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RESEARCH PAPER

Estimation of deteriorative effect of *Aspergillus niger* on onion seed germination and seedling vigour

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SUMMARY

Samples of onion seeds were collected from farmer's houses to prove the pathogenicity of mycoflora, two test were performed *viz.*, Seed and bulb inoculation techniques. Seed inoculation techniques revealed that *Aspergillus niger* reduced seed germination, root and shoot elongation by causing higher percentage of pre and post-emergence mortality and subsequently it also reduced vigor index in comparison to control. The same fungi was also found to cause bulb rot by inoculating the healthy surface sterilized white bulbs and it was observed that *Aspergillus niger* found to be more pathogenic in comparison to *Aspergillus flavus* per cent incidence and per cent intensity and it caused maximum per cent incidence (4.00% / 100.00%) and per cent intensity (2.80% / 30.00%).

Key Words : Seed germination, Onion, Vigour index, Inoculation

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Onion *Allium cepa* L. vernacularly called '*Pyaz*' in Hindi and locally known as '*kanda*', is one of the most important vegetable cum condiment crop of family Alliaceae, grown in all the parts of India. Onion is used throughout the year in the form of salad or condiment or for cooking with other vegetables. Onion has several medicinal uses, its use in the case of sun strokes is known world over (Rai and Yadav, 2005). Onion seeds carry a number of fungi. Although majority of them are saprophytes, a few are potentially pathogenic capable of ruining the crop. Amongst them, *Aspergillus niger* caused black mould disease was reported to be predominant one and causes 30 to 80% loss / spoilage of onion bulb (Quadri *et al.*, 1982). *Aspergillus* sp. attacks / infects bulbs of onion in field/storage, whenever they find injured tissues by producing various enzymes or toxins (Srinivasan and Shanmugam, 2006). Severe incidences of black mould on onion bulbs in storage have been reported from India (Gupta *et al.*, 1991; Maheshwari, 1988), Sudan (Hayden *et al.*, 1994a), Israel (Grienstein *et al.*, 1992), Egypt (Zohri *et al.*, 1992), Australia (Salvestrin and Letham, 1994), Taiwan (AVRDC, 1999), and the United States (Ceponis, 1986). Losses can be as high as 60 per cent during summer storage (Tanaka, 1991). Ko *et al.* (2002) also indicated that Black mould was the major disease during storage under ambient conditions in the tropics. Therefore, the aim of the present study was to investigate the incidence of seed associated fungi and their effect on germination and seedling vigour.

MATERIAL AND METHODS

Samples of onion seeds were collected from farmer's houses, situated in nearby areas of Jobner (District-Jaipur, Rajasthan). Two test were performed for estimation of deteriorative effect of *Aspergillus niger* on onion seed germination and seedling vigour as follows:

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Seed inoculation technique:

(Plated on moist blotter in petri dish and sown in pots in having sterilized soils). Two hundred apparently healthy surface sterilized seeds were taken. The seeds were then rolled, separately on 10 days old sporulating culture of *Aspergillus niger* thriving on PDA contained in Petri dishes. Inoculated seeds were sown in 30 cm. earthen pots (Pre-sterilized and having autoclaved soil) at the rate of 5 seeds per pot. The uninoculated surface sterilized apparently healthy seeds served as control. These pots were kept in cage house (20-25^{0}C). The pots were watered as and when required. Observations on seed germination, pre- and post- emergence mortality and root/ shoot length were recorded on 15th days of sowing, respectively. Seedling vigour was also calculated by following formula suggested by Abdul-Baki and Anderson (1973).

Vigour index = Germination % x (Root length + Shoot length cm.)

Bulb inoculation technique:

The method described by Mirdha and Siddique (1989) was followed to observe the effect of *Aspergillus niger* on bulbs freshly harvested white bulbs of onion (Local variety) were obtained from the farmer's field. The bulbs were surface sterilized with 0.1% $HgCl_2$ solution followed by 3 washing with sterilized distilled water and pricked with a sterilized needle. Fifty bulbs (10 bulbs/ replications x 5) were artificially inoculated with by dipping in spore suspension of a week old culture of *Aspergillus niger* Uninoculated injured and uninjured bulbs served as control. In all four replications were maintained. The bulbs were incubated at 25$\pm$2^{0}C. After one week of inoculation bulbs were examined and isolation were made from inoculated bulbs which showed disease symptoms. Per cent disease incidence and intensity was calculated by using following disease rating scale and formulae supported by Mayee and Datar (1986) after slight modification:

0 = No infection (showing no disease symptoms)

Table 1 : Pathogenicity of seed-borne *Aspergillus* sp. of onion by seed inoculation technique and sown in pots having sterilized soils

Aspergillus sp.	Germination (%)	Per cent seedlings showing symptoms*	Per cent seedling mortality		Elongation (cm)		Vigour index	Type of symptoms
			Pre - emergence	Post-emergence	Root	Shoot		
Aspergillus flavus	63.00	10.00	4.75	8.00	2.50	2.00	283.50	Seed rot, seedling decay
Aspergillus niger	56.00	38.00	15.75	12.75	3.00	3.50	364.00	Seed rot, seedling decay, tip burning
Control (uninocualted Soil)	68.00	0.00	0.00	0.00	3.25	4.00	493.00	-

No. of seed tested 200, * Based on emerged seedlings

Table 2: Pathogenicity of seed-borne *Aspergillus* sp. of onion by seed inoculation technique and plated on moist blotter in Petridish

Aspergillus sp.	Germination (%)	Per cent seedlings showing symptoms*	Per cent seedling mortality		Elongation (cm)		Vigour index	Type of symptoms
			Pre-emergence	Post-emergence	Root	Shoot		
Aspergillus flavus	65.00	20.00	7.75	10.50	3.00	2.50	357.50	Seed rot, seedling decay
Aspergillus niger	58.00	40.00	17.75	15.25	3.50	3.00	377.00	Seed rot, seedling decay, tip burning
Control (uninocualted seed)	70.00	0.00	0.00	0.00	4.00	4.25	577.50	-

No. of seed tested 200, * Based on emerged seedlings

Table 3 : Effect of *Aspergillus sp.* on onion bulb tested by bulb[1] inoculation technique

Aspergillus sp.	No. of fruits inoculated	Per cent disease incidence	Per cent disease intensity
Aspergillus flavus			
With injury	50.00	16.00	6.00
Without injury	50.00	0.00	0.00
Aspergillus niger			
With injury	50.00	100.00	30.00
Without injury	50.00	0.00	0.00
Control			
With injury	50.00	0.00	0.00
Without injury	50.00	0.00	0.00

* Average of 5 replications (10 bulbs/ replication) , [1] = Local cultivars (white bulb)

1 = 1-10% bulb area infected
2 = 10.1-25% bulb area infected
3 = 25.1 -50% bulb area infected
4 = 50.1-75% bulb area infected
5 = 75.1% and above bulb area infected.

Formula to calculate per cent disease incidence and per cent disease intensity were as follows:

$$\text{Per cent disease incidence} = \frac{\text{Total number of infected bulbs}}{\text{Total number of inoculated bulbs}} \text{x100}$$

RESULTS AND DISCUSSION

Inoculation seed plated on moist blotter in Petridish:

In the present investigation, seed and fruit inoculation were used to prove the pathogenicity of *Aspergillus niger* and *Aspergillus flavus* isolated from onion seeds. Findings of seed Inoculation Techniques (Plated and sown in moist blotter / pots, respectively) in present investigation revealed that *Aspergillus niger* reduced seed germination, root and shoot elongation by causing higher percentage of pre- and post-emergence mortality (Table 1) and subsequently it also reduced vigour index. Association of *Aspergillus niger* with seed causing bulb rot of onion was also reported by Gupta *et al.* (1984); Hayden and Maude (1992 and 1994) and Kumar and Lokesh (1999).

Inoculation seed sown in pots having sterilized soil:

Inoculation of healthy seeds sown in pots having sterilized soil with *Aspergillus niger* caused both pre- and post emergence mortality and produce symptoms like seed rot, tip burning of leaves and caused seedling decay in seed inoculation *Aspergillus niger* causes higher pre- and post-emergence mortality (15.75% and 12.75%, respectively) and 38.00 per cent seedling showed symptoms, which *Aspergillus flavus* was proved less pathogenic, as it caused 4.75 per cent pre- and 8.75 per cent post-emergence mortality and only 10.00 per cent seedling showed symptom (Table 2).

Bulb inoculation:

The same fungi was also found to be caused bulb rot by inoculating the healthy surface sterilized white bulbs and it was observed that *Aspergillus niger* found to be more pathogenic in comparison to *Aspergillus flavus* per cent incidence and per cent intensity and it causes maximum per cent incidence (4.00% / 100.00%) per cent intensity (2.80% / 30.00%). Table 3 revealed the susceptibility of white bulb to *Aspergillus flavus and Aspergillus niger,* as they have high per cent disease incidence (16.00% /100.00%) and intensity (6.00 % / 30.00%) in comparison to control (0.00% / 0.00%). Similar observations were also reported by Rathi and Ramalingam (1974), Gupta *et al.* (1994) and Hayden and Maude (1992 and 1994).

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