



Original Paper

Integrated Disease Management, Combining Chemical and Bio-control Agents, is Effective Against *Agroathelia rolfsii* Infecting Eggplants

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Abstract— The seeds of *Guibourtia coleosperma* serve as potential *Agroathelia rolfsii* severely infects 500 plant species causing stem and root rots on plants including eggplants. This study evaluated the compatibility of integration of chemical fungicides x biological agents for the management of recalcitrant disease agents like *A. rolfsii*. A factorial trial was set up using the completely randomized design with each treatment being replicated thrice. Interaction of Mancozeb (at 50 and 100% concentrations) x *Trichoderma* species caused 29.3-100% inhibition of *A. rolfsii* and Ketoconazole (at 50 and 100% concentrations) x *Trichoderma* species caused 95.8-100% inhibition of *A. rolfsii*. Mancozeb (100% concentration) x *T. harzianum* controlled the pathogen most, followed by the *T. viride* combination. The percentage inhibition by chemical fungicide (main effects) ranged from 100% inhibition by Ketoconazole (100% concentration) to 97.9-100% for Ketoconazole (50% concentration), to 65.2-93.6% for Mancozeb (100% concentration) and lastly 23.3-78.6% for Mancozeb (50% concentration) with time. The percentage inhibition (main effects) ranged from 60.3-96.7% for *T. virens*, 60.8-92.1% for *T. harzianum*, and 69.3-95.8% for *T. viride* with time. The chemical fungicides and *Trichoderma* species were highly positively correlated (0.522** at $p \leq 0.05$). The high concentrations of the fungicides antagonized the *Trichoderma* species as well, so more work should be carried out on this aspect. This approach to disease management for eggplants is highly effective using chemical x bio-control agents to safeguard eggplants from *A. rolfsii*. Applying this method can protect crops and result in long-term profitability. The combination of fungicides and biocontrol agents is strongly recommended for the management of this fungal pathogen.

Keywords— *Athelia rolfsii*, Aubergine, *Sclerotium* rots, *Solanum melongena*, *Trichoderma* species

I. INTRODUCTION

Eggplant or brinjal (*Solanum melongena* L. in the family Solanaceae) is a widely consumed fruit vegetable worldwide especially in the tropics and sub-tropics [1] [2]. Eggplant serves many social, nutritional and medicinal purposes like reducing acidity in the body and providing low sugar, zero-fat fruits.

Ingesting of eggplant and tomato fruits can reduce cardiovascular and oncological diseases due to their high content of antioxidants and bioactive agents [3] [4] [5].

Eggplant cultivation encounters many limiting factors among which are pests and diseases [6]. For instance, *Agroathelia rolfsii* (Sacc.) Redhead & Mullineux, (syn: *Athelia rolfsii* (Cruzi) Tu and Kimbrough (syn. *Sclerotium rolfsii* Sacc.) induces the white mold rot on more than 500 plant species. *A. rolfsii* is pervasive in warm humid climates globally [7] [8] [9].

Sclerotium cepivorum Berk the causal agent of white rot, is one of the most destructive soil-borne pathogens that pose a significant threat to the production of *Allium* species all-inclusive [10]. Stem rot of groundnuts caused by *S. rolfsii* is favored by humid and warmer soil conditions at all growth stages [11].

These diseases reduce the yield hence the farmers' income. Coupled with the risk involved in agriculture and the increasing human and animal population, it is eminent that research on disease control be increased. Based on common knowledge, application of chemical pesticides alone can control most pest and disease situations; however these pesticides leave horrible residues in food which damage environment, human, and animal health drastically [12].

We understand the challenges eggplant farmers face when dealing with *A. rolfsii* that significantly damage their crop. Our team has conducted extensive research (this article is one of such trials) and we are pleased to offer an effective solution - integrated disease management strategies that combine reduced chemical and bio-control agents. By adopting this approach, one can protect 500 different crop species including eggplants from harm and ensure a healthy yield.

We are committed to supporting farmers and helping them achieve sustainable profits. Most farmers are resource poor and

borrow money. They commit all their resources into expensive inputs, labor, and land. If they fail we will not be able to feed and have a healthy global population. Above all arable land is limited in supply.

This research should help society to eradicate poverty (UN Sustainable Development Goal (SDG) 1) by ensuring farmers make profit, achieve zero hunger and sustainable agriculture (SDG 2) by reducing the amount of chemical pesticides judiciously and still produce enough yield, achieve good health and wellness (SDG 3) by scientifically reducing the amount of synthetic pesticides, attain decent work and economic growth (SDG 8) by keeping farmers in business through reduction of yield loss. Moreover, we could reduce inequalities in society (SDG 10) and ensure gender equality by keeping youth and women farmers (SDG 5) in business even when they have very little capital and land. Production of eggplant is quite lucrative since it yields early and for long.

Finally, this research aims at decent work and economic growth and empowerment (SDG 8). Moreover, we can achieve responsible consumption and production (SDG 12) through reduction of chemical pesticides which contaminate food. We strongly believe that an empowered farming population can attain these SDG goals by practicing reduced chemical pesticide usage coupled with bio-control agents. Thus, we are offering this effective solution to all and sundry.

Trichoderma harzianum Rifai is very tolerant to some synthetic chemicals which include Sulfur, Mancozeb (64%) + Metalaxyl M (4%) and then Chlorothalonil (40%) + Metalaxyl M (4%) in descending order of magnitude of tolerance. *T. harzianum* also tolerates Copper hydroxide and Propiconazole [13]. Hence the choice of this agent for this trial.

The advantages of using Trichoderma abound. *Trichoderma* species are exploited widely as a bio-pesticide. *Trichoderma* species employ different mechanisms like antibiosis, competition (for nutrients or space), tolerance (to stress through enhanced root and plant development), induced resistance, inactivation of pathogen's enzymes, solubilisation, and sequestration of inorganic nutrients. *Trichoderma* species are used for seed treatment, seed bio-priming, seedling treatment, soil applications, and foliar applications [14].

The information on the use of integrated strategies for the management of *Sclerotium* rots shows that much research still has to be carried out to ensure the reliability and sustainability of the integrated management of *Sclerotium* rots. The objective of this study was to evaluate the compatibility of integration of chemical fungicides x biological agents for the management of recalcitrant disease agents like *A. rolfsii*.

II. MATERIALS AND METHODS

A. Site Study Area

This research was carried out at the Faculty of Agriculture Laboratory complex in Alex Ekwueme Federal University, Abakaliki (at 6.069°N by 8.199°E). The university is situated about 21 kilometres in the outskirts of the State capital.

B. Isolation and identification of the fungi utilized in the trials

Infected eggplant stems utilized for this research were obtained from the University Research and Teaching Farm. The *Trichoderma* isolates were obtained from Bambara groundnut seeds, mushrooms, crop seeds, and farmland soils.

The fungi (*Trichoderma* spp. and *A. rolfsii*) were isolated using potato dextrose agar (PDA) medium which was autoclaved at 120°C and 15 psi for 15 minutes according to the manufacturer's instructions.

The plates were incubated for 7 days at room temperature. The fungus was sub-cultured on PDA to obtain pure cultures. The fungi were identified by microscopy (ZEISS compound microscope) using literature and manuals on fungi [15].

C. Experimental procedures: Effects of synthetic pesticides and biocontrol agents on *A. rolfsii*

The experiment was laid out in petri dishes using a completely randomized design and each of the 13 treatments was replicated three times. The treatment set consisted of two factors (i.e.; 3 isolates of *Trichoderma* species (*T. virens* (J.H.Mill., Giddens & A.A.Foster) isolate BGMZ2, *T. viride* Pers. isolate AIBK, and *T. harzianum* isolate AICV26) x two synthetic fungicides (i.e.; Mancozeb and Ketoconazole)), and a control. These thirteen treatments were already too many, so no other treatments/checks/standards were included.

Mancozeb (i.e.; applied at a rate of 2,000 g/ha - it is a contact fungicide) and Ketoconazole (200 mg per 100 ml H₂O) were utilized to compose the treatments. One litre of each chemical was made. Five grams of Mancozeb or 1,000 mg of Ketoconazole per litre of sterile distilled water were made. Each chemical treatment consisted of two levels (50% and 100% concentrations) and they were applied to the Petri dishes according to the layout. The control was inoculated with the *A. rolfsii* isolate alone. The nutrient agar medium was inoculated with a 2-mm disc of the pathogen placed at the edge of the plate according to the layout.

D. Data collection and analysis

The percentage inhibition of the pathogen was calculated using equation 1.

$$IH = ((C - T) / C) \times 100\% \quad (1)$$

Where:

IH = Percent inhibition of growth of the fungus

C = Perpendicular* radius of fungus colony in the control plate

T = Perpendicular radius of the fungus colony in treated plate

*Perpendicular refers to 'right angle' through the centre of the plate in this context.

The data were subjected to the analysis of variance (ANOVA) using the General Linear Model (GLM) procedure in the Genstat (second edition Discovery version) statistical package and Statistical Package for Social Sciences (SPSS)

version 25. Separation of the means was carried out using the Duncan's multiple range test (DMRT) at $p \leq 0.05$.

III. RESULTS AND DISCUSSION

A. Resulta

The result of the effect of the interaction of synthetic fungicides x *Trichoderma* species deployed against *A. rolfsii* is presented in Fig. 1. The result reveals that combining Ketoconazole (50% and 100% concentrations) x *Trichoderma* species resulted in 95.8-100% inhibition of the pathogen at 192 HAI. The interaction of Mancozeb x *Trichoderma* species resulted in more control of the pathogen at higher concentrations of the synthetic fungicide. The control of the pathogen ranged from 29.3-100% inhibition for all concentrations of Mancozeb x *Trichoderma* species. The interaction of Mancozeb (50% concentration) x *Trichoderma* species was at par all through, while at the higher concentration of Mancozeb, *T. harzianum* interactions controlled the pathogen more, followed by *T. viride* combinations and lastly the *T. virens* combinations. All the chemical x *Trichoderma* species combinations were significantly different ($p \leq 0.05$) compared to the control.

The results revealed the same pattern (from 48 HAI through 192 HAI), whereby Ketoconazole (100% concentration x all the *Trichoderma* species controlled the pathogen most, followed by Ketoconazole (50% concentration x all the *Trichoderma* species, Mancozeb (100% rate) x all the *Trichoderma* species and finally Mancozeb (50% rate) x all the *Trichoderma* species.

The main effect of synthetic chemicals applied against *A. rolfsii* is presented in Fig. 2. The result shows that the effect of Ketoconazole against the pathogen improved with time. The 100% rate of Ketoconazole completely inhibited the pathogen till 192 HAI. However, the effects of the two rates of Mancozeb (50% and 100% concentrations) reduced with time. The effects of both chemicals were consistently significantly different $p \leq 0.05$ compared to the control. The main effect of the chemical fungicides in terms of decreasing level of efficiency could be ranged as Ketoconazole (100% concentration), Ketoconazole (50% concentration), Mancozeb (100% concentration), and lastly Mancozeb (50% concentration). The percentage inhibition by chemical fungicide thus ranged from 100% inhibition by Ketoconazole (100% concentration) to 97.9-100% for Ketoconazole (50% concentration), to 65.2-93.6% for Mancozeb (100% concentration) and lastly 23.3-78.6% for Mancozeb (50% concentration) with time.

Thus, the main effects of chemical fungicides revealed that (between 48-192 HAI) Ketoconazole (100% concentration) controlled *A. rolfsii* most, followed by Ketoconazole (50% concentration), then Mancozeb (100% concentration) and lastly Mancozeb (50% concentration).

The main effect of *Trichoderma* species applied against *A. rolfsii* is presented in Fig. 3. The result shows that the effect of *Trichoderma* species was fairly consistent against *A. rolfsii*. Although at 48 HAI, *T. harzianum* showed less potency than *T. viride* and *T. virens*. The percentage inhibition ranged from 60.3-96.7% for *T. virens*, 60.8-92.1% for *T. harzianum* and 69.3-95.8% for *T. viride* with time. Thus the percentage inhibition by biocontrol species ranged from 60.3-96.7%. The general trend of excellent control (between 48-192 HAI) ranged from *T. viride* and *T. virens* at the top of the peak, and lastly *T. harzianum* which started off poorly.

The effects of chemical fungicides and *Trichoderma* species were highly positively correlated (0.522** at $p \leq 0.05$) at point of termination of the trial. The effects of chemical fungicides were highly positively correlated to the results of the full interaction (0.976** at $p \leq 0.05$) and the effects of *Trichoderma* species on the full interaction were also highly positively correlated to those of the full interaction (0.434** at $p \leq 0.05$) at the termination of the experiment (NB: ** Correlation is significant at the 0.01% level (2-tailed)).

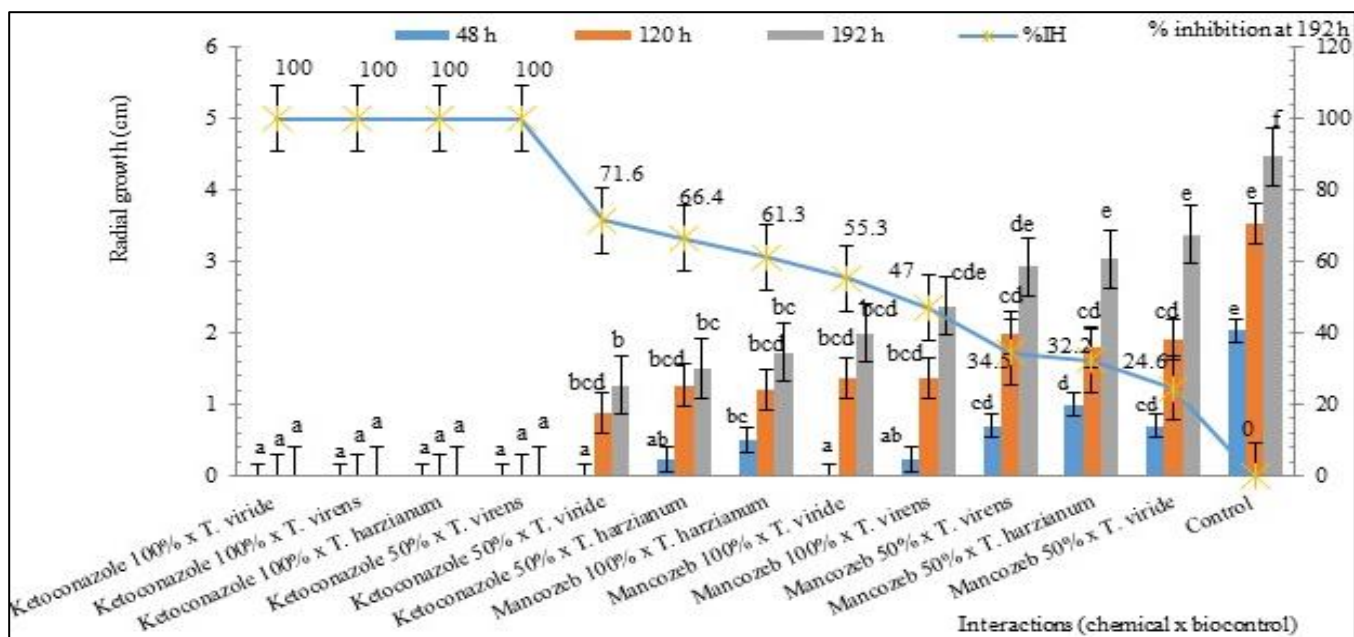
B. Discussions

The result of the application of synthetic fungicides showed that Mancozeb and Ketoconazole are effective agents against *A. rolfsii*. This affirmed the findings of [16] which showed that inhibition of *A. rolfsii* by Mancozeb (at 100% concentration) ranged between 10-90% with time.

Black pod disease (*Phytophthora palmivora*) on cocoa (*Theobroma cacao* L.) was managed using five fungicides. These fungicides include Copper oxychloride, Metalaxyl + Mancozeb, Propiconazole 25%, Mancozeb, Mancozeb + Cymoxanil each combined with cultural practices (cultural practices such as nutrient management, pruning, plant and field hygiene), cultural practices alone, and control were implemented. Copper oxychloride successfully lowered disease severity 4.76 times more than the other treatments and incidence of disease was reduced 50% more than in control plots [17]. The current findings on main effects of the fungicides confirmed these findings of [17].

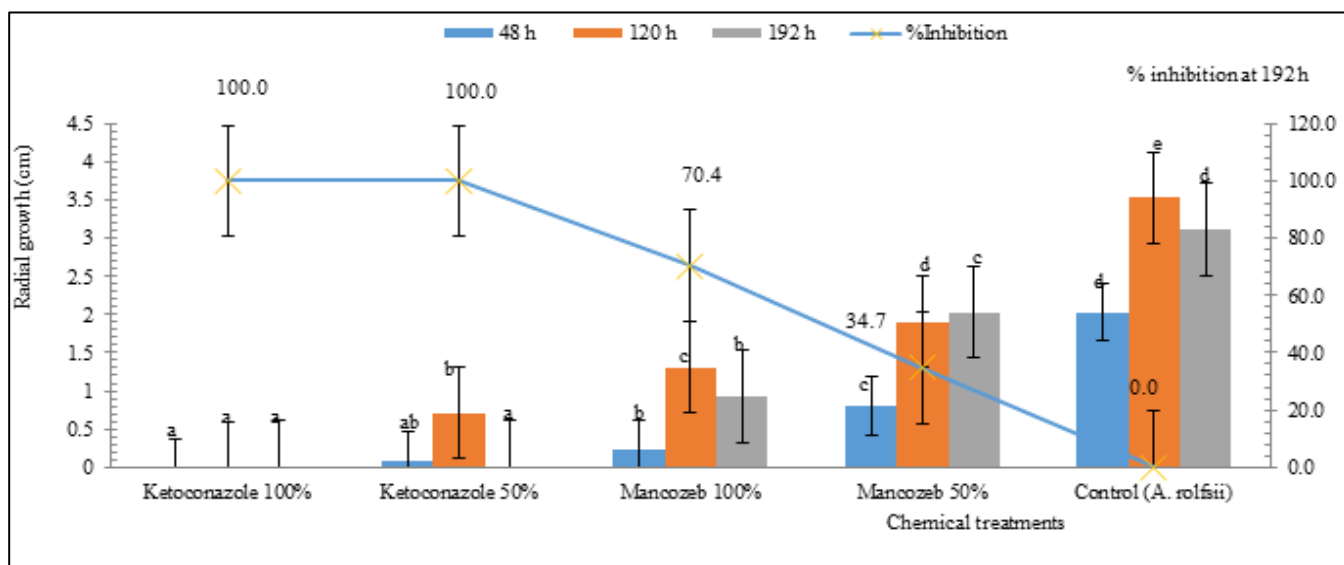
Biochar has been associated with organic agents that suppress plant pathogens (fungi, bacteria, and nematodes) through boosting of the activities of beneficial soil microorganisms, increasing soil fertility, leading to creation of suppressive soils, as well as boosting plant defense mechanisms [18] [19] [20].

The application of rhizobacteria overcame pepper stem rot disease induced by *Phytophthora capsici*, leading to an increase in seedling growth [21]. Using diverse disease management measures is the best principle of plant pathology when it relates to sustainable production. The current study has clearly shown that biocontrol and chemical fungicides can be used simultaneously to boost plant production. This confirms the findings presented in this paragraph.



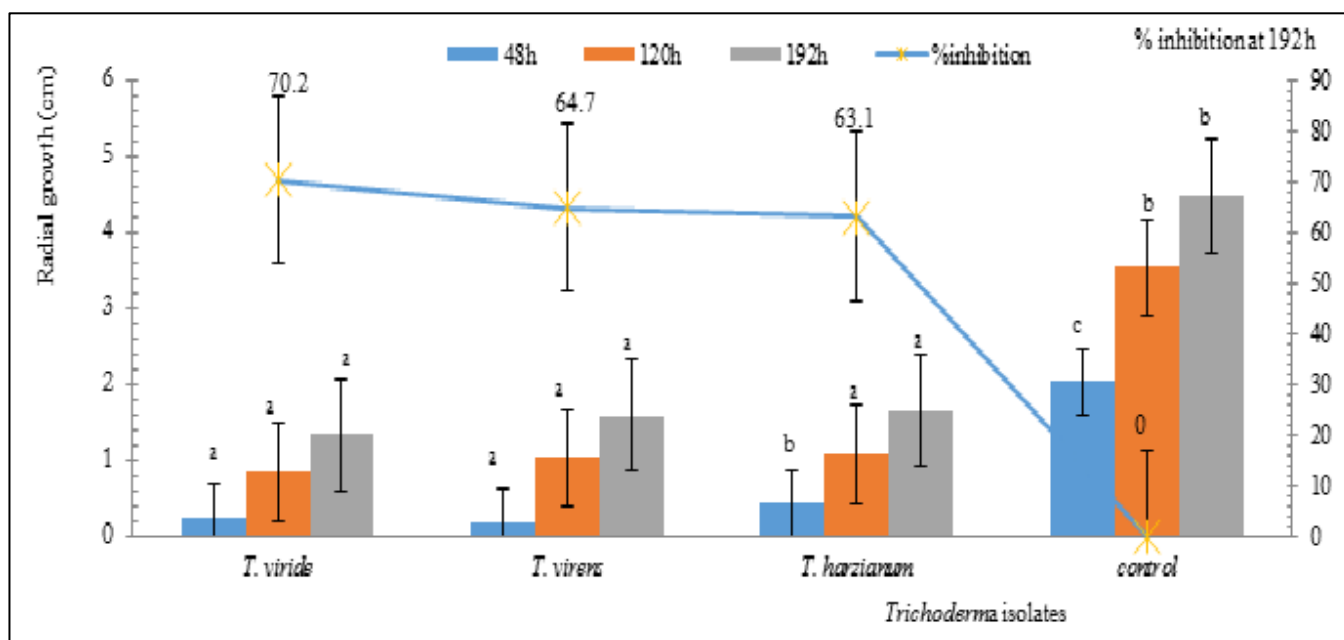
Means over-shadowed by the same letter(s) in a series are statistically similar based on DMRT ($p \leq 0.05$).

Fig. 1. Interaction effect of synthetic fungicides x bio-control agents applied against *A. rolfsii*



Means over-shadowed by the same letter(s) in a series are statistically similar based on DMRT ($p \leq 0.05$).

Fig. 2. Main effect of synthetic fungicides applied against *A. rolfsii*



Means over-shadowed by the same letter(s) in a series are statistically similar based on DMRT ($p \leq 0.05$).

Fig. 3. Main effect of *Trichoderma* isolates applied against *A. rolfsii*

The isolates *Diatrype palmicola* displayed the highest inhibition percentage (49.9%) in comparison to the other isolates applied against *A. rolfsii* from tomatoes. They argued that 8-methoxynaphthalen-1-ol found in *D. palmicola* was the potential bioactive agent inducing fungal control [22]. These findings corroborated the findings herein on the ability of biocontrol agents (e.g. *Trichoderma* species) to inhibit the radial growth of plant pathogens.

Aspergillus terreus extract successfully inhibited *Alternaria solani* in vitro [6]. Granular and liquid formulations of *Trichoderma asperellum* were able to improve its antagonism against *Rhizoctonia solani* (infecting corn seedlings) in vitro and in vivo [23]. The application of biocontrol agents can be carried out in the form of extracts of the biocontrol agents especially where the biocontrol agent (e.g. *Aspergillus*) may be viewed with suspicion by unenlightened users. The formulation problem may also be solved by using extracts. The current study will continue on these trends.

T. harzianum is very tolerant to Sulphur followed by Mancozeb (at 64% WP) but it is not compatible with many other fungicides [13]. All the *Trichoderma* isolates showed a steady linear increase in the inhibition of the growth of *Cylindrocladium* species. This calls for a case-by-case research [24].

T. asperellum Samuels, Lieckf. & Nirenberg was compatible with Mancozeb, Mancozeb+Metalaxyl-M, Copper hydroxide, Copper oxychloride, Metalaxyl. Tebuconazole, Propiconazole, Captan, and Carbendazim were incompatible with *T. asperellum* [25].

Mancozeb (at 100% rate) significantly inhibited the pathogen more than all other synthetic chemical treatments, followed by Mancozeb at 50% concentration, Metalaxyl+Copper(I)oxide (50% and 100% concentrations) (which were at par (at 144 HAI)) [24]. Mancozeb+Carbendazim (50% concentration), Mancozeb+Cu(I)O+Metalaxyl (100% concentration), and Mancozeb (100% concentration) significantly ($p \leq 0.05$) inhibited *Colletotrichum alatae* B.S.Weir & P.R.Johnst more compared to the other treatments. These findings corroborated the findings of this present study [26].

The systemic fungicide - Azoxystrobin (i.e., Azoxystrobin or Amistar®) was highly compatible with *T. viride* and *T. harzianum* followed by Metalaxyl. Concerning non-systemic fungicides, Mancozeb recorded the least inhibitory effect on *Trichoderma* sp. This finding also agrees with the current research whereby Ketoconazole was more potent than Mancozeb [27].

Trichoderma asperellum showed high compatibility with Captan and Mancozeb. The use of antagonistic strains of biocontrol agents together with the Chlorothalonil however resulted in a reduction of the growth of *Trichoderma* species [28]. These findings confirm the findings of this current study whereby higher levels of fungicides inhibited the biocontrol agents.

Additionally, at above 50 ppm, Mancozeb (75% WP) started inhibiting the growth of *T. viride*. Thiram 75% WP (at 1000 ppm fungicide) caused up to 69.3% inhibition of *T. viride*. At 1,000 ppm, Chlorothalonil 75% WP caused 75.3% inhibition

of the radial growth of *T. viride*. Finally, Carbendazim 50% WP, Propiconazole 25% EC, and Hexaconazole 5% EC were not compatible with *T. viride* [14].

Again, these concurs with findings that higher levels of the fungicides were antagonistic to the biocontrol agents. Solutions of Mancozeb (100% rate) and Mancozeb+Carbendazim (50% and 100% rates) were more effective in suppressing the development of *Lasidiopodia theobromae* (Pat.) Griffon & Maubl. mycelia compared to Mancozeb+Metalaxyl+Copper. Mancozeb treatment (alone) inhibited pathogen's growth (8.0–100%) [29].

IV. CONCLUSIONS

Eggplant is a widely consumed fruit vegetable globally. It is severely infected by all kinds of pathogens and pests including *Agroathelia rolfsii*; which causes the stem and root rot diseases. Integrated disease management strategies against *A. rolfsii*, the pathogen that infects eggplants, show efficient compatibility between chemical and bio-control agents. Mancozeb, Ketoconazole, and *Trichoderma* species contributed significantly to the management of *A. rolfsii*. The higher concentrations of the chemical fungicides reduced the radial growth of the pathogen more. Combining the pesticides prolonged the time when the control was effective. Hence combining the control agents is strongly recommended. The fungicides antagonized the bio-control agent as well; thus, research is necessary on this aspect. Just for the sake of completeness we may wish to stress that these excellent results will need to be replicated in other situations and environments.

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