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Invitro Testing of the Trichoderma Species as Bio-control Agents of (*Neofusicoccum. mangiferae* .Crous) Pathogen of Branch Disease in Shade and Fruit Trees

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ABSTRACT

This study carried out to test the fungicidal potentiality of three Trichoderma species under laboratory condition. The laboratory experiments were conducted at the laboratory of plant quarantine-Administration of Plant Protection Ministry of Agriculture. Any Trichoderma specie was considered as treatment in addition to one synthetic fungicide (Amstar-Top) for comparison, each treatment replicated 6 times and arranged in CRD. The result revealed that: Amistar-Top at concentration of 200 ppm, had given significant inhibition compared with the three species of Trichoderma with different isolates of the pathogen. However, there was no significant difference between inhibition percentages from Amistar-Top in some treatments. More over *T. harzianum* with *N. mangiferae* isolated from *Mangiferaindica* and *T. viride* with the fungal isolate from *F. benjamina* were more effective than Amistar-Top.

Keywords: *Trichoderma* spp., Pathogen, mycoparasitism, *Neofusicoccum. mangiferae* .Crous, Inhibition

1. Introduction

Most of the pandemic plant diseases were a result of the infection with a wide spread of microorganism. Besides, excessive usage of pesticides and synthetic fertilizers expose the living organisms to the high toxicity of chemical compounds.In the other hands chemical fungicides are still employed injudiciously as a primary means of disease control. These chemicals are not only expensive, but their application results in the build-up of harmful level of toxins in human beings and in our ecosystem [1] and[2].Biological control mechanisms are contemplated as significant measures for disease managementbecause chemical fungicides adversely affect other non-target organisms [3].One of the strategies used to control pathogens is mycoparasitism whereby a species or strain of fungus directly attacks and feeds on other fungi [4].The antagonistic ability of *Trichoderma* species was discovered 70 years ago. *Trichoedrma* spp. are now the most common fungal biological control agents throughout the world. The primary mechanism of antagonism in Trichoderma is mycoparasitism[5]. *Trichoderma* spp. significantly suppress the growth of plant pathogenic microorganisms and regulate the rate of plant growth.Recent works have shown that common plant disease such as root rotdisease, damping off, wilt, fruit rot and other plant diseases can be controlled by *Trichoderma* spp. [6]; [7] ; [8] ; [9] . *Trichoderma* cultures have been isolate from four marketed bio control brands that claimed to have T. harzianum[10]. Over the past several decades, various attempts to control plant diseases have been made for eradication or prevention through the development of systemic fungicides. Continued and repeated application of fungicides has disturbed the biological control by natural enemies and let to out-break in disease and development of resistance to various types of fungicides toxicity to non-target organisms and environmental problems [11].In addition, use of fungicides is uneconomic due to longevity of trees that necessitates repeated application over a long period of time. Triazoles antifungal such as tebuconazole are now widely used for treatment of fungal infections due to their broad spectra activity and improved safety profile compared to other fungicides [12]. Due to the importance of bio-agents to control plant diseases, this work is considered a contribution in this aspect especially, to test inhibition activity of three *Trichoderma* species to *N. mangiferae*. Many scientist studied the advantage of the genus Trichoderma on controlling and reduction of plant diseases, they found that Trichoderma species affect some plants cause diseases, reduce production and damage the plant such as mashroom[13]; [14]; [15].

2. Materials and Methods

2.1. Effect of Trichoderma species on the growth of *N. mangiferae*

Three species of the genus Trichoderma were obtained from the laboratory of plant quarantine- Administration of Plant Protection, Ministry of Agriculture. Active pure culture from each species was prepared by transferring samples from each species into PDA plates and incubated at 28°c for seven days (until the Petri dishes completely covered). Five mm discs of PDA from seven days old culture of each isolate of *N. mangiferae*and the same size disc from seven days old cultures of *Trichoderma* species were placed 3 cm apart from each other and 3 cm from the edge of the plate. The antagonistic potentiality of the three *Trichoderma* species against *N. mangiferae*isolates was measured as inhibition % considering the radial growth in the opposite directions from the centre of the inoculums discs as control (R) and the growth towards the disc of pathogen as treatment (R_1). The inhibition percent was calculated according to the following formula;

I % = $R\text{-}R_1/R$

I %= Inhibition percentage

Whereas R= the growth length from the centre of the inoculums disc towards the edge of the plate

R_1= the growth towards *Trichoderma* disc

2-2 Effect of Amistar-Top on the radial growth of *N. mangiferae*

Five mm discs of PDA from seven days old culture of each isolate of *N. mangiferae*and placed in the center of PDA media amended with different concentrations of the fungicide (Amstar-Top), clean discs were placed for control treatment. The fungicidal potentiality of Amstar-Top was measured as inhibition % considering the radial growth from the center of the Petri-dish. The inhibition percent was calculated according to the following formula;

I % = D-D1/D

I %= Inhibition percentage

Whereas D= growth diameter in control, D1= growth diameter in the treatment

3- Results

3-1 Effect of *Trichoderma* species on the radial growth of four isolates of *N. mangiferae*

The biological interaction between *N. mangiferae* isolates and *Trichoderma* spp. resulted into reduction of the radial growth of the *N. mangiferaeinvitro.* The inhibition percent ranged between 49.8% and 82.14% (Fig-1). *Trichodermaviride* was the most effective against *N. mangiferae* isolated from *C. lemon*, it has resulted in 82.14% inhibition percent. The least inhibition percent was of *N. mangiferae*was that of *T. koningei* with *N. mangiferae* isolated from *F. nitida*. However the statistical analysis showed no significant differences between the means of inhibition percent regarding different *Trichoderma* spp. (Table 1).

Table 1. Inhibition percentages of radial growth of four *N. mangiferae* isolates treated with three *Trichoderma*species

Treatments (Trichoderma species)	Isolates of *N. Mangiferae* Host plants Inhibition%			
	Citrus lemon	*F. .benjamina*	*F. nitida*	*m. indica*
T.harzianum	78.44a	49.80a	53.02a	66.90 a
T. viride	82.14a	64.30a	68.54a	78.82 a
T. koningei	78.76a	54.96a	50.86a	72.78a
LSD	17.63	18.47	35.77	18.47

Mean with the same letter(s) in the column are not significantly different at P< 0.05.

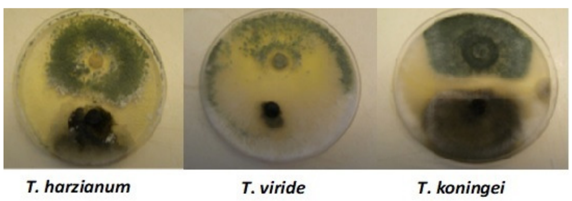

Fig-1.Effect of Trichoderma spp. on the radial growth of *N. mangiferae*

3-2 Effect of different concentration of Amistar-Top on the radial growth of *N. mangiferae* isolates

The systemic fungicide Amistar-top was very effective in reduction of radial growth of *N. mangiferae* isolates. Three concentrations of the fungicide have been tested *Invitro*: 100, 200 and 500 ppm. The results showed that there was no significant different between the two concentrations of 200ppm and 500ppm, but they differed significantly from the concentration of 100ppm. This result means that the concentrations 200ppm and 500ppm have the same efficacy (Table-2) and (Fig-2).

Table-2. Effect of Amistar- Top on the radial growth of four isolates of *N. mangiferae*

Concentrations	Isolates of *N. mangiferae* Host plants			
	C. lemon	*F. benjamina*	*F. nitida*	*M. indica*
	Inhibition %			
100ppm	55.98b	52.96 b	52.04b	51.06b
200ppm	88.08a	85.16a	82.72a	85.00a
500ppm	90.44a	89.64a	90.32a	90.75a
LSD	8.077	19.92	17.30	12.43

Mean with the same letter(s) in the column are not significantly different at P< 0.05.

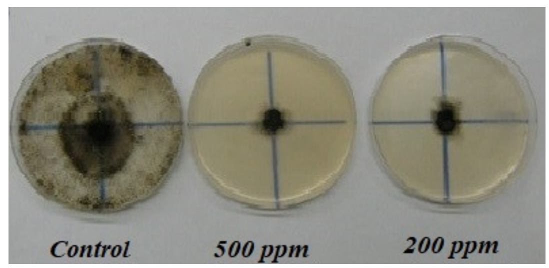

Fig-2. Effect of Amistar Top on the radial growth of *N. mangiferae*

3.3- Effect of three *Trichoderma* species on the growth of *N. mangiferae* isolates compared with Amistar-top.

The bio-agents tested were compared with the fungicide Amistar-Top tested in the laboratory showed that *Trichodema* spp. have approximately similar effect as Amistar-Top on the pathogen isolates radial growth and (Fig-3). Amistar-top with *N. mangiferae* isolated from *M. indica* was significantly better than *T. harzianum*. However, there was no significant differences between Amistar-Top effect and *T. viride* with *N. mangiferae* isolated from *Citruslemon, Ficusnitida*and*mangiferaeindica.* On the other hand Amistar-top showed its significant superior to *T. viride* with *N. mangiferae*isolated from *F. benjamina*.

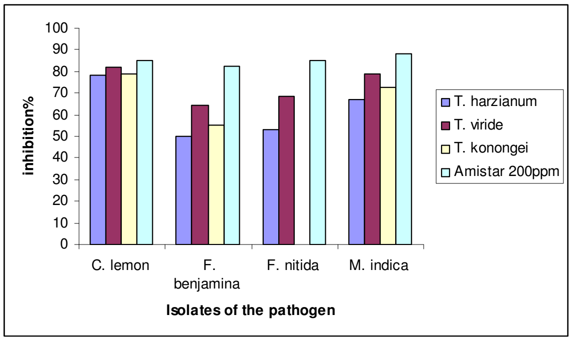

Fig -3.Comperison of inhibition effect (%) of three *Trichoderma*spp. and Amistar on four isolates of *N. mangiferae*

4- Discussion

Trichoerman spp. plays a major role as bio control agents, owing to their capability of improving crop yield by multiple roles, such as bio-pesticide, bio-herbicide and plant growth promotion [16].*Trichoderma*spp. showed a potential bio control efficiency against fungal phyto-pathogens either throughdirect mechanisms of mycoparasitism and antibiosis orby indirect mechanisms as competition for nutrients,enhancing plant defense responses and exhilaratingplant growth [17]. In this regard, *Trichoderma*bio agentssuppress the growth of fungal phyto-pathogens by amixed action through their metabolites inhibitory effectand lytic enzymes which disintegrate the fungal cell wall [18].The roles of BCAs are a well-established fact and become increasingly crucial, and in several cases complementary or even replacing the synthetic chemical components where antagonistic fungi play an important part [19]. Scientist found that the input cost and crop productivity application of BCA are economical and low cost compared to synthetic inputs [20]. *Trichoderma* was found to reduce the incidence and severity of *Fusariumoxysporium* in the cotton seedlingsand was the most efficient product [21].Bio-interaction between *Trichoderma* spp. and plant pathogenic fungi tested in the laboratory showed that *Trichoderma* species were differently effective in inhibiting the different isolates of the pathogen (*N. mangiferae*). *T. viride*gave the highest inhibitory action on *N. mangiferae* isolated from symptomic lime tree with 80% inhibition. In counterpart *T. harzianum* have the least inhibition with *N. mangiferae* isolated from *F. benjamina* with 49.8% inhibition. This inhibition of the pathogen can be attributed to the different biological interactions between *Trichoderma*spp. and the pathogen. The systemic fungicide Amstar-Top was used as reference revealed that it is highly effective in reducing the radial growth of different isolates of the pathogen, exactly 88.08% inhibition. This is due to the fact that in case of synthetic chemical fungicides the formulations applied are very accurately calculated according to the recommendations of the company. On the other hand in case of bio-control agents it is difficult to setup formulations for application as the activity of the control agent is subjected to biological factors and environmental conditions. However, frequent use of fungicides leads to environmental and health problems and results in the development of fungicide resistance [22].This fact may give the farmer evident to rely on chemicals more than bio-agents application. The above mentioned is related to the findings of researchers who stated the differentiation of antifungal activity and secondary metabolites secreted from two different strains of T. harzianum[23]. Similar result explained that T. harzianum, T. viride, and T. atroviride are excellent bioremediation agent of carbendazim. Within 5 days, 85% of carbendazim were degraded by T. harzianum, whereas 20%–50% of carbendazim degraded by the other two species [24]. The above mentioned related to results stated that *T. harzianum*was able to reduce the incidence of *F. oxysporum*f. sp. *phaseoli*by 35.0 to 51.0% in common bean seeds[25]. A similar recent study indicated that the bio agents of *T. harzianum*were found highly effective against the fungal strains causing black point disease of wheat and decrease the incidence of *Fusariumoxysporium* and increased germination rate in *Phaseolus vulgaris* L. so that these bio control agents could be used in formulation of natural fungicides avoiding the harmful impact of the synthetic fungicides[26].

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