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PPR toxin activity of post-harvest sapota fungi under the influence of period, ph and temperature

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ABSTRACT

Seeds of *Vigna aconitifolia* Jacq.(mataki seeds) pre-soaked in cell free culture filtrate of post-harvest sapota fungi viz.*Geotrichum candidum*, *Aspergillus niger* and *Rhizoctonia solani*. Impact of effect of incubation period, pH and temperature on fungal metabolites from culture filtrates on seed germination was studied for toxicity. It is clear from the result that culture filtrate of all the post-harvest sapota fungi hampered percentage of mataki seed germination and showed reduction in root length and shoot length as compared to control.

Introduction

Phytotoxins are other important metabolites of fungi involved in the process of fruit pulp biodeterioration. Harvested grains are colonized by various species of Aspergillus, under such conditions leading to deterioration and mycotoxin production (Reddy et al., 2009). Aspergilli are the most common fungal species that can produce mycotoxins in food and feedstuffs. Mycotoxins are well known for their health–hazardous effects in human beings and animals (Probst et al., 2007; Reddy and Raghavender, 2007). During the metabolic process, fungi secrete their metabolites in medium. These metabolites are of different types and are known to cause diseases in plants, animals and humans who use to eat this infected food (Schumann, 1991; Singh et al., 1991).

Materials And Methods

Storage fungi: Storage fungi (Geotrichum candidum, Aspergillus niger and Rhizoctonia solani) were isolated from post-harvested sapota and maintained on PDA media.

Culture Filtrate Preparation: The test fungi isolated from post-harvest sapota fruits were grown on Glucose nitrate medium. 25 ml of the medium was poured in 100 ml Erlenmayer conical flasks and autoclaved at 15 lbs pressure for 20 minutes. The flask on cooling were inoculated separately with 1 ml standard spore suspension of the test fungi prepared from 7 days old culture grown on PDA slants. The flasks were inoculated for 6 days at 25 ± 1°C. On the 7th day the flasks were harvested by passing the contents through sterile Whatman's filter No.l. The cell free filtrates were collected in the presterlized bottles and termed as crude toxin preparations, which is cell-free filtrate. These preparations were tested for their toxicity. (Umecharuba and Neachukwa, 1997).

Assay of Phytotoxins: The toxicity of culture filtrates was determined by Seed germination method. Surface sterilized 100 seeds of the test crops were soaked in crude toxin preparation for 24 hours. They were then placed on moist blotter in petriplates. Seeds soaked similarly in freshly prepared uninoculated liquid medium served as control. Per cent germination or per cent inhibition of germination, root and shoot length of seedlings were measured after 7 days of incubation at room temperature.

Results And Discussion

From the data given in table 1, it becomes clear that with increase in incubation period, toxicity also increases. It was noticed that after 5 days, Geotrichum candidum could produced toxin. The culture filtrates of three fungi harvested from 10th day onwards showed increase in toxicity with increase in the incubation period upto 25 days. On 25th day, Aspergillus niger (85%), Geotrichum candidum (70%) and Rhizoctonia solani (50%) showed inhibition of seed germination.

The results given in table 2, revealed that toxin production in all the tested fungi was observed at pH 3 and increased gradually with the increase in pH upto 6.Toxin production at pH 9 was not found in Geotrichum candidum, Aspergillus niger and Rhizoctonia solani. It was interesting to note that, optimum pH for toxin production was 6.0 in Geotricum candidum (60%) and Rhizoctonia solani (50%) whereas 4.5 in case of Aspergillus niger (60%) for inhibition of seed germination.

It is evident from table 3 that at 10^{o}C, none of the fungus could produce toxin. Toxin production in all tested fungi was seen at 20^{o}C and increased upto 30^{o}C.The maximum toxin production was at 30^{o}C in all the fungi, Aspergillus niger (70%), Geotrichum candidum (60%) and Rhizoctonia solani (40%). At 50^{o}C, there was low toxin production in Geotrichum candidum (20%) and Aspergillus niger (25%) while no toxin production in Rhizoctonia solani. Optimum temperature for toxin production was between 30^{o}C - 40^{o}C in Aspergillus niger (70%) while 30^{o}C in Rhizoctonia solani (40%) and Geotrichum candidum (60%).

Kakde et al. (2010) reported that the filtrate of A.niger, Penicillium chrysogenum and Macrophomina phaseolina inhibited percentage seed germination, with increase in filtrate age, soaking time in all fungal filtrates. It means that metabolites are discharged by the tested fungi in the media in which they were grown.

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Table 1: Effect of Incubation period on toxin production

Fungi	Days				
	5	10	15	20	25
	% inhibition of seed germination				
Aspergillus niger	20	70	75	80	85
Geotrichumcandidum	15	55	60	65	70
Rhizoctonia solani	00	40	45	50	50
SEm±	6.009	8.6602	8.6602	8.6602	10.1379
CD (P=0.05)	19.1086	27.5394	27.5394	27.5394	32.2385

SEm± - standard error of mean

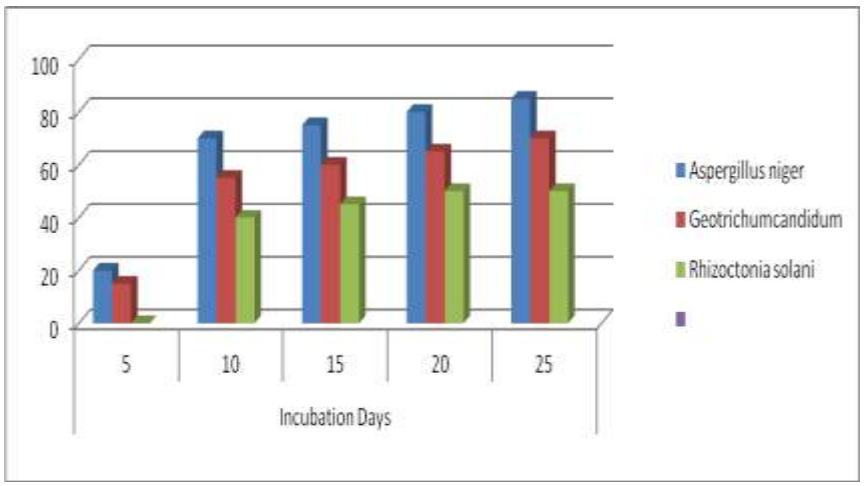


Table 2: Effect of pH on toxin production.]

Fungi	pH				
	3	4.5	6	7.5	9
	% inhibition of seed germination				
Aspergillus niger	25	60	57	45	00
Geotrichum candidum	17	45	60	40	00
Rhizoctonia solani	10	17	50	25	00
SEm±	4.277	10.927	4.940	6.024	0.0000
CD (P=0.05)	13.608	34.74	15.70	19.15	0.000

SEm± - standard error of mean

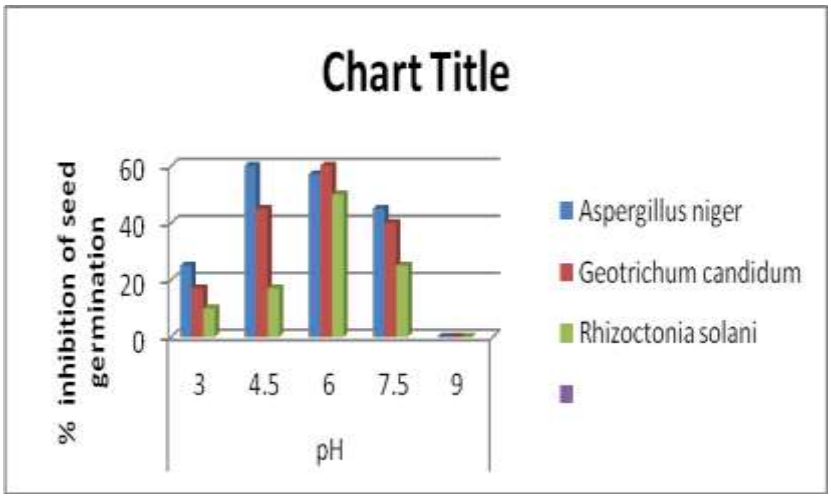
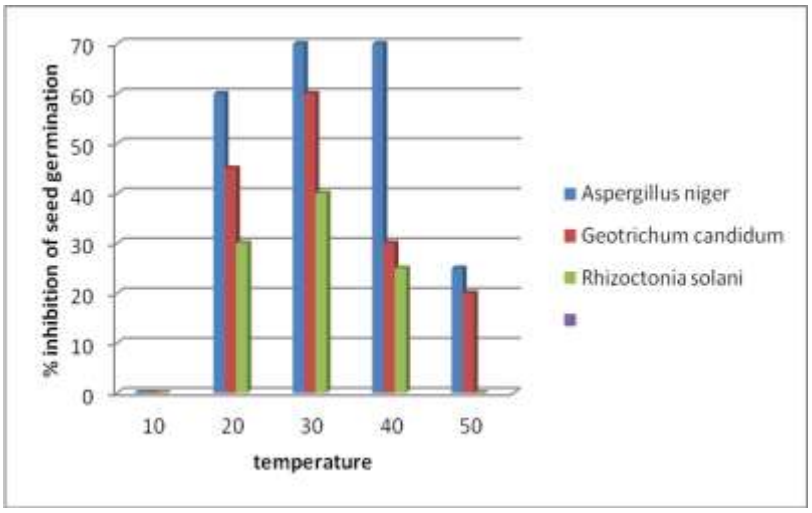


Table 3: Effect of temperature on toxin production

Fungi	Temperature (°C)				
	10	20	30	40	50
	% inhibition of seed germination				
Aspergillus niger	00	60	70	70	25
Geotrichum candidum	00	45	60	30	20
Rhizoctonia solani	00	30	40	25	00
SEm±	0.00	8.066	8.410	12.20	6.623
CD (P=0.05)	0.00	25.63	26.75	38.78	21.28

SEm± - standard error of mean



In present study an optimum pH for toxin production was 6.0 in case of Geotrichum candidum and Rhizoctonia solani whereas 4.5 for Aspergillus niger. While fungi at extreme high pH were unable to produce toxin. This clearly suggests the impact of pH on the metabolic activities of microorganisms.

Inhibitory nature of the fungi was also recorded by different workers. Sinha and Prasad (1981) recorded inhibition of seed germination in mung due to Alternaria alternata, Curvularia lunata and Macrophomina phaseolina. Haikal (2008) reported that toxic metabolites secreted by Aspergillus niger, Fusarium culmorium, Penicillium spp. and R.solani reduced the percentage of seed germination of soyabean. Hilty and Lee (1988) found that filtrate from Phomopsis phaseoli reduced germination of soyabean, this is due to toxins secreted in the media. Madhavrao and Thakur (1978) reported that culture filtrate of Sclerotium rolfsii hampered the germination of Solanum melongena.

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