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Effect of Temperature on the Antagonistic Performance of *Trichoderma*spp Against *Fusariumoxysporum*

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***Abstract* – *Fusariumoxysporum* is a well-known soil-borne fungi and it is difficult to control their pathogenic strains by conventional strategies. Trichoderma is a biological microorganism used for controlling the soil borne plant pathogens and are environmentally acceptable alternative to the existing chemical treatment methods. The cultures of two Trichoderma species (*Trichodermaharzianum* and *Trichodermavirde*) were examined under different temperature in laboratory conditions. The experiment was conducted on complete randomized design with 3 replications with two methods. Two bio-control agent (*Trichodermavirde* and *Trichodermaharzanium*) and one target pathogen (*Fussariumoxysporum*) were used for the experiment. The result revealed that percent inhibition of mycelia growth (PIMG) recorded high in method one *Fussarium* + T. *harzanium* at 30^0C (63.57%) followed by T. *virde* at 30^0C (46.52%), but in that method the lowest colony diameter recorded at 20 and 25 ^{0}C was 4.08 cm and 3.75 cm respectively. The present study proved that *Trichodermaspp* has good antagonistic ability on the mycelia growth of *Fussariumoxysporum*.**

***Keywords* – Bio-control, *Fussariumoxysporum, Trichodermavirde, Trichodermaharzanium*.**

I. INTRODUCTION

Soil-borne fungi have a wide host range and persist for longer periods in soil by means of resistant resting spores. The plant diseases caused by such fungi are among the most difficult to control (Nakkeeran *et al.*, 2002). Application of chemicals for the control of such pathogen is not economical (Yucel *et al*., 2007). Fungicides replacement with bio-control agents is an alternative mean to manage the plant pathogens, produce safe food and reduce environmental pollution (Dubey*et al.*, 2007). Biological control involves the use of biological organisms to control plant pathogens or plant diseases. The microbial inoculants as bio-control agents are effective and attractive alternatives to prevent the deficiencies brought about by the exclusive reliance on chemicals (Cigdem and Merih, 2003).

There are many biological control strategies for the control of soil-borne diseases. Among the potential bio-control agents in the rhizosphere, several species of Trichoderma are reported to be effective in controlling a variety of fungal plant diseases (Dubey*et al.*, 2007). The fungi *Trichoderma* spp. are one of the most important bio-control agents and most frequently isolated from soil and plant root ecosystems (Sharma *et al.,* 2012 and Altinok, 2009). Several strains of Trichoderma have been developed as bio-control agents against fungal diseases of plants (Freeman *et al.,* 2004) Trichoderma are free-living fungi and common in soil and root ecosystems (Nusrat, *et al.,* 2013).

These filamentous fungi are very wide spread in nature, with high population densities in soils and plant litters. They are environmentally safe to control plant pathogen compared to any other pesticides. Farmers can easily use antagonistic fungi commercially to increase their yield of crop and decrease cost of pesticides (Carvalho *et al.,* 2014). Members of Trichoderma particularly *Trichodermaharzianum* and *Trichodermaviride*

are promising biological control agents against plant diseases. Hence, the present study carried to evaluate the effects temperature on two Trichoderma species against *Fusariumoxysporum*.

II. MATERIAL AND METHODS

Description of the Study Area

The study was conducted in the plant pathology laboratory, School of Plant Sciences, Haramaya University, Ethiopia. The area is geographically located 517 km east of Addis Ababa at about $37^0 43`$ latitude to the east and $10^0 25`$ longitude to the north.

Sources of Pathogen and Bio-Agents for Study

*F. oxysporum*pure culture were obtained from plant pathology laboratory of the School of Plant Sciences and two *Trichoderma*spp namely *T. viride* and *T. harzianum* were used for the experiment.

Screening by Dual Culture Methods

Two species of *Trichoderma* with pathogenic *F. oxysporum* were studied in a dual culture assay on PDA medium in 90 mm petri plates as described by (Nakkeeran *et al.*, 2002). The experiment was arranged as a completely randomized design with three replicates. The inoculated plates were incubated at three different temperatures (20, 25, and 30 °C) with two dual culture methods (Figure 1) and at each incubation temperature there was a control. The experimental design was completely randomized design (CRD) with three replications. The radial growth of the pathogen *F. oxysporum* and Percent inhibition of mycelial growth (PIMG) of the *F. oxysporum* was measured with 24 hrs interval after 1 days of incubation by measuring the radial growth of the *F. oxysporum* colony until the mycelia of target pathogen fully cover the control petridish.

Method I

A 5 mm size discs of culture of the two *Trichoderma* spp., (5-7 days old culture) and the same size of another agar disc of *F. oxysporum* was placed opposite to each other and close to the periphery of 90 mm Petriplates containing PDA. For control, *F. oxysporum*was placed in a similar manner on PDA plate. All pairing will be carried out in three replications and incubated at three different temperatures (20, 25 and 30°C).

Method II

A 5-7 days old culture of 5 mm agar discs of the two *Trichodermaspp* were placed on 2 cm away from the periphery of the petriplates and the same size of another agar discs of *F. oxysporum* was placed 2 cm away from periphery of the petriplates in opposite manner separately. Plates were incubated in the same manner as of first method. For control, *F. oxysporum*was placed in a similar manner on PDA plate.

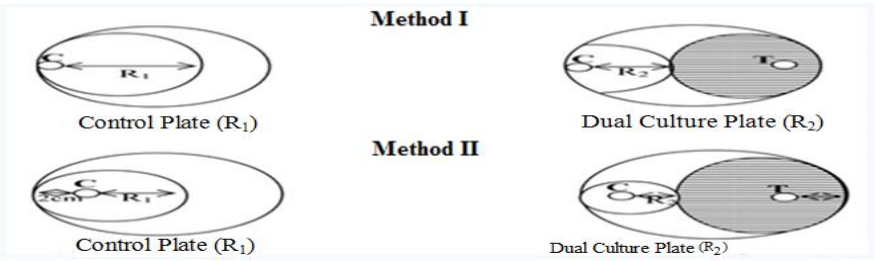

Fig. 1. Pictorial representation of methods in dual cultures.

Data Analysis

The inhibition percent in the mycelial development of the pathogen fungus was calculated by the formula: R1 = (C-T) / C x 100; Where RI is the inhibition percentage of the radial mycelial growth, C is the radial growth of the pathogen in the control (mm), and T is the radial growth of the pathogen in dual culture (Hajieghrari *et al*., 2008). The results were subjected to ANOVA.

The above two readings were transformed in to PIMG using the formula (Skidmore and Dichinson, 1976), where, PIMG= $(A_1 - A_2) / A_1 x100$.

Where

A_1: The diameter of mycelium growth of pathogenic fungus in control.

A_2: The diameter of mycelium growth of pathogenic fungus with Trichoderma fungus.

III. RESULT AND DISCUSSIONS

Two culture methods were applied to the percent inhibition of mycelia growth (PIMG) of *F. oxysporum* at three different temperatures (20, 25 and 30^{o}C). The results obtained are shown in tables and figures as follows.

Table 1. Fusarium radial growth at the end of incubation date on method one dual culture.

Treatments	Incubation temperature (^{o}C)					
	20	% inhibition from the control	25	% inhibition from the control	30	% inhibition from the control
Fusarium +*T. viridea*	4.08	34.72	3.75	45.09	3.92	46.52
Fusarium + *T.harzanium*	4.17	33.28	3.83	43.93	2.67	63.57
Fusarium only (Control)	6.25		6.83		7.33	

On dual culture method one, Fusarium + *T. viridea* the lowest radial growth recorded at 20 and 25 was 4.08 cm and 3.75cm, respectively (Table 1), while in the control treatment it was 6.25cm and 6.83cm, respectively than Fusarium + *T. harzanium*. The highest PIMG was recorded from Fusarium + *T. harzanium* at 30 ^{0}C (63.57%) followed by Fusarium + *T.* viridea 46.52%.This result showed both trichoderma inhibit the growth of *F. oxysporium* to various degree, that was at 20 ^{0}C and 25 ^{0}C the PIMG showed almost the same trend that was approximately the same for both *Trichoderma*spp, this shows has at 20 ^{0}C and 25 ^{0}C no significant difference to each other for the trichoderma but for the control it has significant different. Also indicated *T. viridea* inhibit *F. oxysporium* at low temperature but there was no significant difference and also the highest PIMG was recorded from Fusarium + *T. harzanium* at 30 ^{0}C revealed significance difference.

Table 2. Fusarium radial growth at the end of incubation date on method two dual culture.

Treatments	Incubation temperature (^{o}C)					
	20	% inhibition from the control	25	% inhibition from the control	30	% inhibition from the control
Fusarium +*T. viridea*	4.33	40.28	3.92	54.79	3.83	54.94
Fusarium + *T.harzanium*	4.17	42.48	3.83	55.82	3.92	53.88
Fusarium only (Control)	7.25		8.67		8.50	

From the table 2 above, percent inhibition result showed that there was no significance different on both incubation temperatures. This showed that dual method two has no significance difference with each other but for the control it has significance difference. And also the results indicated both *Trichodermaspp* have ability to inhibit *F. oxysporum*radial growth.

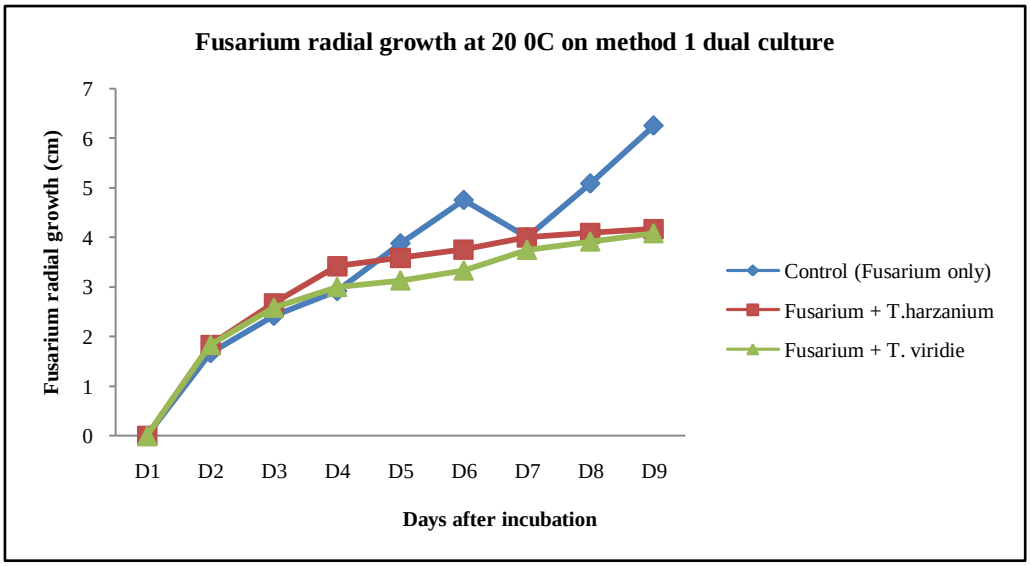

Fig. 2. Fusarium radial growth at 20 ^{0}Cafter incubation in method one.

The radial growth Fussarium at each day after incubation to the 9th day depicted on figure 2. The control (Fussarium only) grows from day 1- day 6 increasing in increasing rate, but with unknown reason at the 7th day the growth was decreased; this could be data recording problem. The growth of control should have to be in straight radial growth otherwise. The *F. oxysporum* within *T. viridea* and *T. harzanium* increases its growth only up to day 4; means they inhibit its growth starting from day 4, but without both Trichoderma the growth was continuous up to day 9. This showed that both *Trichoderma*spp has ability to inhibit F. *oxysporum* growth. When we compare *T. viride* with *T. harzanium* there is a little bit significance difference between them, means *T. viridea* was better.

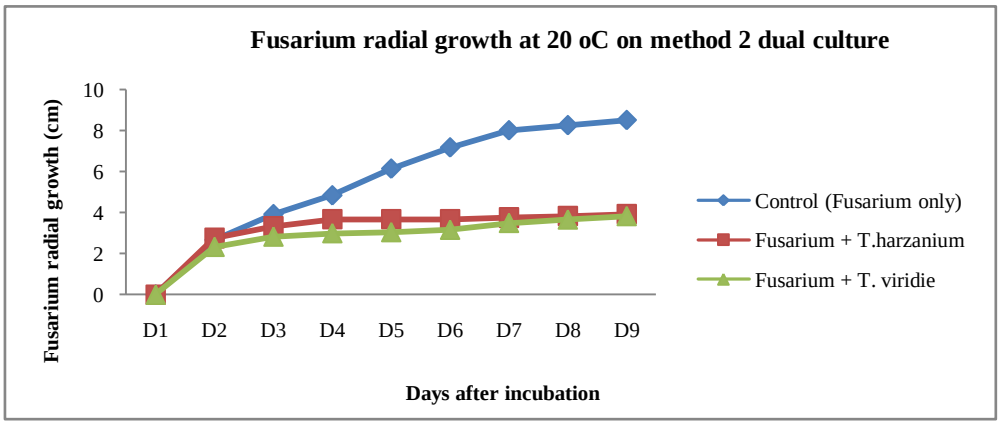

Fig. 3. Fusarium radial growth at 20 ^{0}Cafter incubation on method II.

F. oxysporum radial growth showed a continuous increasing in control from day after day, but the *F. oxysporum* with in *Trichoderma*spp the radial growth was stopped at the 3rd day (Figure 3). After day 3, the *F. moniliformae* radial growth showed a constant growth at both *Tricoderma*spp, that means both *Trichoderma*spp inhibit *F. oxysporum* radial growth with approximately similar level.

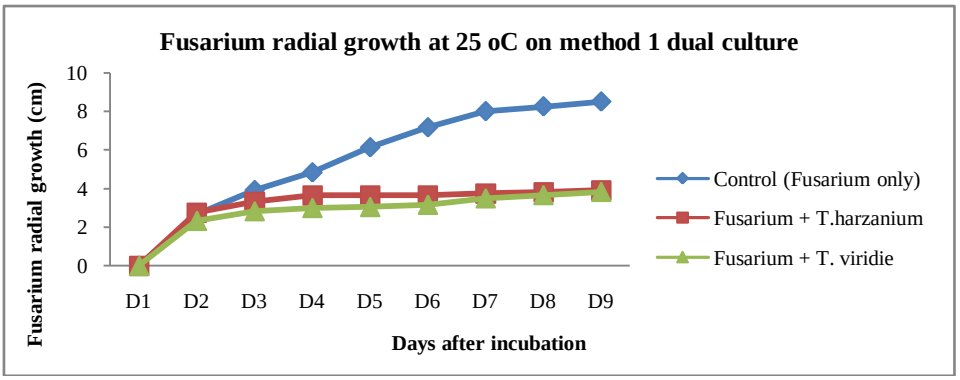

Fig. 4. Fusarium radial growth at 25 ^{0}Cafter incubation on method I.

F. oxysporum radial growth was increased at increasing rate from day after day at 25 ^{0}C (Figure 4). But *F. oxysporum* with in *Trichoderma*spp, the growth was continuous up to day 3. After day 3, the growth of *F. oxysporum* was constant; meaning that *Tricoderma*spp inhibited the growth and also the graph indicate *T. viridiea* showed significant difference.

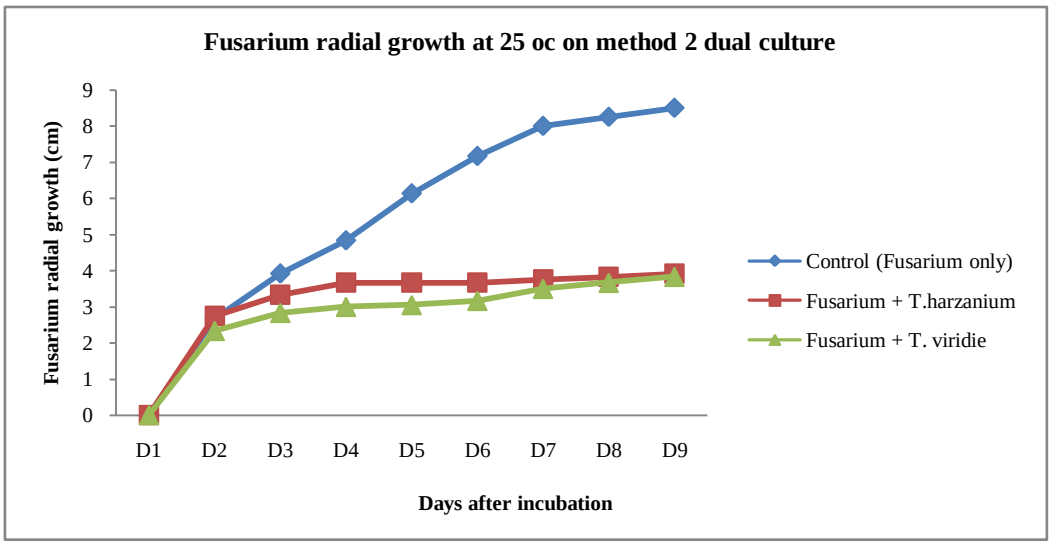

Fig. 5. Fusarium radial growth at 25^{0}C after incubation on method II.

As depicted on figure 5, the *F. oxysporium* growth showed continuous increase at control from day after day, but the *F. moniliformae* with in *Trichoderma*spp growth stopped from day 3. After day 3 the *F. oxysporium* growth constant at both *Trichoderma*spp that means both *Trichoderma*spp inhibited *F. moniliformae* growth with approximately similar level. This showed that the *Trichoderma*spp for each other non-significance difference. *F. oxysporum* growth show continuously increasing at control from day after day, but the *F. oxysporum*with in *F. oxysporum*growth stopped from day 3. After day 3 the fussarium growth constant at both *F. oxysporum* that means both *Trichoderma*spp inhibit *F. oxysporum* growth with same or approximately similar level. This shows that the *Tricoderma*spp for each other non-significance difference.

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performance. Etaborian (2006) reported that the *T. virdie* reduced the colony area of macro phominaphaseoli by 19.2 and 34.9% using the dual culture. Ghisalberti and Rowland (1993) reported that other than mycelium interaction and hyper parasitism by the *Tricoderma* species, scientists have also considered the action use of antibiotic metabolites as contributing mechanism in the bio control of plant pathology. The ability of *trchoderma* species to produce inhibitory substances against microorganism has been described by Dennis and Webster (1971) and Jnatura (1995).The 3 different temperature 20,25 and 30^0C of two *Trichoderma* species were tested in two dual culture methods applied to the PIMG of *F. oxysporum.* In method 1 PIMG was at 30^0C (68.37%), at 25^0C (45.09%) and at 20^0C (34.72%). The highest PIMG at 30 ^{0}C (68.37%) for T. *harzanium.* In method 2 the highest PIMG was at 25^0C (55.82%) for *T. harzanium* and at 30 ^{0}C (54.94%) for *T. viride*.

IV. Conclusion

It may be concluded from the present study that the two *Trichoderma* spp. incubated with different temperature and method to inhibit or prevent mycelia growth of *F. oxysporum.* At law temperature (20 and 25^oc) *F. oxysporum* + *T. virde* could prevent or inhibit the mycelia growth of *F. oxysporum*in method one, but at highest temperature (30^oC) *F. oxysporum* + *T. harzanium* could prevent or inhibit mycelia growth of *F. oxysporum*in method one; this means *F. oxysporum* + *T. harzanium* at 30^oC has highest PIMG. *Trichoderma*spp has a large potential effect as bio-control agent against *F. oxysporum.*

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