 0.3311 mm, and 0.5489 mm), Citrus (0.4644 mm, 0.4789 mm, and 0.5056 mm), Aleo vera (1.156 mm, 1.2367 mm, and 1.2689 mm), Moringa (1.4667 mm, 1.5667 mm, and 1.622 mm) after 48, 72, 96 hrs. respectively as compared to control treatment (Fig. 1c)

### *B. In-vitro assessment of fungicides against C. gloeosporioides*

Among all the 6 treatments, Dithane showed minimum fungal growth (5.408 mm) followed by Aliette (6.964 mm), Amistar (9.880 mm), Topsin M (11.043 mm) and Blitox (13.786 mm), respectively as compared to control treatment (Fig. 2a). Among the interaction between concentration and treatments, Dithane showed minimum fungal growth (7.619 mm, 5.517 mm and 3.088 mm), followed by Aliette (9.252 mm, 7.066 mm and 4.576 mm), Amistar (12.196 mm, 9.793mm and 7.652 mm), Topsin M (13.346 mm, 10.909 mm and 8.873 mm), Blitox (16.049 mm, 13.508 mm and 11.801 mm) at 50 ppm, 100 ppm and 150 ppm concentrations respectively as compared to control treatment (Fig. 2b). The interaction between treatments and duration expressed that Dithane showed minimum fungal growth (6.879 mm, 5.011 mm and 4.333 mm), followed by Aliette (8.392 mm, 6.810 mm and 5.691 mm), Amistar (11.027 mm, 9.652 mm and 8.962 mm), Topsin M (12.316 mm, 10.579 mm and 10.053 mm), Blitox (15.04 mm, 13.724 mm, 12.590 mm) after 48 hrs, 72 hrs and 96 hrs respectively as compared to control treatment (Fig. 2c)

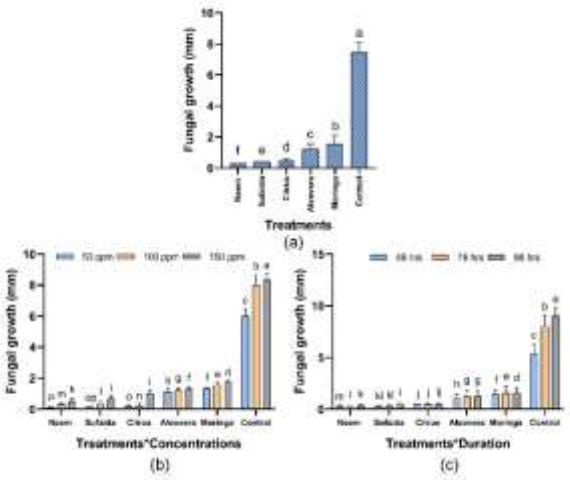


Fig. 1: Effect of plant extract on (a) fungal growth against *C. gloeosporioides*, (b) interaction between treatments and concentration, (c) interaction between treatments and duration

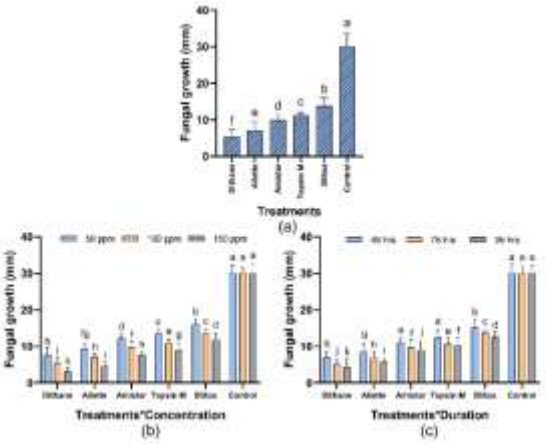


Figure 2: Effect of fungicides on (a) fungal growth against *C. gloeosporioides*, (b) interaction between treatments and concentration, (c) interaction between treatments and duration

***C. In-vivo assessment of fungicides and plant extract alone and in combination against C. gloeosporioides***

Among all the 4 treatments, Dithane + Neem showed maximum disease incidence (14.844 %), followed by Dithane (13.841%) and Neem (8.168%), respectively, as compared to the control treatment (Fig. 3a). Among the interaction between concentration and treatments, the Dithane + Neem showed maximum disease incidence (21.572%, 14.370% and 8.591%), followed by Dithane (18.599%, 13.344% and 9.580%) and Neem (12.341%, 8.478% and 3.684%) at 0.5%, 1%, and 1.5% concentrations respectively as compared to control treatment (Fig. 3b). The interaction between treatments and duration expressed that Dithane + Neem showed maximum fungal growth (16.872%, 14.981% and 12.680%), Dithane (15.436%, 13.722% and 12.366%) and Neem (9.701%, 8.239% and 6.563%) after 1, 2 and 3 weeks respectively as compared to control treatment (Fig. 3c)

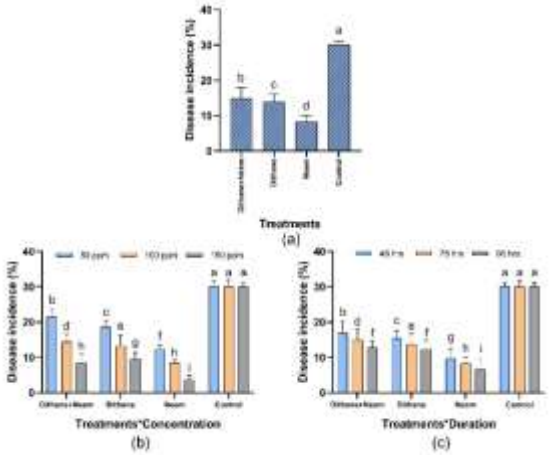


Figure 3: (a) Graphical representation of disease incidence, (b) interaction between treatments and concentration, (c) interaction between treatments and duration against *C. gloeosporioides*

## IV. DISCUSSION

Fungi, bacteria, viruses, nematodes, and various other organisms can cause plant diseases (Hussain et al., 2020). Fungicides in agriculture are used to combat fungus disease and fungal infections. They can be applied as seed treatments, in-furrow treatments, or foliar sprays. Among them are synthetics and non-synthetics (Shuping and Eloff, 2017; Thambugala et al., 2020). Biological control can also activate a plant's immune response. Plants have an innate immune system to defend themselves against pathogens (Joshi et al., 2020). Generally, fungicides inhibit spore germination and the growth of hyphae in fungi by blocking specific metabolic pathways.

Mechanisms that perform these different functions are known as "modes of action" (Velivelli et al., 2020). There have been various management strategies used for the control of leaf spot disease, such as chemicals, plant extracts, etc., but chemical control is the most effective because it is cheap, readily available, and gives quick results (Ons et al., 2020). Leaf spot disease has been controlled using different management strategies, such as chemicals, plant extracts, etc. Still, the chemical has proven to be the most effective since it is inexpensive, accessible, and gives results quickly (Bhagat and Kumar, 2022). The study was based on identifying the pathogen causing leaf spot disease of *P. pinnata* to find better management through fungicides and plant extract. Current research proves that *C. gloeosporioides* was the causal agent for *P. pinnata* leaf spot disease. The efficacy of fungicides is dependent on the dosage used, the sensitivity reaction of embattled fungus, and the storage of administration. The present research applied five chemicals Mancozeb, Fostyl-aluminum, Thiophonate-methyl, Azoxystrobin, and Copper-oxychloride, to estimate their antifungal potential against the leaf spot of *P. pinnata*. The result indicated that Dithane (mancozeb) showed the best result and significantly reduced the mycelial growth of *C. gloeosporioides*. Mridha et al. (2007) reported that spraying Dithane-45 (mancozeb) effectively treated leaf spot disease caused by *C. gloeosporioides.*

In managing plant disease, most chemicals are effectively used but are injurious to human beings and plant and animal vigor (Kumar et al., 2019). On the other hand, plant extracts are believed to be botanical pesticides that can replace chemicals due to their non-harmful effects on human, plant, and animal health (Dev et al., 2016). Plants contain several bio toxicants which may be useful against the pathogen (Dev et al., 2016). Antifungal properties of several plants have been reported by several researchers (Hu et al., 2019).

Natural materials such as plant extracts and microbes have been proposed as alternatives to synthetic pesticides. Due to their high efficacy, plant extracts are very promising alternatives. Natural plant extracts have few or no health concerns since they are derived from natural resources because they are low-cost, risk-free, nontoxic, and ecologically friendly. Preservatives and pesticides from plant extracts can be a good alternative to chemical ones (Shahbaz et al., 2022). The study's main objective was also to find better management of *C. gloeosporioides* by using plant extract and chemicals. In the present research, five plants extract, Neem, Sufaida, Citrus, Aleo vera, and Moringa, were applied to evaluate the antifungal potential against leaf spots of *P. pinnata*. The results indicated that Neem showed the least amount of mycelial fungal growth and produced the best control results. Vegetables, grains, legumes, and perennial crops are only a few examples of Colletotrichum's enormous variety of hosts. *Azadirachta indica and Nicotiana tabacum* ethanol extracts are antifungal against Colletotrichum (Haider et al., 2020). *Azadirachta indica* and *Nicotiana tabacum* plant extracts are safe and effective alternatives to conventional fungicides (Haider et al., 2020). The above-mentioned plants act as a preservative, exhibit antibacterial activity against some fungal and bacterial species, and act as an agriculture pest control agent (Ahmed et al., 2022).

Chemicals are well understood to be hazardous to human plant and animal health and are not environmentally friendly, but when fungal infections develop rapidly in the field, there is no option other than the application of fungicides (Veliz et al., 2017). On the other hand, plant extracts are biodegradable and environmentally friendly. They have antimicrobial properties (Patil and Kim, 2017). In the research described above, certain chemicals and plant extracts were analyzed. Dithane chemical and Neem plant extract showed significant results against leaf spots of *P. pinnata* under in vivo conditions. Neem recorded the lowest disease incidence, and the highest disease incidence was recorded by the Dithane+Neem combination.

## V. CONCLUSION

It is concluded that synthetic drugs and phytoextracts were shown to have varied degrees of antifungal activity against the pathogen responsible for the disease. The maximum antifungal activity was identified in the case of Dithane among the synthetic chemicals, while the highest antifungal activity was observed in the case of neem extract among the phytoextracts. The study demonstrates the potential of using synthetic chemicals and phytoextracts as affordable alternatives to chemical fungicides to manage *P. pinnata* leaf spot disease. Furthermore, the study provides valuable insights into the mode of action of the tested synthetic chemicals and phytoextracts, which could help in the development of more effective antifungal agents. The results also indicate that the antifungal activity of the tested compounds is concentration-dependent, which could have practical implications for their use in the field. Notably, the tested synthetic chemicals and phytoextracts were found to be safe for the environment and human health, making them sustainable options for disease management. Despite the promising results, further research is needed to optimize the application methods and concentrations of the tested compounds for their effective use in the field. Moreover, future studies should evaluate the long-term effects of synthetic chemicals and phytoextracts on plant growth and productivity. Overall, the study provides a foundation for developing sustainable and effective management strategies for leaf spot disease in *P. pinnata*.

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