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INTEGRATED MANAGEMENT OF FUNGAL PATHOGENS OF YAM (*DIOSCOREA ALATA* L.) USING *TRICHODERMA* SPECIES

Bikila Wedajo[1] and Tesfaye Alemu[1,*]

ABSTRACT: Plant pathogens are the major constraints that decrease the productivity and post-harvest deterioration of yam crop. Among the plant pathogens, fungal pathogens are major agents responsible for the infection of yam crop. Therefore, the present study was carried out to manage fungal pathogens of yam (*Dioscorea alata* L.) crop using *Trichoderma* spp., fungicides and their combination for integrated disease management. *In vitro* evaluation of dual culture test of AUT1 (*Trichoderma harzianum*) and AUT2 (*Trichoderma viride*) obtained from Addis Ababa University against AUF1 (*Fusarium* isolate 1), AUA1 and AUA2 (*Alternaria* isolate 1 and 2) and AUV1 and AUV2 (*Verticillium* isolate 1 and 2) revealed that both biocontrol agents showed 60.42% to 76.95% and 68.82% to 75.75% of mycelial growth inhibition, respectively. From *in vitro* evaluation of fungicides (curzate, 43.95% WP and sancozeb, 80% WP) tested at concentrations of 100, 200, 400, 600, 800 and 1000 ppm based on inhibitory concentration against fungal pathogens of yam. Sancozeb was more effective than curzate in terms of percent mycelial growth inhibition of fungal pathogens. For this study the minimum concentration of fungicide, 600 ppm (curzate) and 400 ppm (sancozeb) were recommended. From the study of combined efficacy of *Trichoderma* species with fungicides, the highest percent of inhibition of mycelial growth of fungal pathogens was 85.6%, 79.7%, 87.5%, 89.3% and 80.2% when AUT2 was combined with sancozeb at 400 ppm for AUF1, AUA1, AUA2, AUV1 and AUV2, respectively. The results showed that combination and integration of AUT1 and AUT2 with chemical fungicides (curzate and sancozeb) at lower concentrations may offer a promising management strategy of fungal pathogens of yam compared to fungicides and *Trichoderma* species separately.

Key words/phrases: Fungicides, Integrated fungal pathogen management, *In vitro*, *Trichoderma* species.

[1] Department of Microbial, Cellular and Molecular Biology, College of Natural and Computational Sciences, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia. E-mail: bikilawedajo@yahoo.com; tesfayealemu932@gmail.com, tesfaye.alemu@aau.edu.et
* Author to whom all correspondence should be addressed.

INTRODUCTION

Biological control is an environmentally friendly, scientifically proven and effective means of mitigating pathogens or pests through the use of useful microorganisms (Campbell, 1989). A world estimated loss due to crop diseases was up to 12%, while a loss due to post-harvest food spoilage was between 10 and 50% (Agrios, 2005). Effective control of crop losses due to pests therefore holds the key for steady and stable food supply of the world. Amongst all effective and recommended control of the crop pests, biological control holds a great promise for the future. Basically, biological control has the advantages of being environmentally friendly and not hazardous to the health of human beings, livestock and wildlife (Omer and Shahzad, 2007). There are several limiting factors for the production, processing and quality of yam in the world. Among these constraints, fungal diseases contribute greatly to high loss of yield before and after harvest of yam (Adesiyan and Odihirin, 1975). The most important biological control agents (BCA) belong to the genus *Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp. and *Streptomyces*. Biological control of plant pathogens is an attractive alternative to decrease heavy dependence of modern agriculture on costly chemical fungicides, which not only cause environmental pollution but also lead to the development of resistant strains (Harman *et al*., 2004). Apart from biocontrol ability, the BCAs possess other advantages such as rhizosphere competence, tolerance of fungicides, saprophytic competitive ability, ability to tolerate high and low temperatures, adaptability to different edaphic conditions, good searching ability, host specificity, high reproduction rate, short life cycle, adaptability, well adapted to different stages of life cycle of target host, and ability to maintain itself after reducing host population (Harman *et al*., 2004). Okigbo and Ikediugwu (2000) have shown that *Trichoderma viride* displaced the naturally occurring mycoflora on the surface of the yam tuber.

Some soil-borne root infecting fungi are difficult to eradicate by fungicides because they produce resting structure like sclerotia, chlamydospores or oospores for their survival for a longer period of time under adverse environmental conditions (Omer and Shahzad, 2007). Therefore, the combined use of BCAs and chemical fungicides has attracted much attention in order to obtain synergistic effects in the control of soil-borne diseases (Locke *et al*., 1985). Reduced amount of fungicide can stress and weaken the pathogen and render its propagules more susceptible to subsequent attack by the antagonists (Hjeljord and Tronsmo, 1998). It has been reported that many *Trichoderma* spp. have an induced resistance to

many fungicides but the level of resistance varies with fungicide (Omer and Shahzad, 2007).

Most of the fungal pathogens of yam were known in many countries, but in Ethiopia there is few report concerning fungal pathogens of yam and their management. Therefore, this study was designed to evaluate *in vitro* potential of the BCAs and fungicides (curzate and sancozeb) against fungal pathogens of yam (*Dioscorea alata*). *Trichoderma harzianum* and *Trichoderma viride* were screened for tolerance to fungicides (curzate and sancozeb) and integrated management of fungal pathogens of yam used in combination with fungicides.

MATERIALS AND METHODS

Preparation of *Trichoderma* species and fungicides

Two species of *Trichoderma* (*Trichoderma harzianum*, AUT1 and *Trichoderma viride*, AUT2) were used to evaluate their antagonistic potentials against the fungal pathogens. The fungal cultures (AUT1 and AUT2) were obtained from Mycology Laboratory, Department of Microbial, Cellular and Molecular Biology (DMCMB), Addis Ababa University which were isolated from the soil samples collected from Gera, Gomma, Mana, Kossa and Seka Chokeressa woredas of Jimma Zone, Ethiopia by Yonas Urbanos (2010). The fungicides used were curzate (43.95% WP) and sancozeb (80% WP) obtained from Mycology Laboratory DMCMB, Addis Ababa University.

Isolation and identification of disease causing fungal pathogens of yam

Five fungal pathogens were isolated from infected leaves and tubers of yam (*Dioscorea alata*) from Seka Chekoressa Woreda, Jimma Zone, Ethiopia. The fungal isolates were designated as AUF1 (Addis Ababa University *Fusarium* isolate 1), AUA1 and AUA2 (Addis Ababa University *Alternaria* isolates 1 and 2), and AUV1 and AUV2 (Addis Ababa University *Verticillium* isolates 1 and 2) which were employed throughout the present study.

In vitro evaluation of antagonistic activity of *Trichoderma* species

Dual culture method

Dual culture method (Hajieghrari *et al*., 2008) was employed to evaluate the antagonistic potential of AUT1 and AUT2. A 5 mm diameter mycelial disc from the periphery of 7 day old culture of biocontrol was placed on the opposite side of the fungal isolates on potato dextrose agar (PDA). The

fungal isolates were inoculated for 12 hours prior to the placement of the *Trichoderma* spp. to establish the growth of the test fungus. The experiment was arranged in three replicates. Plates having only the test fungal isolates were used as control and incubated at 25±1°C for eight days. Growth inhibitions were recorded every day and the final measurements were recorded at the 8th day of inoculation. Radial growth reduction (percentage of inhibition) was calculated in relation to growth of the control by the following formulae (Montealegre *et al*., 2003):

$$\text{Percent of inhibition} = \frac{(C-T) \times 100}{C}$$

Where C is radial growth measurement (mm) of the fungal isolates in the control plates and T is radial growth of the fungal isolates in the experimental plates.

In vitro evaluation and testing of fungicides against fungal isolates

The poisoned food technique

The purpose of this experiment was to evaluate the efficacy of curzate and sancozeb fungicides at different concentrations against the fungal pathogens which were available currently on market to control pathogens. Evaluation and testing the effect of fungicides against the fungal isolates was done according to Nene and Thapliyal (1993). The fungicides (sancozeb and curzate) were obtained from Mycology Laboratory, Department of Microbial, Cellular and Molecular Biology, Addis Ababa University. The fungicide concentrations were prepared as follows: If the formulated product (fungicide) has 50% active ingredient, for 1 ppm solution 2 mg of the formulated product should be dissolved in a litre of solvent (Nene and Thapliyal, 1993). Therefore, curzate (Copper Oxychloride 39.75% + Cymoxanil 4.2%) has 43.95% WP, for 100 ppm solution 0.175 g, 200 ppm (0.35 g), 400 ppm (0.7 g), 600 ppm (1.05 g), 800 ppm (1.4 g) and 1000 ppm (1.75 g) was added in a litre of solvent. For preparation of sancozeb (mancozeb 80% WP) 0.32 g, 0.64 g, 1.28 g, 1.92 g, 2.56 g and 3.2 g were used for 100, 200, 400, 600, 800 and 1000 ppm, respectively, and dissolved in a litre of distilled sterilized water. The fungicides were added to the autoclaved PDA medium (to prevent denaturation of the fungicides) cooled to 45°C with the amount of 2 ml per plate, so that the required concentrations were obtained. Triplicate culture plates, each containing 20 ml of the test medium, were used to test each fungal isolates at different concentrations of fungicides. Potato dextrose agar (PDA) plates inoculated

with fungal isolates without fungicide were used as control and replicated three times.

Mycelial plugs of fungal isolates, 5 mm in diameter were cut from 7 days actively growing margins of the fungal isolate culture by sterile cork borer and transferred aseptically into the centre of the Petri dish containing PDA medium with different concentrations of fungicide. Inoculated plates were incubated at 25°C for 10 days. Growth of fungal isolates at each concentration was determined by measuring mycelial growth diameters in two perpendicular directions on each culture plate. Measurements were averaged in triplicates, and the diameters of the plugs used to inoculate the plates were subtracted from each measurement. The relative growth reduction for each fungicide was calculated according to Rita and Tricita (2004) as follows:

$$L = \frac{(C - T) \times 100}{C}$$

Where L is percent of inhibition; C is radial growth of the fungal isolates in control; and T is radial growth of the fungal isolates in the presence of the fungicides.

Tolerance of BCAs (*Trichoderma* species) to fungicides

Species of *Trichoderma harzianum* (AUT1) and *Trichoderma viride* (AUT2) were evaluated for tolerance to fungicides (curzate, 43.95% WP and sancozeb, 80% WP) by using food poison method (Nene and Thapliyal, 1993) at 100, 200, 400, 600, 800 and 1000 ppm concentrations as previously. Fungicides (curzate and sancozeb) were added to potato dextrose agar medium (PDA) to get final concentration of 100, 200, 400, 600, 800 and 1000 ppm active ingredient. Potato dextrose agar medium without fungicide served as control. A 5 mm inoculum disc of *Trichoderma* species was cut from the margin of actively growing colony and placed in the centre of each Petri plate. Petri plates were incubated at 25±1ºC. Three replications were maintained for each treatment. Percent reduction in radial growth over control was calculated by using the following formulae (Rita and Tricita, 2004):

$$L = \frac{(C - T) \times 100}{C}$$

Where L = Percent reduction in growth of *Trichoderma* species; C = Radial growth (mm) of *Trichoderma* species in control; and T = Radial growth

(mm) *Trichoderma* species in treatment.

Combination of *Trichoderma* species with fungicides against fungal isolates

The combined use of biocontrol agents and fungicides was applied by the method of Nene and Thapliyal (1993). In this technique, the growth medium was poisoned with fungal toxicants. The fungicides used were sancozeb and curzate. The fungicide concentrations of 600 ppm for curzate and 400 ppm for sancozeb were prepared on which both the test fungal isolates and antagonists can grow, and added to the autoclaved PDA medium after cooling to 45°C, so that the required concentration was obtained for both fungicides. Triplicate culture plates, each containing 20 ml of the test medium were poured and after solidification of medium, the test fungal isolates were inoculated 12 hours prior to the placement of the *Trichoderma* spp. to establish the growth of the test fungal isolates following the dual culture method. Fungal pathogens grown on potato dextrose agar plates without BCAs and fungicides were used as a control. After screening the minimum concentration of fungicides (curzate at 600 ppm and sancozeb at 400 ppm) at which the biocontrol survive were determined by measuring mycelial growth diameters and percentage inhibition of radial growth was calculated following the formulae suggested by Rita and Tricita (2004):

$$L = \frac{(C - T) \times 100}{C}$$

Where L is mean inhibition percent of radial mycelial growth; C is radial growth measurement of the test fungal isolates in control; and T is radial growth of the test fungal isolates in the presence of BCAs and fungicides.

Data analysis

The statistical analysis of mycelial growth diameters of fungal isolates and percent of inhibition were tested by One-way ANOVA. Mean comparisons of *Trichoderma* species, fungicides and combination of *Trichoderma* species and fungicides were conducted using SPSS statistical analysis software version 16. Mean separation was determined according to Duncan's multiple range test ($P<0.05$).

RESULTS

In vitro evaluation of antagonistic activity of *Trichoderma* species

Dual culture test of each *Trichoderma* spp. (AUT1 and AUT2) showed different degrees of inhibition against the mycelial growth of fungal pathogens of yam crop. It is clearly indicated in Table 1 that the mycelial growth of fungal pathogens showed significant differences in comparison to control after seven days of incubation at 25°C. Similarly, growth inhibition was recorded for both antagonistic over the mycelial growth of AUA2 by AUT1 (76.95%) and AUT2 (75.75%) (Table 1). *Verticillium* isolate 2 (AUV2) measured 58.33±2.08 mm radial growth extension in the control whereas 14.25±1.00 mm towards the antagonistic fungus AUT2 with percentage of mycelial growth inhibition of 75.57%. *Trichoderma viride* (AUT2) revealed paramount performance on the mycelial growth inhibition of the fungal isolate AUF1 (14.67±2.52 mm) compared to control treatment (Table 1). *Trichoderma viride* (AUT2) reduced the mycelial growth of AUF1 by 74.62% whereas AUT1 showed 72.15% of inhibition. *Trichoderma harzianum* (AUT1) (62.35%) followed AUT2 (68.82%) in performance against AUA1. From comparison of means, AUT2 (73.30%) was more effective than AUT1 (69.12%) in terms of percentage of mycelial growth inhibition of fungal isolates as indicated in Table 1.

Table 1. *In vitro* evaluation of antagonistic activities of *Trichoderma* species against mycelial growth of fungal isolates.

Fungal isolates	Control growth (mm)	*Trichoderma harzianum* (AUT1)		*Trichoderma viride* (AUT2)	
		Growth of pathogen (mm)	% of inhibition	Growth of pathogen (mm)	% of inhibition
AUF1	57.67±2.52a	16.00±2.00a	72.15a	14.67±2.52b	74.62b
AUA1	67.67±2.52c	24.81±2.00b	62.35b	20.43±1.53a	68.82c
AUA2	50.67±3.06b	11.67±0.58c	76.95a	12.33±2.08c	75.75d
AUV1	86.00±2.00d	34.03±1.53d	60.42b	25.59±2.65d	70.24c
AUV2	58.33±2.08a	15.33±1.53a	73.72a	14.25±1.00b	75.57b
Mean±SD	64.06±12.82	14.26±2.49	69.12	13.13±2.32	73.30

Each value is an average of three replicates ± standard deviation. Means followed by the same letters within a column are not significantly ($p<0.05$) different, according to Duncan's multiple range test.
AUF1=*Fusarium* isolate 1; AUA1=*Alternaria* isolate 1; AUA2=*Alternaria* isolate 2; AUV1=*Verticillium* isolate 1; AUV2=*Verticillium* isolate 2; mm=millimetre

In addition to the numerical results obtained in Table 1, *in vitro* evaluation of *Trichoderma harzianum* (AUT1) and *Trichoderma viride* (AUT2) against fungal isolates after seven days of incubation by comparing with the control (fungal isolates without AUT1 and AUT2) is shown in Fig. 1.

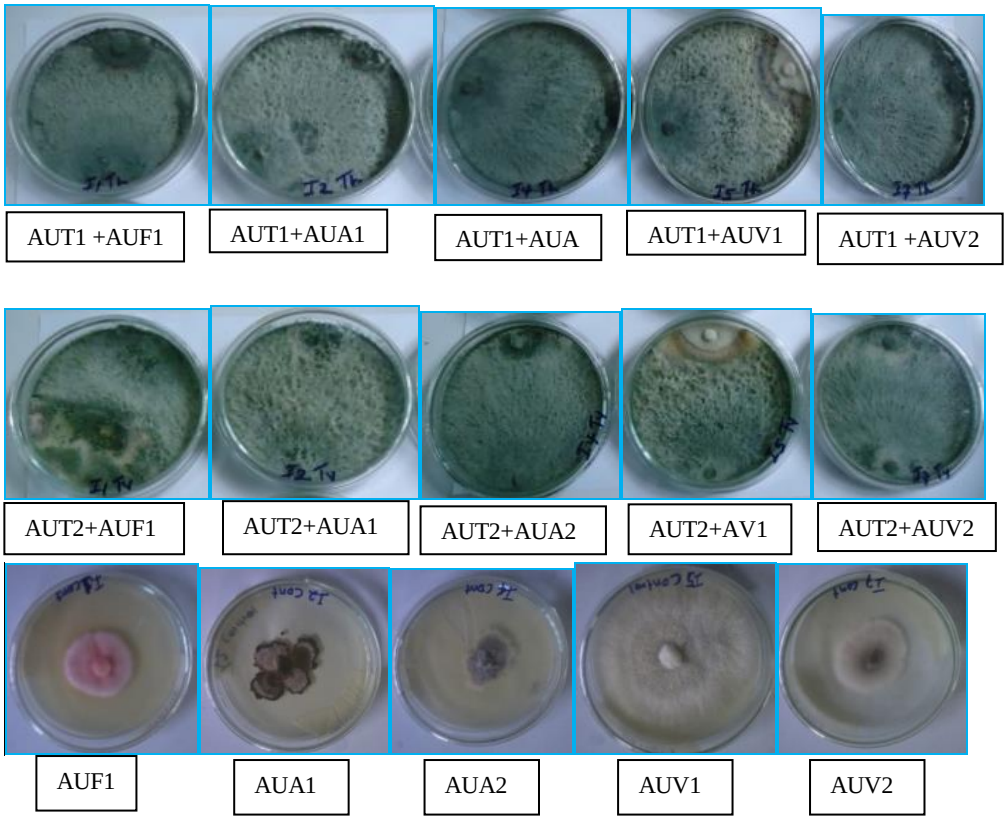

Fig. 1. *In vitro* evaluation of *Trichoderma harzianum* (AUT1) and *Trichoderma viride* (AUT2) against fungal isolates after seven days of incubation.
(AUT1 + AUF1; AUT1 + AUA1; AUT1 + AUA2; AUT1 + AUV1; AUT1 + AUV2) = Dual culture of *Trichoderma harzianum* with fungal pathogens separately
(AUT2 + AUF1; AUT2 + AUA1; AUT2 + AUA2; AUT2 + AUV1; AUT2 + AUV2) = Dual culture of *Trichoderma viride* with fungal pathogens separately
(AUF1; AUA1; AUA2; AUV1; AUV2)=controls or fungal pathogens without *Trichoderma* species

In vitro evaluation of fungicides on mycelial growth of fungal isolates

In vitro evaluation of the inhibitory effect of two fungicides curzate (43.95% WP) and sancozeb (80% WP) on the test fungal isolates were evaluated using different concentrations of 100, 200, 400, 600, 800 and 1000 ppm (Tables 2 and 3). There were significant ($p<0.05$) differences among the concentrations of curzate and sancozeb on the mycelial growth inhibition of fungal isolates on the growth medium (Tables 2 and 3). But a significant ($p<0.05$) difference was observed between concentrations of curzate and the mycelial growth of all the fungal isolates for up to ten days of incubation at 25°C with the exception of AUA2 in which the complete inhibition was

observed at concentrations of 800 and 1000 ppm. Also significant ($p<0.05$) difference of inhibition was recorded for sancozeb fungicide with regard to concentration ranges from 100-1000 ppm on all the mycelial growth of fungal isolates.

Table 2. *In vitro* evaluation of curzate at different concentrations on mycelial growth of fungal isolates after ten days of incubation at 25°C on potato dextrose agar.

Concentration (ppm)	Mean inhibition percentage of fungal isolates					Mean±SD
	AUF1	AUA1	AUA2	AUV1	AUV2	
100	37.8d	42.2g	75.7d	24.4f	20.7e	40.1±3.8
200	40.0d	47.8f	81.1c	39.6e	37.4d	49.1±4.1
400	44.2c	55.9e	84.2c	47.3d	46.7c	55.6±5.6
600	48.9b	63.0d	90.1b	62.2c	57.2b	64.2±3.9
800	59.6a	69.6c	100a	71.8b	65.4a	73.2±6.2
1000	63. 3a	76.9b	100a	79.0a	69.6a	77.7±8.2
Control	62.7e	46.0a	50.7e	87.3g	53.7f	60.1±4.1
Mean ±SD	50.8±4.1	57.3±4.9	83.1±6.4	58.8±8.5	50.1±6.3	70.4±5.1

Each value is an average of three replicates. Means followed by the same letters within a column are not significantly ($p<0.05$) different, according to Duncan's multiple range test. Control was measured in mm.
AUF1=*Fusarium* isolate 1; AUA1=*Alternaria* isolate 1; AUA2=*Alternaria* isolate 2; AUV1=*Verticillium* isolate 1; AUV2=*Verticillium* isolate 2; mm=millimetre

Table 3. *In vitro* evaluation of sancozeb at different concentrations on mycelial growth of fungal isolates after ten days of incubation at 25°C on potato dextrose agar.

Concentration (ppm)	Mean inhibition percentage of fungal isolates					Mean±SD
	AUF1	AUA1	AUA2	AUV1	AUV2	
100	61.2a	67.4a	87.6a	85.9b	77.1c	75.8±6.1
200	63.3a	75.4b	92.1b	89.7a	78.4c	79.7±6.5
400	69.2b	80.4c	100c	90.1a	81.4c	84.2±6.4
600	71.3b	84.1d	100c	100d	100b	91.1±7.3
800	100c	86.0d	100c	100d	100b	97.2±2.5
1000	100c	89.1d	100c	100d	100b	97.8±2.1
Control	62.7d	46.0e	50.7f	87.3c	53.7a	60.1±7.4
Mean±SD	75.3±6.5	75.4±5.6	90.1±6.8	93.2±2.4	84.3±6.4	83.7±2.7

Each value is an average of three replicates. Means followed by the same letters within a column are not significantly ($p<0.05$) different, according to Duncan's multiple range test. Control was measured in mm.
AUF1=*Fusarium* isolate 1; AUA1=*Alternaria* isolate 1; AUA2=*Alternaria* isolate 2; AUV1=*Verticillium* isolate 1; AUV2=*Verticillium* isolate 2; mm=millimetre

The response of individual fungal isolates to the two fungicides at different concentration is shown (Table 2 and 3). Relatively maximum percent of inhibition (100%) was recorded between 400-1000 ppm against all the fungal isolates, except that AUA1 showed growth throughout the concentrations of sancozeb compared to the rest of fungal isolates. The highest (75.7%) percentage inhibition of mycelial growth of the test fungal isolate was displayed against AUA2 at the lowest concentration of 100 ppm by curzate and 87.6% in case of sancozeb with complete inhibition between 400-1000 ppm (Table 2 and 3). Likewise, AUV1 and AUV2 were

completely inhibited (100%) by sancozeb with the range of concentrations between 600-1000 ppm. The same pattern of inhibition was displayed on AUF1 at the concentrations of 800 and 1000 ppm by sancozeb.

Also, *in vitro* evaluation of sancozeb 80% WP and curzate 43.95% WP at 100 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm and 1000 ppm concentrations on mycelial growth of fungal isolates after ten days of incubation at 25°C as compared to the growth of control (fungal isolates without the addition of sancozeb and curzate) is shown in Fig. 2 and Fig. 3.

In vitro evaluation of *Trichoderma* species for tolerance to curzate and sancozeb

The results of Table 4 and 5 showed that *Trichoderma* spp., AUT1 and AUT2 were evaluated for tolerance to fungicides like curzate and sancozeb. Incorporation of curzate and sancozeb in growth medium did not affect the growth of *Trichoderma* spp. instead fungicides favoured the growth of antagonistic fungi at lower concentrations of 100 and 200 ppm. However, by increasing the fungicidal concentrations to 400 and 600 ppm, the antagonists tolerated the fungicides to some extent but were reduced slightly at higher concentrations of 800 and 1000 ppm compared to control. The highest (74.1%) percent of inhibition was recorded at high concentration of 1000 ppm for curzate and (78.9%) for sancozeb fungicides. From comparison of means, AUT1 was more inhibited than AUT2 by both fungicides. AUT2 was more tolerant of both fungicides than AUT1 (Table 4 and Table 5).

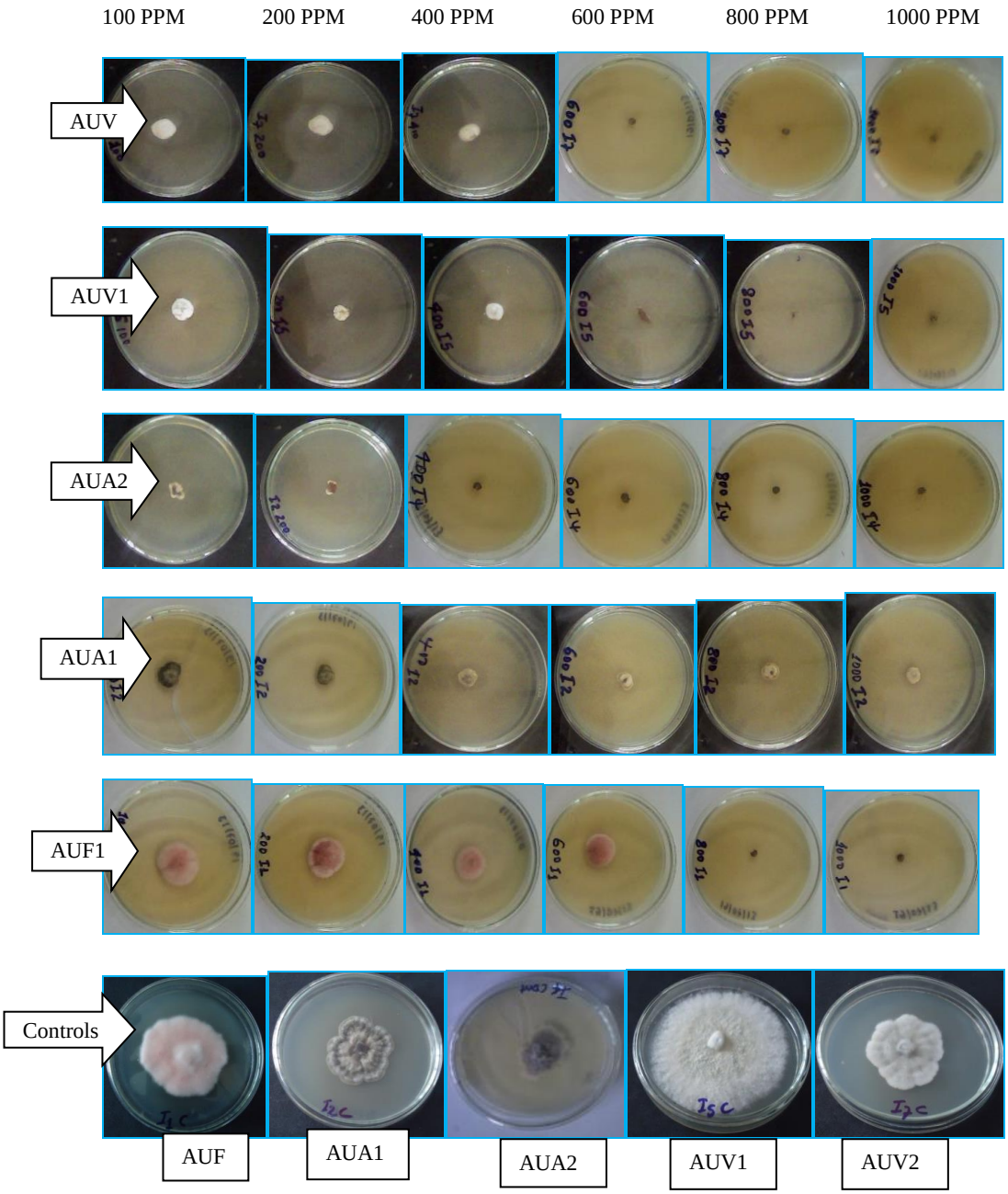

Fig. 2. *In vitro* evaluation of sancozeb, 80% WP at different concentrations on mycelial growth of each of the fungal pathogens after ten days of incubation at 25°C.
AUV2; AUV1; AUA2; AUA1; AUF1=each fungal pathogen from left to right growing at different concentration of sancozeb (100 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm and 1000 ppm)
Controls= from left to right are without the addition of fungicides

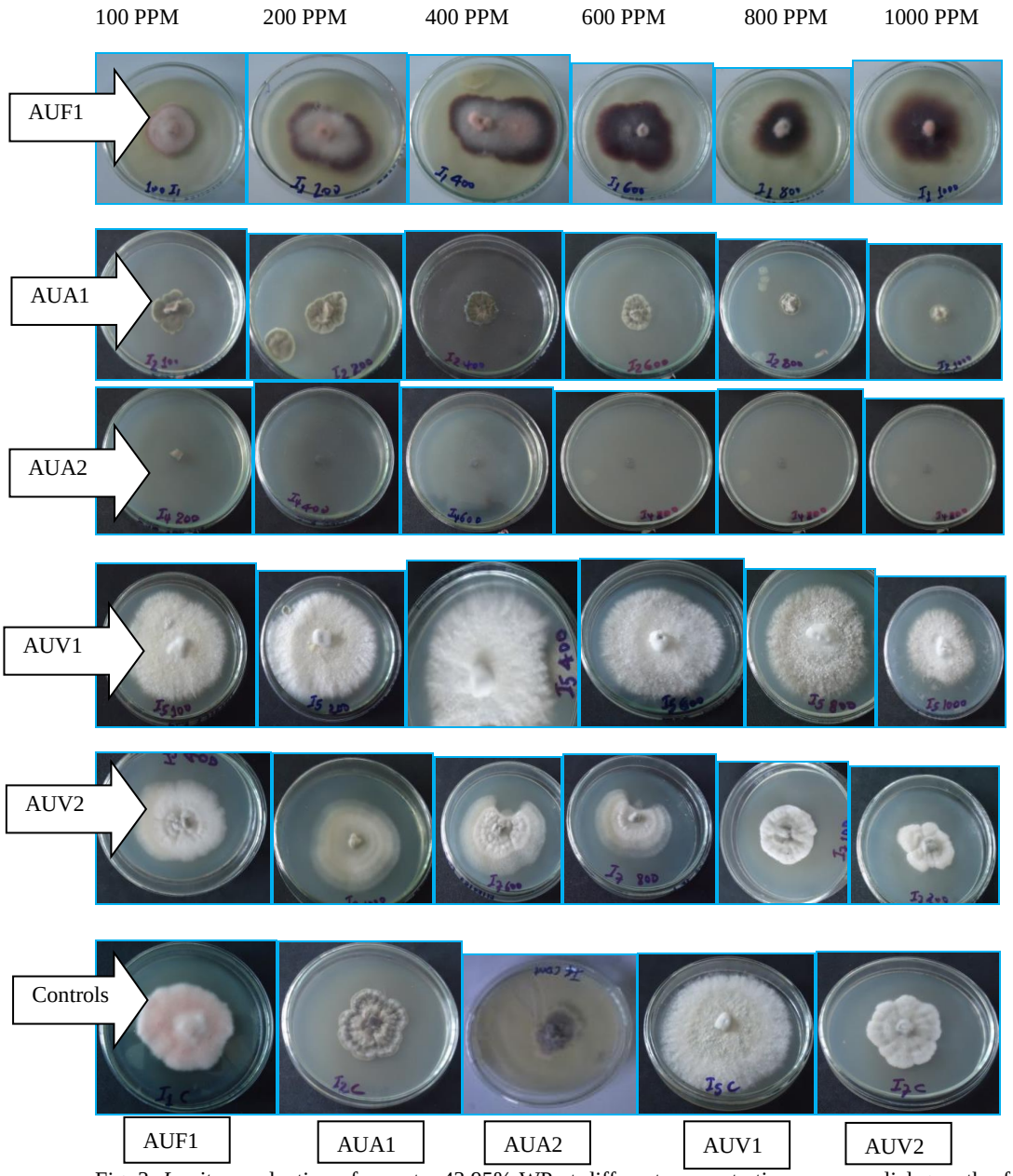

Fig. 3. *In vitro* evaluation of curzate, 43.95% WP at different concentrations on mycelial growth of fungal isolates after ten days of incubation at 25°C.

AUF1; AUA1; AUA2; AUV1; AUV2 =each fungal pathogen from left to right growing at different concentration of curzate (100 ppm, 200 ppm, 400 ppm, 600 ppm, 800 ppm and 1000 ppm)

Controls= from left to right are without the addition of fungicides

Table 4. Evaluation of *Trichoderma* spp. for tolerance to curzate at different concentration after seven days of incubation at 25°C.

Concentration (ppm)	*Trichoderma harzianum* (AUT1)		*Trichoderma viride* (AUT2)		Mean±SD
	Growth (mm)	% inhibition	Growth (mm)	% inhibition	
100	87.0±0.57a	3.3b	88.0±0.57f	2.2a	45.1
200	71.0±0.57b	21.1c	81.0±0.57e	10.0b	45.7
400	62.6±0.66c	30.4d	71.0±0.57d	21.1c	46.2
600	44.3±0.57d	50.7e	42.3±1.20c	52.9d	47.5
800	31.0±0.57e	65.5f	31.0±0.57a	65.5f	48.3
1000	23.3±0.88f	74.1a	28.6±0.88b	68.1e	48.4
Control (mm)	90.0±0.0g	0.0g	90.0±0.0f	0.0a	90.0
Mean±SD	53.2±5.43	40.84	57.0±5.80	36.7	53.02

Each value is an average of three replicates ± standard deviation. Means followed by the same letters within a column are not significantly ($p<0.05$) different according to Duncan's multiple range test. mm=millimetre

Table 5. Screening of *Trichoderma* spp. for tolerance to sancozeb at different concentration after seven days of incubation at 25°C.

Concentration (ppm)	*Trichoderma harzianum* (AUT1)		*Trichoderma viride* (AUT1)		Mean±SD
	Growth (mm)	% inhibition	Growth (mm)	% inhibition	
100	85.0±0.57f	5.5b	88.0±0.57e	2.2a	45.2
200	66.6±0.88e	25.9c	71.6±0.88d	20.3b	46.1
400	43.0±0.57d	52.2d	43.0±1.52c	52.2c	47.6
600	30.0±0.33c	66.3e	33.0±1.15b	63.3d	48.2
800	22.0±0.57b	75.5f	26.6±0.88a	71.4e	48.6
1000	19.0±0.57a	78.9g	25.6±0.33a	70.3e	48.7
Control (mm)	90.0±0.0g	0.0a	90.0±0.0e	0.0a	47.4
Mean±SD	50.8±5.85	50.7	54.0±5.75	46.7	47.4

Each value is an average of three replicates ± standard deviation. Means followed by the same letters within a column are not significantly ($p<0.05$) different according to Duncan's multiple range test. mm=milimeter

In vitro combination of *Trichoderma* species with fungicides against the test fungal isolates

It is clearly observed that an *in vitro* studies of combined use of biocontrols (AUT1 and AUT2) with chemical fungicides (curzate at 600 ppm and sancozeb at 400 ppm) against the fungal isolates revealed that it was more efficient than fungicides (Table 2 and 3) and *Trichoderma* individually (Table 1). At these concentrations (400 ppm and 600 ppm), the fungal antagonists were 50% compatible with both fungicides (Table 6). The combined efficacy of biocontrols with fungicides in response to individual isolates revealed that the highest percentage inhibition of mycelial growth were recorded as 85.6%, 79.7%, 87.5%, 89.3% and 80.2% when AUT2 was combined with sancozeb against AUF1, AUA1, AUA2, AUV1 and AUV2, respectively (Table 6). In the same pattern, the lowest percent of inhibition of mycelial growth of 77.6% and 71.1% was observed when AUT1 was combined with curzate for AUV2 and AUA1, respectively, whereas AUT2 with curzate recorded the lowest percentage inhibition against AUA1

(76.0%). AUV2 revealed the lowest percent of inhibition of 78.7% when AUT1 was combined with sancozeb. Similarly, the lowest percent (79.7%) of inhibition was exhibited for AUA1 when AUT2 was combined with sancozeb.

Table 6. *In vitro* evaluation of combination of fungicides with *Trichoderma* spp. at a concentration of curzate (C) 600 ppm and sancozeb (S) 400 ppm on mycelial growth of fungal isolates.

Fungal isolates	Control (mm)	Mean % inhibition of fungicide + biocontrol				Mean±SD
		AUT1+ C	AUT1+ S	AUT2+ C	AUT2+ S	
AUF1	62.7c	79.2b	82.9a	82.4a	85.6a	78.5±2.5
AUA1	46.0d	71.7c	78.9a	76.0b	79.7a	70.4±3.4
AUA2	50.6c	81.6a	86.7b	80.2a	87.5b	77.3±2.5
AUV1	87.3d	85.5b	84.7c	87.1b	89.3a	86.7±3.5
AUV2	53.7a	77.6b	78.7b	77.6b	80.2b	73.5±3.3
Mean±SD	60.1±4.3	79.1±5.3	80.6±3.2	80.6±4.1	84.4±3.9	77.2±3.0

Each value is an average of three replicates. Means followed by the same letters within a row are not significantly ($p<0.05$) different according to Duncan's multiple range test.
AUF1=*Fusarium* isolate 1; AUA1=*Alternaria* isolate 1; AUA2=*Alternaria* isolate 2; AUV1=*Verticillium* isolate 1; AUV2=*Verticillium* isolate 2; mm=millimetre
AUT1+ C=*Trichoderma harzianum* combined with curzate; AUT1+ S=*Trichoderma harzianum* combined with sancozeb; AUT2+ C=*Trichoderma viride* combined with curzate; AUT2+ S=*Trichoderma viride* combined with sancozeb

DISCUSSION

In the present study, the results of dual culture revealed that the rapid colonization of the medium by AUT1 and AUT2 (*Trichoderma* spp.) were effective in controlling mycelial growth of fungal isolates isolated from infected yam leaves and tubers. The previous findings of Okigbo and Ikediugwu (2000) have reported that *Trichoderma viride* controls post-harvest rot of yams, presumably by direct parasitism and antibiotic production. Similarly, Sahi and Khalid (2007) have also reported inhibition of 62% and 36% for *Trichoderma viride* and *Trichoderma harzianum* against *Fusarium oxysporum*, respectively.

Both species of *Trichoderma* spp. showed best performance against AUA1 (62.35±1.33% and 76.95±0.52%) and AUA2 (68.82±1.53% and 75.75±1.71%) inhibition by AUT1 and AUT2, respectively (Table 1). It is clearly indicative that this was due to *Trichoderma harzianum* which inhibited *Alternaria alternata* hyphae and exhibited morphological changes such as deformation, increase of cellular vacuolization, cell wall disintegration and dissolution of cytoplasm (Sempere and Santamarina, 2007). Similarly, Pandey (2010) reported that *Trichoderma harzianum* caused 67.07% inhibition of *Alternaria alternata*, while an inhibition of 66.67% was recorded by using *Trichoderma viride*. Our result indicated inhibition by AUT2 (75.57±0.63%) and AUT1 (73.72±1.27%) inhibition

percentage after seven days of incubation against AUV2. Hanson (2000) reported that biological control of *Verticillium dahliae* Kleb. in cotton with a mixture of lignin and *Trichoderma viride*.

As shown from an *in vitro* evaluation of the inoculation of fungicides, curzate and sancozeb decreased mycelial growth of fungal isolates compared to the control. Isolate AUA2 was found to be the most sensitive to both fungicides. Sancozeb was highly toxic to the fungal isolates tested compared to curzate. Therefore, sancozeb was effective and retained its efficacy even at the lowest concentrations by achieving 100% complete inhibition over the control compared to curzate. Similarly, Nisa *et al*. (2011) also observed that from a study amongst non-systemic fungicides, mancozeb was the most effective (14.20 mm) in reducing mycelial growth of *Fusarium oxysporum*. Harish *et al*. (2007) reported that mancozeb (0.2%), a main component of sancozeb, was observed to be the most effective, and significantly reduced the spore germination of *Helminthosporium oryzae*.

From the treatment of the fungal pathogens using biocontrol agents and fungicides, AUT1 and AUT2 showed potential antagonistic activity against the fungal isolates of yam. The results showed that no inhibition of mycelial growth was observed at 100 ppm level of both fungicides compared to the control and a gradual increase was observed in percent of inhibition as the concentration increased, but inhibition was lower than that of sancozeb. Papavizas (1985) has reported that the differentiating response of antagonists to various fungicides might be due to their inherent resistance to the fungicides and their ability to degrade chemicals. Thus, the results of the present screening would help in the selection of biocontrol agents which can be used with reduced dose of selected fungicides (curzate and sancozeb) for the control of yam fungal isolates.

The results from this experiment showed that at the concentrations 400 ppm and 600 ppm, the *Trichoderma* spp. was 50% compatible with both fungicides. This showed that the antagonists were able to utilize the fungicides as a source of nutrient, but above these concentrations it may weaken the efficacy of *Trichoderma* spp. (AUT1 and AUT2). Likewise, below these concentrations, the test fungal pathogens compete with the antagonists for nutrient and space, because these concentrations were not significantly effective in inhibition of mycelial growth for both test fungal isolates and biocontrols.

The results showed that effects of antagonists and fungicides and the interaction effect between antagonists and fungicides were significant ($p<0.05$) against fungal isolates. The maximum percentage inhibitions of the mycelial growth of the five fungal isolates were recorded when AUT2 was integrated with sancozeb followed by combination of AUT1 with sancozeb. Therefore, combination of sancozeb with AUT1 and AUT2 was more effective than combination of curzate with AUT1 and AUT2 in terms of mycelial growth inhibition percentage. The result of this experiment clearly showed that integration of fungicides and biological treatments against fungal isolates under *in vitro* condition displayed potential for inhibiting the mycelial growth when compared to the application of biological or chemical treatments alone.

Similarly, Srinivas and Ramakrishnan (2002) have reported that integration of biocontrol agents and commonly used fungicides showed positive association by reducing the seed infection compared to fungicide and the fungal antagonists individually. Silimela and Korsten (2001) have reported that the efficiency of the biocontrol agent could further be improved when it was applied with the recommended fungicide and used at a lower concentration. Thus, the antagonistic potential of *Trichoderma* spp. in terms of enhanced modes of action as increased hyperparasitism activity in the present study.

The present study implied that an *in vitro* application of fungicides (curzate and sancozeb) was effective in controlling fungal isolates, but they are not environmentally friendly for managing the fungal isolates. Therefore, rather than applying these chemical fungicides alone, it is very important to use *Trichoderma* species (AUT1 and AUT2) for effective management of fungal isolates of yam since they do not have side effect on the environment. From the result of this study, it is clearly indicated that the combination of *Trichoderma harzianum* and *T. viride* with the lower concentration of curzate and sancozeb reduced the fungal mycelial growth to some extent, which shows that they are very important to apply as an integrated fungal disease management of yam.

Among the two fungicides tested, sancozeb was effective in terms of reduction of mycelial growth inhibition. But, the biocontrol agents were used as an alternative to chemical fungicides to reduce the mycelial growth of the fungal isolates due to their side effect on human and animal health, normal flora of soil and also pathogenic fungi became very fast resistant to them. In the present study, antagonists were evaluated for their compatibility

with curzate and sancozeb as fungicidal tolerant biocontrol to manage the yam fungal isolates through integrated approach by combining biocontrols and chemical fungicides. Therefore, strategies for managing yam fungal isolates in combinations of *T. harzianum* and *T. viride* and curzate and sancozeb fungicides practice together to manage effectively is very important and relevant in an *in vitro* condition.

CONCLUSION

It can be concluded that the *Trichoderma* species reduced the growth of all fungal pathogens: AUF1, AUA1, AUA2, AUV1 and AUV2 significantly at different levels and, therefore, can be incorporated for integrated fungal pathogens management of yam. Hence, *Trichoderma* species (AUT1 and AUT2) can be used as a potential biocontrol agent to manage these pathogens. Also, its efficacy for managing yam fungal pathogens by combination of *Trichoderma* species (AUT1 and AUT2) with fungicides (sancozeb and curzate) was found to be more effective in comparison to the fungicide alone. Therefore, this research can have promising potential in agricultural fields to protect yam affected with various fungal pathogens.

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