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RESEARCH NOTE

Effect of pathogenic seed mycoflora of ajwain (*Trachyspermum ammi* L.) on volatile oil content of seeds

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ABSTRACT

The estimation of deteriorative effect of seed mycoflora on volatile oil contents of ajwain seeds, samples collected at post-storage (just before next sowing) stage were isolated using blotter and agar plate methods. Two separate inoculations were made with seven different mycoflora and seed inoculated with *Drechslera australiensis* showed increase in per cent volatile oil content. Seed inoculated with rest of the fungi *viz., Aspergillus flavus, Aspergillus niger, Aspergillus ochraceous, Drechslera australiensis, Fusarium sporotrichioides, Rhizopus oryzae* and *Trichoderma viride* showed reverse/equal in per cent volatile oil contents of seeds.

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Ajwain (*Trachyspermum ammi* L.) also known as Bishop's weed and carum, is one of the most important seed spice crops. It belongs to family Apiaceae and is believed to have originated from India and Egypt. States in India where cultivation of this crop is most widespread are Rajasthan, Gujarat, Uttar Pradesh, Punjab, Tamil Nadu and Andhra Pradesh. Ajwain seeds contains moisture 8.9 per cent, protein 15.4 per cent, fat 18.1 per cent, minerals 7.1 per cent, fibre 11.9 per cent, carbohydrates 38.6 per cent, calcium 1.42 per cent, phosphorus 0.30 per cent and iron 14.6 per cent (Dashora *et al.*, 2006). Several factors limiting the production of the crop in which poor health of the seed is one of the major factors which takes heavy toll of the crop at all the stages right from seedling to harvest and also during transport and storage. Ajwain seeds carry a number of mycoflora. Although majority of them are saprophytes, a few are potentially pathogenic capable of ruining the crop. One of the factors responsible for this is the use of contaminated seeds by farmers for the sowing purpose because several fungi are responsible for deterioration of ajwain grains/seeds in storage causing reduction in the germination potential and chemical constituents of seeds (Sharma and Sharma, 2006). Therefore, the pathogenic effect of mycoflora on volatile oil contents of ajwain (*Trachyspermum ammi* L.) seeds was investigated.

Seed samples were collected and from different mandies (godowns/shops) situated in different major ajwain growing regions of the Rajasthan. Mycoflora associated with seeds samples collected at post-storage (just before next sowing) stage were isolated using two incubation methods *i.e.* Blotter method and Agar plate method.

Blotter method :

Three circles of blotter papers were placed at the bottom of sterilized Petri dishes aseptically and moistened by sterilized distilled water. Ten seeds were placed at an equal distance in each Petri dish. These dishes were incubated at 22±1 ^{0}C with 12 hours of light alternating with 12 hours of dark period. The seeds were examined on 7th day of incubation for emanating fungal colonies.

Agar plate method:

Two hundred seeds from each sample were taken for isolation of internally seed mycoflora. Seeds were surface sterilized with 0.1 per cent mercuric chloride solution for 1-2 minutes followed by 3 washings with sterilized distilled water. Sterilized Petri dishes each containing 20 ml Potato dextrose

agar (PDA) medium was used for incubation of seeds. Ten seeds per Petri dish were equispaced/aseptically and incubated at 22±1 ^{0}C with 12 hours of light alternating with 12 hours of dark period. The fungal colonies emanating from seeds were examined from 3rd and 8th day of incubation. Isolation of mycoflora from ajwain seeds was carried out and maintained on 2 per cent ootato dextrose agar (PDA) medium. Observations on per cent incidence of seed mycoflora were recorded in both Blotter and Agar plate methods.

Oil estimation method :

In 20 ml sterilized distilled water, 100 g apparently healthy surface sterilized seeds of highly contaminated seed sample BHIR was placed in 500 ml conical flask and autoclaved for 15 minutes at 1.045 kg/cm2 pressure. Autoclaved seeds were inoculated separately with each fungus *viz., Aspergillus flavus, A. niger, A. ochraceous, Trichoderma viride, Drechslera australiensis, Fusarium sporotrichioides and Rhizopus oryzae.* Inoculated seeds were incubated at 25±1 ^{0}C for 15 days. After 15 days of incubation, oil was estimated by essential oil distillation assembly *i.e.* clevenger apparatus (AOAC, 1970). One hundred gram inoculated seeds with each fungus under test were ground finely with electrical grinder. The seed powder was transferred in assembly flask (1 litre) and 540 ml water was added to fill the flask up to half of its capacity and placed on heating mantle. Heating was done for 5 to 6 hrs continuously. The volatile oils were collected in the graduated side arm of the assembly. Two consecutive readings were taken at 30 minutes until there was no change in oil content. The volume of volatile oil obtained in terms of millilitre 100 g seed sample directly reveals per cent oil content in the seeds.

During the present investigation, Blotter method and Agar plate method were employed for detection of fungi associated with seed of samples collected and also with infected seedlings raised from them at storage stage of their collection. The studies revealed that in all seven fungi were detected in both Blotter and Agar plate methods from almost all the seven seed samples and these fungi were *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceous, Drechslera australiensis, Fusarium sporotrichioides, Rhizopus oryzae* and *Trichoderma viride* (Table 1 and 2). Amongst these, some of the fungal species have already been reported on seeds of ajwain including *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceous* and *Rhizopus* sp By Swarup and Mathur (1972), Manjari *et al.* (1996) and Sharma and Sharma (2006). The association of *Drechslera australiensis, Fusarium sporotrichioides* and *Trichoderma viride* were found to be as the new fungi on Ajwain seed in the present study. In general, little variation was observed in Blotter and Agar plate method in incidence of seed mycoflora on Ajwain seeds. This variation might be due to reason that some of the weak and slow growing saprophytic fungi on pre surface sterilization of seed and different substratum used in the methods employed (Neergaard and Saad, 1962 and Jain, 1990). Neergaard and Sadd (1962) also observed that Blotter method and Agar plate method were equally voluable and supplementary to each other.

Table 3 : Effect of different seed mycoflora on the volatile oil content of seed

Sr. No.	Storage fungi	Volatile oil content (%)
1.	*Aspergillus flavus*	3.10 (10.14)
2.	*Aspergillus niger*	3.20 (10.30)
3.	*Aspergillus ochraceous*	3.80 (11.24)
4.	*Drechslera australiensis*	4.20 (11.82)
5.	*Fusarium sporotrichioides*	3.60 (10.94)
6.	*Rhizopus oryzae*	3.80
7.	*Trichoderma viride*	3.40 (10.62)
8.	Control (uninoculated)	3.80 (11.24)
	S.Em±	0.25
	C.D.at 5%	0.73

The per cent volatile oil content of the Ajwain seed inoculated with seven mycoflora *viz., Aspergillus flavus, A. niger, A. ochraceous, Drechslera australiensis, Fusarium sporotrichioides, Rhizopus oryzae* and *Trichoderma viride* were assessed and expressed as per cent of dry weight of seeds. Out of seven spices of fungi tested for mycoflora *i.e. Drechslera australiensis* (4.20%) increased no significantly oil content of seed in compared to control (3.80%). Oil content was observed to be reduced in seed inoculated with rest of the mycoflora *i.e. Aspergillus flavus* (3.10%), *A. niger* (3.20%), *A. ochraceous* (3.80%), *Fusarium sporotrichioides* (3.60%), *Rhizopus oryzae* (3.80%) and *Trichoderma viride* (3.40%). However, significant difference was observed between *Aspergillus flavus* and *A. niger* as compared to control (Table 1). Seed inoculated with rest of the fungi *viz., Aspergillus flavus, Aspergillus niger, Aspergillus ochraceous, Drechslera australiensis, Fusarium sporotrichioides, Rhizopus oryzae* and *Trichoderma viride* showed reverse/equal in per cent volatile oil contents of seeds. Increase and/or decrease in oil contents of groundnut, mustard, soybean, ajwain, cumin and fennel due to fungal invasion with different fungi *viz., Alternaria brassicicola, Aspergillus flavus, Cladosporium herbarum, Fusarium* sp., *F. oxysporum, Phoma* sp. and *Rhizoctonia* sp. have been also reported by Shivpuri *et al.* (1990), Lalithakumari *et al.* (1971), and Anonymous (2005). Increase / decrease in oil contents of seed may due to utilization of proteins in preference to oil, synthesis of oil in mycelium as secretion of lipase which activated the formation of oil from seed tissues (Pattinson and Thornton, 1965 and Sharma and Chauhan, 1976). Invasion of fungi on grains or seeds are

reported to cause biochemical deterioration and change in the quality and quantity of seed nutrient (Lalithakumari *et al.*, 1971 and Dharm, 2002).

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