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Toxic effect of secondary metabolites secreted by rhizospheric fungi isolated from Bt-cotton

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Abstract
In present investigation, toxic effect of secondary metabolites secreted by rhizospheric fungi isolated from Bt-cotton against seed germination and seedling growth was tested in Wheat and Jowar. The culture filtrate of *Penicillium islandicum*, *Trichoderma viride*, *Aspergillus niger*, *Fusarium oxysporum* and *Alternaria solani* was obtained. Seed germination percentage and growth was affected by all five fungal secondary metabolites. The secondary metabolites of *Trichoderma viride* and *Penicillium islandicum* enhances seed germination percentage while it was least by *Aspergillus niger*, *Fusarium oxysporum* and *Alternaria solani*.

Keywords: secondary metabolites, rhizosphere fungi, Bt-cotton, seed germination, wheat and jowar

Introduction

Transgenic crops are new products of agriculture biotechnology. The environmental risks and benefits of transgenic crops are topic of hot debate. Very few reports are available on the impacts of transgenic/GM plants specially cotton or their / it's products (that they release in soil) on soil microorganism such as fungi.

Fungal metabolites are those substances which are produced by fungi during their metabolic processes. The constituents of metabolites are phenols, terpenoids, amino acids and plant growth regulators (Griffin, D.H., 1981) [6]. Aspergellic acid, aflatoxin B1 and B2, cyclopiczonic acid, fusaric acid, naphthoquinones and fumonizin are some of those substances which threaten the health of plants and animals, (Singh *et al.* 1991) [13].

On the other hand, *Penicillium* and *Aspergillus* species have been reported to produce gibberellin which is a growth regulating hormone in higher plants (Hasan, 2002; Hamayun et al., 2009) [7]. *Penicillium* species, a large number of micro-organisms are known to produce toxic metabolites when cultivated on synthetic media. Fungal metabolites are substances discharged by fungi in their metabolic processes. The metabolites are products of some amino acids, cyclic peptides, aromatic, phenols, terpenoids and plant growth regulators (Graffin, 1981; Madhosing, 1995; Nema, 1992) [6]. Fungi may play an important role in plant survival by enhancing nutrient uptake and producing growth promoting metabolites such as gibberellins and auxins. *Penicillium* and *Trichoderma* species are known to produce a variety of beneficial compounds to suppress the pathogens (Hyakumachi *et al*., 1994; Narisawa et al., 2004; Dubey et al., 2007) [9] and stimulate plant growth by the production of phytohormones (Hasan, 2002) [7] and/or degradation of complex substrates (Altmore *et al.*, 1999) [1]. Seed-borne fungi are responsible for inhibiting normal growth of seedlings in various crops (Howlett, 2006). The mycotoxin produced by *Fusarium proliferatum* reduces seed germination (Kritzinger *et al*. 2003) [12]. Enzymes of fungi are known to be involved in the breakdown of cell wall and maceration of plant tissue, which play an important role in invasion of plants by pathogens (Gothoskar *et a1*., 1955) [5].

In the present study, the seed samples of *Triticum aestivum* and *Sorghum vulgarae* were treated with culture filtrates of *Penicillium islandicum, Trichoderma viride, Aspergillus niger, Fusarium oxysporum and Alternaria solani.* and their effect on percentage of seed germination and seedling growth was studied.

Materials and methods

Isolation of rhizosphere fungi: rhizosphere fungi were isolated from rhizosphere of bt cotton (Jalander and Gachande, 2011) [4] and five fungi were selected for the present study.

Collection of seed samples: Seed samples of *Triticum aestivum* and *Sorghum vulgarae* were collected from adjoining of Parbhani district market area.

Production of fungal metabolites: five different rhizosphere species of bt cotton namely *Penicillium islandicum, Trichoderma viride, Aspergillus niger, Fusarium oxysporum and Alternaria solani.* were grown in 250 ml conical flask containing 100 ml Czapek's liquid medium for ten days at 25±2 ºC. On incubation culture filtrates were filtered in pre sterilized flasks by using What man filter paper No.50 and stored at 4ºC. Effect of culture filtrates on seed germination and seedling growth of seeds of each seed sample were surface sterilized with 0.1% mercuric chloride. seeds of each sample were suspended in culture filtrates of selected fungi and incubated at room temperature (28±2ºC) for 24 hours. Seeds were plated at equidistance on moist blotters (9+1) according to ISTA (1966) [11]. At the same control was also maintained with distilled water. After seven days of incubation period, plates were observed and germination percentage, root length and shoot length was measured.

Result and Discussion

From table. 1 of above research it is found that the All the

five fungal secondary metabolites affected germination percentage and seedling growth of *Triticum aestivum*. The maximum seed germination at 15 days shown by *Trichoderma viride* (92.4%), *Penicillium islandicum* (89.4%), and there is decrease in seed germination shown by *Alternaria solani*., *Fusarium oxysporum* and *Aspergillus niger*. Similarly all the five fungal secondary metabolites affected germination percentage and seedling growth of *Sorghum vulgare* (Table.2). The maximum seed germination at 15 days shown by *Trichoderma viride* (90.5%), *Penicillium islandicum* (88.5%) and there is decrease in seed germination shown by *Alternaria solani*., *Fusarium oxysporum* and *Aspergillus niger* over control.

From the table 1 and 2 it is found the shoot length and root length also influenced by culture filtrate of fungi. The increase in shoot length by *Penicillium islandicum* and *Trichoderma viride* and least increase in other namely *Alternaria solani*, *Fusarium oxysporum* and *Aspergillus niger* over control.

According to the results, all studied seed growth parameters were affected by the treatments and there (Ibatsam *et al*., 2013) [10] the formation of toxins by fungi has also been reported, which act on cereals growth in soil (Krasil'nikov, 1958) [15]. Current findings confirmed the previous reports of shoot length promotion by fungal culture filtrate application (Choi *et al*., 2005; Khan *et al.*, 2008) [3]. *Penicillium* sp. can stimulate plant growth in cereal crops (Whitelaw *et al*., 1997) [16]. *Alternaria alternata* produced several toxic metabolites of major toxicological importance including, HST-toxin, AAL-toxins, tenuazonic acid, alternariol monomethyl ether, alternariol, altenuene, and altertoxin I in artificial medium during its growth period (Holensein and Stoessi, 2008) [8]. There is close relationship between the duration of treatment and process of inhibition of seed germination and seedling emergence in crop plants (Sung *et al*., 2011; Bhajbhuje, 2014) [14, 2]. From the study it was found that some toxic fungal metabolites promote and some affect seed germination and emergence of *Triticum aestivum* and *Sorghum vulgarae* seeds.

Table 1: Seed viability and seedlings length in Wheat (*Triticum aestivum*) seeds after treatment of fungal secondary metabolites.

		Seed viability		Seedling emergence	
Rhizosphere fungi	**Duration of treatment (Days)**	**Seed germination (%)**	**Dormant seeds (%)**	**Shoot length (cm)**	**Root length (cm)**
Penicillium islandicum	5	79.5	20.5	4.3	1.8
	10	87.6	12.4	10.5	2.9
	15	89.4	10.6	12.8	4.6
Aspergillus niger	5	79.4	20.6	2.5	1.5
	10	72.2	27.8	3.2	1.9
	15	69.6	30.4	3.5	2.3
Trichoderma viride	5	82.8	17.2	3.6	1.2
	10	89.2	10.8	8.7	1.9
	15	92.4	7.6	11.2	3.7
Fusarium oxysporum	5	72.3	27.7	1.8	1.4
	10	67.4	32.6	4.2	3.6
	15	62.3	37.7	2.7	3.9
Alternaria solani.	5	70.2	29.8	2.5	1.4
	10	72.9	27.1	4.6	2.8
	15	60.4	39.6	3.8	3.9
Control		80.3	19.7	7.4	5.8

Table 2: Seed viability and seedlings length in Jowar (*Sorghum vulgare*) seeds after treatment of fungal secondary metabolites.

		Seed viability		Seedling emergence	
Rhizosphere fungi	**Duration of treatment (Days)**	**Seed germination (%)**	**Dormant seeds (%)**	**Shoot length (cm)**	**Root length (cm)**
Penicillium islandicum	5	78.2	21.8	3.8	2.4
	10	81.4	18.6	6.9	3.4
	15	88.5	11.5	13.4	4.8
Aspergillus niger	5	78.5	21.5	1.8	1.2
	10	74.5	25.5	2.8	1.8
	15	72.8	27.2	3.6	2.1
Trichoderma viride	5	78.8	21.2	2.9	1.8
	10	83.8	16.2	7.6	2.6
	15	90.5	9.5	12.5	4.2
Fusarium oxysporum	5	74.6	25.4	2.4	1.8
	10	78.4	21.6	5.3	2.1
	15	68.8	31.2	5.6	2.4
Alternaria solani.	5	77.6	22.4	1.8	1.4
	10	67.5	32.5	2.5	2.8
	15	65.7	34.3	3.2	3.2
Control		81.5	18.5	8.9	5.8

Conclusion

On the basis of results, it is evident that *Penicillium islandicum* and *Trichoderma viride* are effective for growth promotion in *Triticum aestivum* and *Sorghum vulgarae*. While *Alternaria solani., Fusarium oxysporum and Aspergillus niger* affect the seed germination, root and shoot growth over control. The toxic effect of fungal metabolites was attributed to release of secondary metabolites which act as growth inhibitor, reduced seed germination and seedling growth.

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