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Evaluation of Phytotoxic Activity and Antagonism of *Trichoderma koningii*

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ABSTRACT

Trichoderma species are important biocontrol agent plasticized in present agriculture for the management of crop plant diseases. In the present research study, phytotoxic activity of *T. koningii* on crop plant seeds and antagonism of *T. koningii* against plant pathogenic fungi were tested. Treatment of seeds of crop plants with culture filtrate of *T. koningii* indicated the germination potentiality of treated seeds were increased. There was significant increase in germination of seeds of rice(96%), jowar(94%), and wheat(91%) while moderate increase in germination of seeds of mung(82%), groundnut(81%), sunflower(77%). *Trichoderma koningii* was highly antagonistic over plant pathogenic fungi such as *Alternaria alternata* (71.1%), *Geotrichum candidum* (67.7%), *Rhizoctonia solani* (67.7), *Fusarium proliferatum* (64.4%) and *Aspergillus niger* (57.7%). *Trichoderma koningii* is not phytotoxic in nature for crop plants but it is antagonistic against plant pathogenic fungi.

Key words: *Trichoderma koningii* phytotoxic activity, antagonistism.

INTRODUCTION

Trichoderma species are used wprldwide as a most effective biocontrol agent against number of crop plant pathogens. It reduces growth, survival of plant pathogens by different mechanisms like competition, antibiosis, mycoparasitism and enzyme secretion. The soil borne fungus *T. koningii* is a biocontrol agent with ability to produce enzyme, parasitize pathogenic fungi and induce systemic resistance to crop plants. Antagonistic ability of *Trichoderma* species is very effective nd it inhibits the colony formation of plant pathogenic fungi. Many workers contributed in researching antagonistic activities of *Trichoderma* species. They are closely related with both biocontrol activity and plant growth

(Chet et al., 2006; Howel, 1998).The effect of *T. harzianum* and *T. viride* on cumin seeds after priming resulted increased germination, shoot: root ratio and plant height (Sherma et al., 2009).

Trichoderma species stimulated the growth of tomato plants (Ozbay et al., 2004). Treatment with *Trichoderma* species not only checked the disease but it helps to agriculture in many ways such as soil improvement biological activity of soil, soil fertility and mobilization of minerals (Kulisler, 1997). The *Trichoderma* species can bebeneficial to the plants causing growth stimulation (Kleifield and Chet, 1992; Ousley et al, 1994). *Trichoderma harzianum* was found to be antagonistic against many soil borne pathogens (Papavizas, 1985; Pan et al., 2001; Jash and Pan, 2004). In modern period of plant pathology and agriculture, number of formulations were available in market are made by the application of *T. viride* and *T. harzianum*. But it is necessary to determine the ability of other species of *Trichoderma* for the management of crop plant diseases. Therefore in the research paper, performance of *T. koningii* with respect to phytotoxic activity and its antagonistic potentiality over plant pathogenic fungi was considered.

MATERIALS AND METHODS

1. Isolation of *Trichoderma koningii*:
Trichoderma koningii was isolated from the rhizosphere soil of various crop fields. Isolated *Trichoderma koningii* was maintained on potato dextrose agar (PDA) medium.

2. Phytotoxin activity:
Isolated *T. koningii* was grown on Richard's solution (broth). Twenty five ml of Richard's broth was poured in 100 ml conical flasks. The flasks along with medium were incubated at 15 lbs for 20 minutes. The flasks were allowed to cool and inoculated with 1 ml spore suspension of *T. koningii* from 7 days old culture. The flasks were incubated for 9 days at 27±2^{0}C. after the flaks were harvested by filtration through Whatman filter paper No. 1. The filtrate were collected in pre sterilized conical flaks and considered as crude toxin preperations. These were tested for their toxicity.

For the determination toxicity the crude toxin was treated with seeds of wheat, rice, jowar, gram, mung, moth, groundnut, soybean, sunflower, methi, spinach and shepu. Seeds were sterilizes by treating with 0.1% Mercury Chloride solution and followed by repeated washing with sterilized distilled water. The seeds were soaked in crude toxin for 24 hours. Then they were placed in moist blotter paper in sterilized petriplates. Seed soaked in similarly in uninoculated medium served asa control. Percent germination of seeds was observed and data were recorded (Haikal, 2008)

3. Isolation of pathogenic fungi:
Similarly pathogenic test fungi such as *Alternaria alternata, Rhizoctonia solani, Geotrichum candidum*, *Aspergillus niger, Fusarium oxysporum f. sp. Spinacae, Macrophomina phaseolina, Pythium* spp, *Alternaria tenuissima* and *Fusarium proliferatum* were isolated from naturally infected crop plant. These isolated pathogenic fungi were maintained on PDA slants.

4. Dual culture technique (Morton and Stroube, 1955)**:**
Twenty five ml of sterilized melted PDA was poured in sterilized petriplates and allowed to solidify. Seven days old culture of *Trichoderma koningii* was maintained and inoculated with test fungi about 6 cm away from each other and incubated at 28 ± 1°C. Three replications were maintained for each treatment. Observations of the antagonistic effects of *Trichoderma koningii* were recorded after 7 days. When the growth of pathogenic fungi became static the inhibition over control was calculated (Vincent, 1947).

RESULT

Production of phytotoxin
The obtained results indicated that culture filtrate of *Trichoderma koningii* proved to be stimulatory for the germination of crop plant seeds (Table 1). Seed germination was increased in case of rice (96%), jowar (94%), methi (92%) and wheat (91%) followed by mung (82%), groundnut (81%), and moth (80%). Less increase in germination percent was observed in sunflower (77%), soybean (72%), gram (68%), shepu (67%) and spinach (64%).

Table 1: Effect of culture filtrate of *Trichoderma koningii* on seed germination of crop plants.

Crop plants	Seed Germination (%)		
	control	Treatment	Difference
Wheat (*Triticum aestivum*)	65	91	26
Rice **(***Oryza sativa*)	76	96	20
Jowar **(***Sorghum vulgare*)	80	94	14
Gram **(***Cicer arietinum*)	62	68	06
Mung (*Vigna radiata*)	61	82	21
Moth **(***Phaseolus aconitifolius*)	82	80	02
Groundnut(*Arachis hypogeal*)	75	81	06
Soybean (*Glycine max*)	70	72	02
Sunflower(*Helianthus annus*)	65	77	12
Methi(*Trigonella foenum graecu*)	83	92	09
Spinach *Spinacea oleracea*	62	64	02
Shepu *Anethum graveolens*	60	67	07
Mean	70.08	80.33	10.25

Table No.2: Antagonistic nature of *T. koningii* against phytopathogenic fungi

Sr. No.	Test Fungi	Colony diameter (mm)	Inhibition (mm)	Inhibition (%)
1	Control	90	-	-
2	*Alternaria alternata*	26	64	71.1
3	*Rhizoctonia solani*	29	61	67.7
4	*Aspergillus niger*	38	52	57.7
5	*Geotrichum candidum*	30	60	67.7
6	*Fusarium oxysporum f. sp. Spinacae*	35	55	61.1
7	*Macrophomina phaseolina*	40	50	55.5
8	*Pythium* spp	37	53	58.9
9	*Alternaria tenuissima*	38	52	57.7
10	*Fusarium proliferatum*	32	58	64.4

Antagonistism against pathogenic fungi

Results in the table. 2 indicated that antagonistic potentiality of *T. koningii* against pathogenic fungi. *Trichoderma koningii* inhibited the mycelial growth and sporulation of all tested pathogenic fungi. Its antagonism is maximum in case of *Alternaria alternata* (71.1%), followed by *Rhizoctonia solani* (67.7%), *Geotrichum candidum* (67.7%) and *Fusarium proliferatum* (54.4%). Minimum inhibition on colony formation was observed in case of *Macrophomina phaseolina* (55.5%).

In the present research paper phytotoxic effect of *T. koningii* was tested and results indicated that in case of germination of seeds of crop plants were increased without any adverse effect. However the effect was variable but increase in germination percent is a important for the crop plants that should be advantage for their establishment in the field. *Trichoderma koningii* inhibited the sporulation and mycelial growth of plant pathogenic fungi. The antagonistic nature of *T. koningii* is useful in disease management strategy as its application reduces the use chemical based fungicides which caused many hazards such as pollution problems and induction of physiological resistance in pathogens to fungicides. The use of *T. koningii* can be practiced as supplementary method for disease control. Reports on phytotoxicity by *Trichoderma* species were published

by many workers but *Trichoderma* was not exhibiting phytotoxic effect on crop plants. *Trichoderma harzianum* was found enhanced the growth of lettuce (Caporel et al., 2014). Maka and Alimova (2008) also reported that *T. harzianum* decreased the phytotoxic effect in the soil caused by the use of agrochemicals. *Trichoderma* species secreted metabolites in the soil those were found to be antibacterial as well as antitoxidants of mycotoxins in the soil ecosystem (Zhang et al., 2017). In this study, *T. koningii* investigated as an important antagonistic soil fungus having ability to reduce disease incidence caused by the phytopathogenic fungi. Biological control in present times have been acceptable alternative to the existing chemical treatments (Elad, 2000; Eziashi et al., 2007; Shalini and Kotasthane, 2007). Rajkonda and Bhale (2011) reported effect of *T. harzianum* on seed germination and vigour index in pigeon pea was significant. Bhale et al., (2013) reported that *Trichoderma* species were promising biocontrol agents against post harvest disease in fruits. *Trichoderma viride, T. harzianum* and *T. hamatum* were antagonistic over *Fusarium oxysporum* and *F. proliferatum* (Bahareh et al., 2014). *Trichoderma viride* showed antagonistic effects on *Botrytis cinera, F. oxysporum, Macrophomina phaseolina* and *Rhizoctonia solani* (Sridhar et al., 2015).

CONCLUSION

From the results, it is concluded that *T. koningii* exhibited effective antagonism against phytopathogenic fungi. Its antagonism was observed as low as 55.5% in case of *Macrophomina phaseolina* therefore the biocontrol activity was opportunistic to control various fungal diseases. The germination of treated crop plant seeds was better than control indicating that *T. koningii* was useful for promoting growth of crop plants because it does not exhibited phytotoxic effect. From the results two statements can be cleared firstly the bioagents *T. koningii* was highly antagonistic against plant pathogenic fungi and second it decreases phytotoxicity in the soil and makes the soil more fertile. Thus with the disease management of crop plant diseases the *T. koningii* helps the establishment of seedlings.

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