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Production of toxins by seed borne fungi of groundnut

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SUMMARY

Toxins are important metabolites of seed moulds as they cause loss in seed germinability. Therefore, eight pathogenic seed borne fungi of groundnut were screened for their toxin production *in vitro* under different nutritional and environmental conditions. The seed borne fungi selected for study were *Aspergillus flavus*, *Aspergillus niger*, *A. fumigatus*, *Alternaria tenuis*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Rhizoctonia solani* and *Rhizopus nigricans*. The culture filtrates of the fungi grown on GN medium and Substrate medium were tested against seed germination and radicle elongation. These fungi produced maximum toxin in GN medium while most of them produced poor or nil when grown on substrate medium. The toxin from both medium proved inhibitory for radicle elongation. Effect of different substrates like carbohydrates, nitrogen, amino acids on toxin production was also studied. Carbohydrates like fructose and starch, nitrogen sources like peptone and urea and amino acids like alanine, glycine and glutamic acid were found to be stimulatory for toxin production in most of the fungi.

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India is striving hard to increase agricultural production with a view to accelerate food production to feed the ever increasing population through an integrated approach towards the application of farm technology. Seed play an important role in disseminating pathogenic organisms to areas from hitherto, they have been absent. To check the spread of such pathogens, seed health testing procedure is necessary.

Toxins are another important metabolites of seed moulds as they cause loss in seed germinability. Some selected fungi were screened for their toxin production *in vitro* under different nutritional and environmental conditions. Therefore, eight seed borne fungi obtained from groundnut seeds were, further studied for their ability to produce toxins under different physico-nutritional conditions.

MATERIALS AND METHODS

Production of phytotoxins:

The test fungi isolated from legume seeds were grown on liquid medium containing glucose 1 %, KNO_3 0.25 %, KH_2PO_4 0.1 % and $MgSO_4.7H_2O$ 0.05 %, 25 mL of the medium was poured in 100 mL conical flask and autoclaved at 15 lb pressure for 15 min. On cooling, flasks were inoculated separately with one mL of spore suspension of test fungi prepared from 7 day old cultures grown on PDA slants. The flasks were incubated at 25 ± 2^0C for nine days and were harvested by filtering their contents through Whatman filter paper No. 1. The filterates were collected in presterilized culture bottles and termed as crude toxin preparations. The preparations were tested for their toxicity.

Assay of phytotoxins:

Seed germination method:

Hundred seeds of test crop were soaked in crude toxin preparation for 24 hours. The seeds were then placed on moist blotters in sterilized Petriplates. Per cent germination / per cent inhibition of germination was recorded after a period of 10 days. The seeds soaked in freshly prepared liquid medium and germinated after 10 days served as control.

Inhibition of seedling vigour:

The method for this was adopted from Luke and Wheeler (1955) for studying the toxic effect of culture filterates on root length inhibition. It involved the use of germinated legume seeds of uniform root length, kept at

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25 ± 2^{0}C in 90 mm sterilized petriplates containing 2.5 mL of crude toxin preparation. The root length was measured after 72 hours of incubation. The amount of reduction in treated seedling root length in comparison to the control (sterilized water) was calculated. This reduction was expressed in terms of per cent inhibition of seedling vigour.

RESULTS AND DISCUSSION

The results obtained from the present investigation have been discussed in the following sub heads :

Toxin production in seed-borne fungi of groundnut seeds:

Totally eight fungi from groundnut were studied for their nature of toxin production. The culture filterates (CF) of the fungi grown on GN medium and substrate medium were tested against seed germination and radicle elongation. It is clear from the data that, the fungi isolated from groundnut produced maximum toxin in GN medium while in most of them produced toxin poor or nil when grown on substrate medium. On the other hand, *A. niger* and *R. nigricans* produced more toxin on substrate medium than on GN medium. The toxin from GN medium as well as substrate medium proved inhibitory for radicle elongation. *M. phaseolina* did not produce toxin in substrate as well as non-substrate medium (Table1).

Effect of carbohydrates on toxin production:

Six selected seed borne fungi were further studied for their ability to produce toxins under the influence of different carbohydrates.

It is clear from the results that, *A. flavus* produced maximum toxin in presence of fructose and starch where as, no toxin production occurred in presence of mannitol. Similarly fructose, glycogen and starch for *A. tenuis*, glycogen, galactose, fructose, starch and mannitol for *F. oxysporum*, sucrose, glycogen and mannitol for *R. nigricans*, glycogen and starch for *S. rolfsii* proved stimulatory sources for toxin production (Table 2).

Effect of nitrogen sources on toxin production:

Six different nitrogen sources were tested against toxin production in the seed borne fungi. It is clear from the results that, peptone supported toxin production in all but not in *M. phaseolina*. Similarly, urea proved superior

Table 1: Toxin production in seed borne fungi of groundnut seeds

Fungi	Toxin production in			
	GN Medium		Substrate medium	
	% inhibition of germination	Radicle length (mm)	% inhibition of germination	Radicle length (mm)
Ground nut				
A. flavus	46	4.2	40	6.0
A. niger	18	10.4	34	6.8
A. fumigatus	18	9.5	Nil	10.2
A. tenuis	38	10.0	Nil	9.0
F. oxysporum	30	10.9	Nil	10.5
M. phaseolina	Nil	11.4	Nil	5.8
R. solani	24	9.3	50	5.0
R. nigricans	28	10.9	60	9.9

Table 2 : Effect of carbohydrates on toxin production by seed borne fungi of groundnut seeds

Carbohydrates (1 %)	Toxin production in					
	A. flavus	*A. tenuis*	*F. oxysporum*	*M. phaseolina*	*R. nigricans*	*R. solani*
	% inhibition of seed germination of groundnut					
Fructose	60	44	45	40	20	30
Galactose	40	20	40	45	25	38
Lactose	35	38	30	10	20	40
Sucrose	40	35	25	50	40	32
Glycogen	50	52	60	20	50	65
Starch	65	45	70	32	30	50
Mannitol	10	30	55	50	40	30
Control	Nil	Nil	Nil	Nil	Nil	Nil

Table 3: Effect of nitrogen sources on toxin production by seed borne fungi of groundnut seeds

Nitrogen sources (0.25%)	Toxin production in					
	A. flavus	*A. tenuis*	*F. oxysporum*	*M. phaseolina*	*R. nigricans*	*R. solani*
	% inhibition of seed germination of groundnut					
Pot. nitrate	10	70	45	35	45	35
Sod. nitrate	45	65	55	45	50	45
Amm. nitrate	55	40	40	42	40	65
Urea	80	35	80	25	30	35
Peptone	72	60	65	15	45	65
Casein	30	40	15	35	60	60
Control	Nil	Nil	Nil	Nil	Nil	Nil

Table 4 : Effect of amino acids on toxin production by seed borne fungi of groundnut seeds

Amino acids	Toxin production in					
	A. flavus	*A. tenuis*	*F. oxysporum*	*M. phaseolina*	*R. nigricans*	*R. solani*
	% inhibition of seed germination of groundnut					
Alanine	50	82	90	70	45	10
Cystine	35	55	25	65	60	60
Glutamic acid	50	50	55	80	10	75
Glycine	50	42	48	74	50	16
Lysine	60	65	70	40	55	40
Tryptophan	85	40	30	85	20	72
Control	Nil	Nil	Nil	Nil	Nil	Nil

only in case of *A. flavus* and *F. oxysporum*. Sodium nitrate and potassium nitrate proved to be good source for toxin production (Table 3).

Effect of amino acids on toxin production:

Six amino acids were tested to see their effect on toxin production in seed borne fungi. Among the different amino acids, alanine and glycine proved highly stimulatory for toxin preparation in all the tested fungi except *S. rolfsii*. Similarly, glutamic acid supported toxin production in all the fungi except *R. nigricans*. Lysine was found to be a good source for toxin production in all the fungi (Table 4).

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