Received: 15 November 2022 | Accepted: 19 January 2023 | Published Online: 03 April 2023
Recoletos Multidisciplinary Research Journal Vol. 11 no. 1 (June 2023)
DOI: https://doi.org/10.32871/rmrj2311.01.02

Bio-efficacy of *Trichoderma harzianum* Rifai and Botanical Extracts against Major Fungal Pathogens of Tamarind (*Tamarindus indica* L.) under Field Condition

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Abstract

Domestic production of tamarind has decreased in the country due to diseases. Tests were made on the individual efficacy of botanical extracts like plant growth promoter (PGP) and oriental herb nutrient (OHN), as well as the Trichoderma harzianum Rifai as a possible biocontrol agent against major fungal tamarind diseases. Using Randomized Complete Block Design (RCBD), a field setup was established with five treatments: untreated (negative control), synthetic fungicide, OHN, PGP, and T. harzianum having three replications per treatment. Results revealed that T. harzianum is an effective biocontrol agent against major fungal tamarind diseases. PGP having similar efficiency with T. harzianum, significantly inhibited the infection and provided immediate defense from diseases. OHN is less effective due to delayed reduced infection. The research output is a significant help in the management of various diseases that is cost-effective and safe.

Keywords: biocontrol, botanical extracts, major fungal diseases, tamarind, Philippines

1.0 Introduction

Tamarind, scientifically known as *Tamarindus indica Linnaeus*, is a leguminous fruit-bearing tree and is widely distributed in Africa and Asia. It is extensively utilized for its fruit. It can be consumed as raw or cooked, used as a condiment or spice, or the fruits and seeds can be processed for non-food purposes (El-Siddig et al., 2006). To date, there is low domestic production of tamarind in the country. In the Crop Statistics of the Philippines (2016-2020), it was shown that the volume of production in 2016 at 7,128 mt continued to plummet down to 6,259 mt in 2020 (Philippine Statistics Authority, n.d., p.12).

Gatan (2021) conducted an initial identification and assessment of major phytopathogens of tamarind leaves in Central Luzon, Philippines, revealing *Colletotrichum* leaf spot (100%), *Cercospora* leaf spot (93.75%), black leaf spot (100%), and powdery mildew (100%) are the major occurring fungal diseases. Like any other fruit tree, tamarind, when infected with disease, can result to yield reduction and post-harvest losses. Tamarind is susceptible to various fungal diseases. An estimated 40% yield reduction is recorded due to plant diseases particularly leaf spot, powdery mildew, sooty molds at flowering to maturity stage, and white molds on fruits during pre- and post-production. This is strongly supported by the recent findings of Gatan (2021) and further suggests that plant disease identification and monitoring of its occurrences particularly noting its incidence and severity. Assessment of these parameters are vital in the quantification of disease estimates and yield loss to come up with an effective with an effective management strategy.

In the market, synthetic fungicides have been considered as one of the most commonly available control measures. However, high reliance on synthetic fungicides has negative consequences on the environment, animals, and consumers. Use of biological control agents instead of chemicals in controlling fungal diseases of plants are rather deemed more effective because of their ability to interact with crop plants and their secondary metabolites that accounts for their biocontrol capabilities (Elnahal et al., 2022). This increases their defense against diseases, as well as their growth and development. Prior to the alteration of natural control by synthetic chemicals that are lethal in an environmental context, plant-based fungicides also offer an alternative. Among farmers, adaptability in the use of botanicals is more acceptable which has already established a tradition of use in the early days.

Biological control is a nature-based solution designed to be an alternative measure in controlling plant diseases. *Trichoderma harzianum Rifai* is one of the most commonly used microorganisms as a biocontrol agent. Another species of *Trichoderma* is the *Trichoderma viride Pers* is also used for seed and soil treatment to inhibit different diseases caused by fungal pathogens. Through methods of competition, antibiosis, and mycoparasitism, *Trichoderma* fungi can combat plant diseases. This confers from the pattern of pathogen-host plant interaction from different crops through induction of antifungal compounds, secondary metabolites, mycelial and hyphal changes or destruction and nutrient competition (Anal et al., 2020). Recent studies associated with *Aspergillus niger* van Tieghem infection, *T. harzianum* exhibited maximum mycelial growth inhibition of 65% - 77.67% using dual culture method (Gawarkar et al., 2022; Ren et al., 2022; Saran et al., 2022). Likewise, it is also effective in the inhibition of *Colletotrichum spp.* at a range of 81%-87.8% (Abdulkadir et al., 2022; Muliani et al., 2022; Triasih et al., 2022). Antagonistic potential is also seen against *Fusarium spp.* in mycelial inhibition (Davari & Ezazi, 2022) and further testing of biological management through in vitro and *in planta* under inoculated conditions (Hussain et al., 2016) revealed its efficiency as a biocontrol agent through enzymatic secretions that enabled cell wall degradation (Davari & Ezazi, 2022). Another study specifically involving *Podosphaera xanthii*, which causes powdery mildew, effectively controlled by *Trichoderma spp.* when applied (Hafez et al., 2018).

The possible threats of synthetic fungicides to the environment and to the consumers over the past year have been a growing interest in the investigation of natural materials as sources of new fungicidal properties (Redo et al., 1989) which may solve crop losses. The utilization of botanical extracts such as Oriental Herbal Nutrient (OHN) (Abdullahi et al., 2020; Agi & Azike, 2019; Darmadi et al., 2021; Habsah et al., 2000; Moenne & González, 2021; Petnual et al., 2010; Sani & Yong, 2022; Sarfraz et al., 2020), and Plant Growth Promoter (PGP) are some of the alternatives to suppress disease infections from *Aspergillus*, *Colletotrichum, Fusarium*, and *Podosphaera* species. PGP acts through the induction of chitinase that degrades fungal cell walls (Soares et al., 2016). The mode of action of herbal extracts is through the alteration or degradation of the structure of the cell wall. This will cause an imbalance between the components contained within and outside the cell. The cell contents will lyse and eventually cause fungal cell death (Darmadi et al., 2019).

Fresh ginger root (*Zingiber officinale* Roscoe), turmeric root (*Curcuma longa* L.), garlic cloves (*Allium sativum* L.), licorice root (*Glycurrhiza uralensis*

Fisch. ex DC.), cinnamon bark (*Cinnamomum* spp.), vinegar, and molasses are among the herbs needed in the preparation of OHN. Likewise, gin is also added to hasten the extraction of the bioactive compounds present in the plant. (Castleman, 2010; Chow, 2002; Jayaprakasha & Rao, 2011; Maekawa et al., 2013; Ming & Yin, 2013; Sarker & Nahar, 2004; Yadav et al., 2013). Compounds from these combined extracts have been found to contain antifungal properties (Cho et al., 2006; Darmadi et al., 2021; Habsah et al., 2000; Hojo & Sato, 2002; Petnual et al., 2010; Sarfraz et al., 2020; Teles et al., 2020). Some natural materials from different extracts have been tested and approved as new fungicidal agents. Knowing the antifungal properties of these extracts, as well as the *Trichoderma harzianum* as biocontrol agent to some diseases, this study will serve as a baseline information of its potential to minimize or prevent various plant diseases that can be used by our growers and farmers. Hence, this study was conducted to determine the bio-efficacy of *T. harzianum*, PGP, and OHN against major fungal diseases of tamarind.

This study aimed to determine the individual efficacy of PGP and OHN as botanical extracts as well as the *T. harzianum* as possible biocontrol agent for the suppression of fungal tamarind diseases specifically, Black leaf spot (*Aspergillus niger* van Tieghem), Fusarium leaf spot, *Colletotrichum* leaf spot, and powdery mildew (*Podosphaera* sp.)

2.0 Methodology

The study utilized the experimental method of research where Randomized Complete Block Design (RCBD) was used for the setup. Initially, an equal distribution for the selected 60 disease-free seedlings divided into 15 seedlings per setup. Subsequently, disease infection was adapted from the methods of Gatan (2021) following Koch's Postulates. A total of 4 setups categorized according to the 4 identified fungal disease infections with five treatments as follows: Untreated (negative control), synthetic fungicide, oriental herb nutrient, plant growth promoter, and *T. harzianum* having 3 replications each. Commercial fungicide was used along with 2 organic extracts and 1 biocontrol agent. The efficiency of treatments were evaluated through statistical comparison with the control group (untreated) through a series of verification trials under field conditions.

Experimental site and location

The study was conducted under field conditions at the Crop Science Department of the Pampanga State Agricultural University, PAC, Magalang, Pampanga, Philippines, from October 2020 to December 2020.

Treatments

Sixty disease-free tamarind seedlings were randomly selected from the Tamarind Nursery of Pampanga State Agricultural University PAC, Magalang, Pampanga, Philippines for the set-up under field conditions. Distribution of 15 seedlings ranging from 5-6 months of age on each of the 4 setup was done. Allotment of 3 seedlings as replications per treatment was arranged accordingly. The following are the treatments:

T_1 – Untreated (Negative Control)
T_2 – Synthetic Fungicide
T_3 – Oriental Herb Nutrient (OHN)
T_4 – Plant Growth Promoter (PGP)
T_5 – *Trichoderma harzianum*

Disease inoculation

For the disease inoculation process, methods of Gatan (2021), was adopted with modifications with reference to wounding methods (Savatin et al., 2014) which provides nutrients to pathogens and facilitates their entry into the plant tissue. In the procedure, through the use of a sterilized needle, the leaf surface of 60 disease-free seedlings were pricked to create wounds which served as the entrance of the pathogen and stimulate the infection process.

Subsequently, seven-day-old pure cultures of major fungal tamarind diseases (*Aspergillus* leaf spot, *Fusarium* leaf spot, *Colletotrichum* leaf spot, and powdery mildew) were inoculated individually on the 60 disease-free seedlings divided into 4 setups, respectively. Each setup having 15 pricked healthy seedlings were infected with each disease using cotton swabs dipped on fungal spore solution prepared initially prior to inoculation. Thereafter, all 60 disease-inoculated seedlings were enclosed with plastic covering for 7 days to provide a suitable environment for fungal disease development. Each seedling was submerged in water with the use of a plastic basin to aid in the development of the disease as fungal species tend to thrive faster on moist environment.

Treatment Preparation, Application and Disease Assessment

Application on 15 seedlings per treatment was applied using spray bottles individually containing water, inorganic fungicide, OHN, PGP and BCA at a weekly interval. Specifically, assessment was done at initial and simultaneous at every interval. Consequently, treatments were applied during the morning between 8:00am to 9:00am. Farmer's rate of application for the inorganic (approximately 3.5g per 1L of water) fungicides was adopted in the course of the study trial.

OHN

In the preparation of OHN, the method of Chang et al. (2014) were adopted with minimal modifications. The treatment prepared consists of herbs extracted with liquor and allowed to ferment separately with the addition of brown sugar/ molasses. Fresh ginger (1kg) and garlic (1kg) sliced and mixed with 1L of molasses and 1L of vinegar. The container with the mixture was then tightly sealed with masking tape. It was left to ferment for 3-5 days. After the first stage of fermentation, 1L of gin was added and the mixture was sealed and left for 10 days. Subsequently, the extracted liquid was transferred to another container, 1kg chili was then added and was set aside for another 10 days. The extraction procedure was repeated 2 times until use.

The extracted liquid contains 1000ppm garlic, 435ppm of ginger, 356,000,000ppm of molasses, and 40,000ppm of gin. Mixture was diluted to 0.5ml of extract per 100ml of water for 50ppm concentration and 0.10ml of extract per 100ml for 100ppm concentration and applied using spray bottles. Approximately each seedling received 10-15ml of OHN treatment at 50 and 100ppm concentrations. For field application, 1tbsp or 2tbsp of OHN in 1gal of water is equivalent to dilution of 0.5ml/100ml and 0.10ml/100ml water, respectively.

PGP

Readily available PGP was used and diluted at 100 and 150ppm concentration rates. Each seedling applied with the treatment received an approximate equivalent of 10-15ml of diluted extract at 100 and 150ppm concentrations per plant.

Trichoderma harzianum

Spore suspension of *T. harzianum* was prepared using distilled water and harvested viable spores. Approximately, 10^8/ml of spores was counted using the Neubauer counting chamber and was applied on each seedling.

Data gathering

In the gathering of data, the following parameters were used to obtain results:

1. **Disease Severity**

   The severity of each particular disease was assessed to determine the performance and efficacy of the treatments applied using the subsequent scale rating below:

Scale Description

1 - No infection or absence of any leaf disease

2 - 1-5% infection, presence of leaf spot/ sooty mold/black spot/ powdery mildew in 1-5% surface area of the leaf

3 - 6-25% infection, presence of leaf spot/ sooty mold/black spot/ powdery mildew in 6-25% surface area of the leaf

4 - 26-50% infection, presence of leaf spot/ sooty mold/black spot/ powdery mildew in 26-50 % surface area of leaf

5 - More than 50% infection, more than 50% of the surface area of the leaf is infected with leaf spot/sooty mold/ black spot/ powdery mildew

2. **Percentage of Disease Infection Reduction**
   The percentage of disease infection reduction (DIR) was determined using the formula below:

$$\% \text{ DIR} = \frac{\textit{initial disease infection} - \textit{final disease infection}}{\textit{initial disease infection}} \times 100$$

3. **Agrometeorological data**
   Agrometeorological data such as temperature (minimum and maximum), relative humidity, and rainfall were gathered from DOST-PAGASA at Clarkfield, Pampanga, during the whole duration of the study. The data was used to correlate disease occurrence.

Statistical Analysis

Analysis of Variance (ANOVA), Kruskal-Wallis, and Post Hoc Dunn test were used to statistically analyze the data. ANOVA was used to measure the differences between means of the five treatment groups. Post Hoc Dunn test was also used to determine which specific means are different on the multiple comparison test of the treatments.

3.0 Results and Discussion

Bio-efficacy Trial on Selected BCA under Field Condition

Statistical data were analyzed using Post hoc Dunn test on samples to compare multiple means of the five treatment groups and determine significant differences to answer which treatments are effective compared with the untreated group (negative control). Infected seedlings with black leaf spot, *Colletotrichum* leaf spot, and *Fusarium* leaf spot that were left untreated revealed that the inorganic fungicide has a significantly higher score than untreated (negative control) from week 1 up to week 10 ($p<0.05$) (Tables 1, 3-4). Moisture and temperature have a significant impact on fungi growth (Talley et al., 2002); hence, weather conditions with a minimum temperature range of 24.0-24.5°C, maximum temperature range of 31.0-31.6°C, 66-72°C RH range, and average rainfall ranging from 26.2-312.2mm (taken from DOST-PAGASA at Clarkfield, Pampanga) during the whole duration of the research (October 2020 to December 2020), significantly support the growth and development of the fungal infection. This signifies that the inorganic fungicide used is sufficiently effective in the control of major fungal diseases as it successfully managed the diseases.

Likewise, the effectiveness of *T. harzianum* against black leaf spot, powdery mildew, *Colletotrichum* leaf spot, and *Fusarium* leaf spot as a potential biocontrol agent was statistically observed consistent and efficient (Tables 1-4). Disease infection reduction was seen from week 2 to week 8 for black leaf spot (Figure 1). Manifestations of optimum resistance appeared at week 9 but eventually halted at week 10 (Figure 1). For powdery mildew, the disease infection trend was observed on week 3 to week 10 (Figure 2). *Colletotrichum* leaf spot disease infection suppression was noted at week 3 to week 6 as it developed an optimum resistance at week 7 (Figure 3). Although, the trend lowered at week 8, the disease infection was consistent from week 9 up to week 10 (Figure 3). Lastly, Figure 4 shows the *Fusarium* leaf spot reduction at week 2 to week 4. Likewise, minimal resistance was observed at week 5 to week 6 but with a lowering trend pattern at week 7 to week 9 (Figure 4). The mycoparasitism ability of *T. harzianum* is reduced due to the induction of systemic resistance in plants which is a primary method of pathogen control that ensues through the capability of the fungi to reprogram plant gene expression (Shoresh & Harman, 2008).

The analysis proves that the application of *T. harzianum* Rifai significantly effected on the disease suppression (Figure 5). Significantly manifested on the disease symptoms observed in untreated black leaf spot having circular black spots vs the treated black leaf spot with reduced black spots (Figures 5a and 5b); untreated powdery mildew having powdery patches vs the treated powdery mildew with lesser powdery patches (Figures 5c and 5d); untreated *Colletorichum* leaf spot with black patches spread out on the front and back surface of leaflets vs the treated *Colletorichum* leaf spot with decreased black patches (Figures 5e and 5f); and lastly, *Fusarium* leaf spot forming yellowish halo with a brownish spot on the center vs the treated with less appearance of the leaf spot (Figures 5g and 5h).

The result conforms to the report of Gwa and Ekefan (2017) indicating that the antagonist has the potential for biological control of *Colletotrichum* spp., and when *T. harzianum* tested against *Fusarium* spp., a significant disease reduction, was obtained in cotton, melon and tomato (Panwar et al., 2014; Sivan & Chet, 1986). As a biological control agent, it is potent and can be used to combat a variety of agricultural diseases of various crops. Furthermore, it can also be used to reduce the use of inorganic fungicide and lessen chemical dependency to combat fungal diseases of tamarind.

Table 1. *Dunn's Post Hoc Comparisons on black leaf spot*

Comparison of treatments	*p-values*									
	BLS WK1	BLS WK 2	BLS WK3	BLS WK4	BLS WK5	BLS WK6	BLS WK7	BLS WK8	BLS WK9	BLS WK10
Negative Control - IF	0.021	< .001	0.003	0.001	0.002	0.002	0.002	0.003	< .001	0.002
Negative Control - OHN	0.244	0.321	0.319	0.257	0.161	0.186	0.051	0.003	0.004	0.002
Negative Control - PGP	0.391	0.035	0.003	0.007	0.002	0.002	0.002	0.003	0.056	0.017
Negative Control - TH	0.409	0.035	0.066	0.035	0.011	0.009	0.051	0.071	0.106	0.002

Table 2. *Dunn's Post Hoc Comparisons on powdery mildew*

Comparison of treatments	*p-values*									
	PM WK1	PM WK2	PM WK3	PM WK4	PM WK5	PM WK6	PM WK7	PM WK8	PM WK9	PM WK10
Negative Control - IF	0.123	< .001	0.002	0.002	0.003	0.003	0.003	0.002	0.002	0.003
Negative Control - OHN	0.002	0.008	0.06	0.06	0.061	0.019	0.061	0.002	0.002	0.012
Negative Control - PGP	0.154	0.069	0.027	0.027	0.015	0.015	0.015	0.017	0.002	0.017
Negative Control - TH	0.123	0.106	0.049	0.049	0.003	0.003	0.003	0.002	0.002	0.017

Table 3. *Dunn's Post Hoc Comparisons on Colletotrichum leaf spot*

Comparison of treatments	*p-values*									
	LSC WK 1	LSC WK2	LSC WK 3	LSC WK 4	LSC WK 5	LSC WK 6	LSC WK 7	LSC WK 8	LSC WK 9	LSC WK 10
Negative Control - IF	0.002	< .001	0.001	< .001	0.002	0.005	0.004	0.004	0.003	0.002
Negative Control - OHN	0.021	0.039	0.082	0.04	0.016	0.117	0.01	0.015	0.003	0.009
Negative Control - PGP	0.059	0.032	0.02	0.07	0.214	0.09	0.054	0.024	0.02	0.019
Negative Control - TH	0.04	0.083	0.031	0.029	0.035	0.005	0.318	0.25	0.05	0.051

Table 4. *Dunn's Post Hoc Comparisons on Fusarium leaf spot*

Comparison of treatments	*p-values*									
	LSF WK 1	LSF WK2	LSF WK 3	LSF WK 4	LSF WK 5	LSF WK 6	LSF WK 7	LSF WK 8	LSF WK 9	LSF WK 10
Negative Control - IF	0.006	0.001	0.003	0.003	0.004	0.013	0.012	0.013	0.031	0.007
Negative Control - OHN	0.006	0.004	0.003	0.003	0.004	0.013	0.012	0.013	0.031	0.007
Negative Control - PGP	0.112	0.08	0.179	0.179	0.059	0.004	0.036	0.019	0.039	0.012
Negative Control - TH	0.112	0.08	0.04	0.04	0.084	0.079	0.024	0.024	0.002	0.068

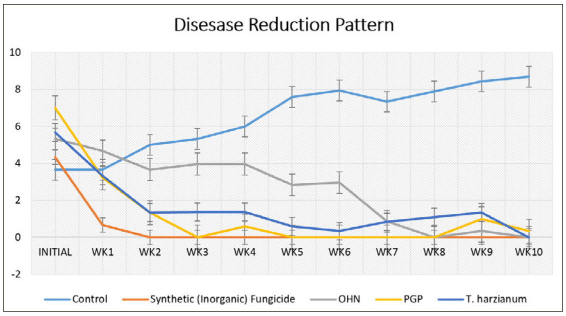

Figure 1. *Disease reduction pattern on all treatments against black leaf spot*

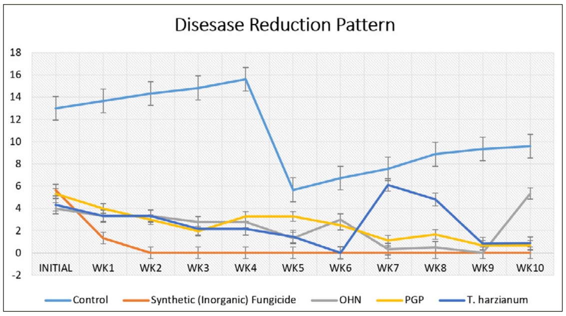

Figure 3. *Disease reduction pattern on all treatments against Colletotrichum leaf spot*

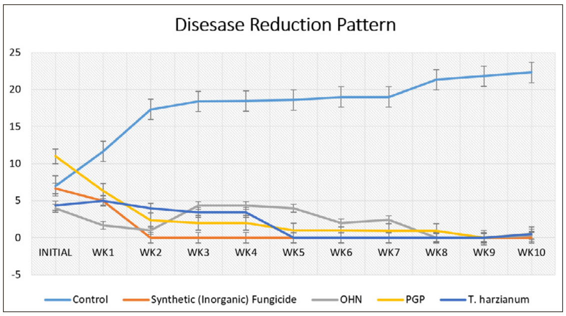

Figure 2. *Disease reduction pattern on all treatments against powdery mildew*

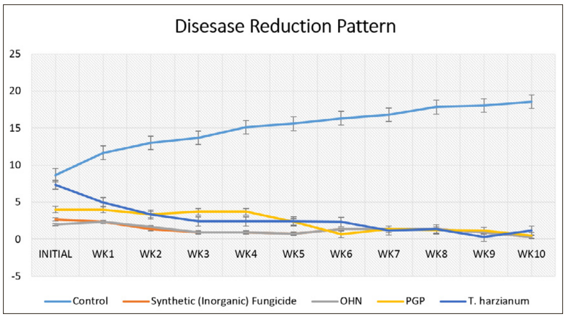

Figure 4. *Disease reduction pattern on all treatments against Fusarium leaf spot*

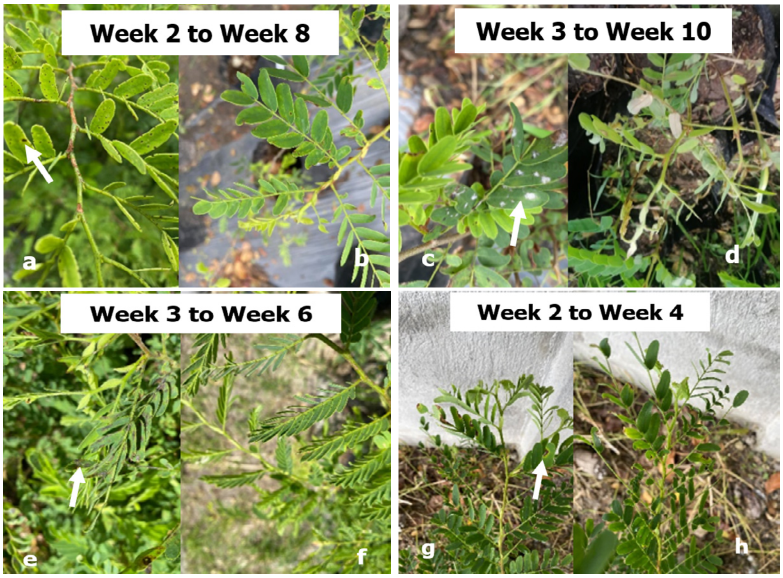

Figure 5. *Representations of disease infection reduction on seedlings treated with T. harzianum: a) black leaf spot infected - untreated; b) black leaf spot - treated; c) powdery mildew infected; d) powdery mildew reduced; e) Colletotrichum leaf spot infected; f) Colletotrichum leaf spot reduced; g) Fusarium leaf spot infected; h) Fusarium leaf spot reduced*

Bio-efficacy Trial on Selected Extracts under Field Condition

The application of PGP to major fungal diseases of tamarind shows that it is an effective control measure. Statistical analysis revealed that samples infected with black leaf spot, *Colletotrichum* leaf spot, and *Fusarium* leaf spot, treated with the negative control vs the inorganic fungicide has revealed that the inorganic fungicide has a significantly higher score than untreated (negative control) from week 1 up to week 10 ($p<0.05$) (Tables 1 and 3-4).

Correspondingly, as shown in Figure 1, upon PGP application, black leaf spot disease infection was inhibited from week 2 to week 8 but with reduced inefficacy at week 9-10. The disease infection trend for powdery mildew continuously increased at week 3 up to week 10 (Figure 2). As exhibited on Figure 3, the Colletotrichum leaf spot infection was efficiently suppressed only at weeks 8-10. Although there was an evident decreased infection at weeks 2-3, but with fluctuating efficiency was recorded at week 4 up to week 7 (Figure 3). PGP was significantly potent against Fusarium leaf spot at weeks 6-10 (Figure 4). Generally, PGP significantly reduced the disease infection of the four major fungal pathogens of tamarind. Efficacy is linked to the capability of PGP extract to provide secondary metabolites that promotes better plant growth and tolerance to abiotic stresses (Moenne & Gonzalez, 2021). Likewise, environmental conditions possibly affects fungal disease development wherein the recorded minimum temperature ranges from 24.0-24.5°C while the maximum temperature range from 31.0-31.6°C having 66-72°C RH, and average rainfall of 26.2-312.2mm. The recorded environmental conditions favored fungal development. According to Talley et al. (2002), relative humidity is the most important environmental factor affecting plants' occurrence and severity of fungal infections. In spore germination and infection, the high relative humidity for several hours of free surface water is essential (Harrison et al., 1994). Infection (i.e., fungus invasion of plant tissue) and disease (i.e., the manifestation of symptoms such as lesions or necrosis) on plants caused by airborne fungi are also promoted by temperatures of 15–40°C (Agrios, 1988; Cooke & Whipps, 1993).

The efficiency of OHN against black leaf spot and powdery mildew was noted on week 8 to week 10 (Figures 1-2). Although a significant decrease was recorded at weeks 1-2 and week 6 for the powdery mildew, an unstable pattern of disease reduction was noted. *Colletotrichum* leaf spot was controlled from weeks 7-10 and a significant reduction at weeks 1-2 and 4-5 (Figure 3). Remarkably, *Fusarium* leaf spot was effectively reduced from weeks 1-10 (Figure 4).

PGP and OHN are both promising extracts for significantly reducing fungal diseases on tamarind (Figure 7). It is evident in the disease symptoms seen in untreated black leaf spot, which has circular black spots, compared to the treated black leaf spot, having reduced black spots (Figures 6a and 6b; Figures 7a and 7b); untreated powdery mildew, which has powdery patches, compared to the treated powdery mildew, having fewer powdery patches (Figures 6c and 6d; Figures 7c and 7d); and untreated *Colletorichum* leaf spot, which has black patches scattered across the front and back surfaces of leaflets (Figures 6e and 6f; Figures 7e and 7f). Finally, untreated *Fusarium* leaf spot, which forms a brownish area in the center with a yellowish halo around vs the treated with decreased formation of the leaf spot (Figures 6g and 5h; Figures 7g and 7h).

Both contain compounds that elicit pathogen protection through the ability to degrade fungal cell walls (Agi & Azike, 2019; Darmadi et al., 2019; Moenne & González, 2021; Soares et al., 2016). However, PGP is notably valued as more effective than OHN at reducing disease infection. It reduces black leaf spot, powdery mildew, *Colletotrichum* leaf spot, and *Fusarium* leaf spot infections after a range of 1-3 weeks of application. It continues to provide protection until the 10th week, unlike OHN, which takes 7 weeks to show a positive response to the disease infection with the exception of *Fusarium* leaf spot infection that was immediately controlled at week 1.

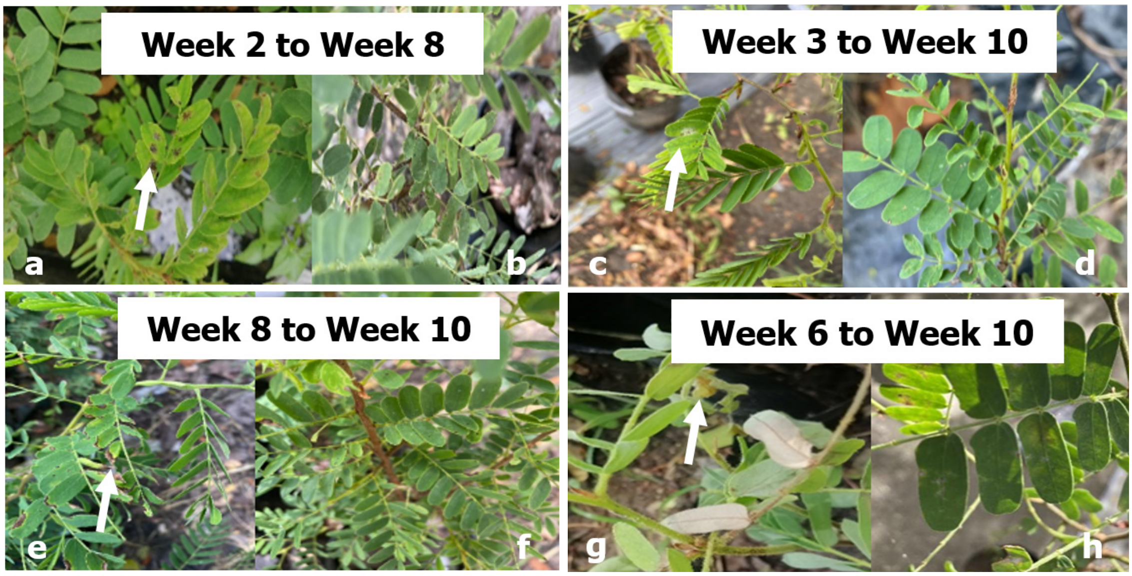

Figure 6. *Representations of disease infection reduction on seedlings treated with PGP: a) black leaf spot infected - untreated; b) black leaf spot - treated; c) powdery mildew infected; d) powdery mildew reduced; e) Colletotrichum leaf spot infected; f) Colletotrichum leaf spot reduced; g) Fusarium leaf spot infected; h) Fusarium leaf spot reduced*

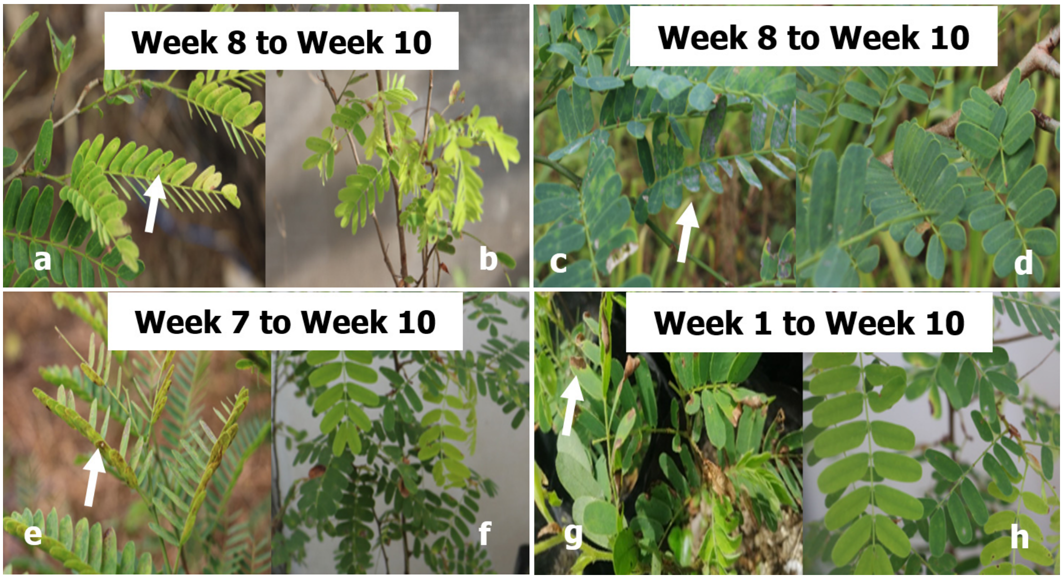

Figure 7. *Representations of disease infection reduction on seedlings treated with OHN: a) black leaf spot infected - untreated; b) black leaf spot - treated; c) powdery mildew infected; d) powdery mildew reduced; e) Colletotrichum leaf spot infected; f) Colletotrichum leaf spot reduced; g) Fusarium leaf spot infected; h) Fusarium leaf spot reduced*

4.0 Conclusion

From the results, it is evident that the *T. harzianum* is an effective biocontrol agent against black leaf spot, powdery mildew, *Colletotrichum* leaf spot, and *Fusarium* leaf spot of tamarind. In the same way, among the extracts used, PGP efficiently inhibited and provided immediate protection against the major fungal diseases of tamarind. OHN can also be used as an alternative control; however, its efficacy can be observed after some time. Thus, *T. harzianum* and PGP (vs the negative control) is recommended as cost-effective measures in the management of tamarind fungal diseases because of its low-cost, availability, and fast-action disease infection reduction. Meanwhile, to achieve the optimum efficiency, *T. harzianum* and PGP should be applied when there is no strong wind or rain to avoid leaching and preferably in the morning or late in the afternoon. The use additional botanical extracts and other microorganism with promising antifungal properties is needed to come up with an alternative management measure in controlling plant disease that can be cost-effective as well as environment and consumer safe.

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