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Influence of Plant Oils and Bio-Fungicides on Seed *mycoflora* of Chilli (*Colletotrichum capsici*)

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ABSTRACT

Production of chilli is constrained by several diseases, of which fungal diseases are more prominent and cause seedling rot and fruit rot. Use of chemical fungicides is a common practice in managing the fungal diseases. However, indiscriminate use in a long run leads to residual toxicity, induced resistance in pathogens and environmental pollution. Therefore, identification of eco-friendly measures would be highly useful to control the seed borne mycoflora. Hence, two experiments were conducted to identify the plant oils and bio-fungicides in comparison with chemical fungicide, the carbendazim. The seed mycoflora incidence was minimum with neem oil (5 ml kg^{-1} seed) and it decreased the mycoflora incidence by 70 per cent compared to the untreated seed. Seed dressing with Trichoderma viride (10 g kg^{-1} seed) or Trichoderma viride (5 g kg^{-1} seed) + Pseudomonas fluorescens (5 g kg^{-1} seed) decreased the mycoflora incidence by 81.8 per cent compared to untreated seed. Both these seed treatments are comparable to or better than carbendazim (0.2 %) treatment. Hence, neem oil or Trichoderma viride can be effectively used to control seed mycoflora in place of carbendazim.

Key words: *Mycoflora, Carbendazim, Trichoderma viride, Fungal diseases*

INTRODUCTION

Chilli (*Capsicum annum* L.) is a vital spice component in Indian food as green chilli and dried chilli. In India, green chilli is grown in an area of 2.92 lakh hectare with an annual production of 33.90 lakh tonnes and; the dried chilli in an area of 8.45 lakh hectare with a production of 21.26 lakh tonnes[15]. In terms of green chilly production, the state of Haryana stands 5th in the country after Karnataka, Bihar, Andhra Pradesh and Jharkand. Haryana has an area of 0.17 lakh hectare and production of 1.134 lakh tones[15]. Chilli production of India was dropped from the 1st place (2011) to 3rd position at present[24] due to diseases caused by fungus, bacteria and viruses. Among fungal diseases, fruit rot is very important as it reduces the market value of fruit and seed quality, in addition to yield loss up to 50 per cent[18]. The seedling rot and fruit rot of chilli is caused by different species of *Colletotrichum*[6,11,12,23].

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The *Colletotrichum* sp. and other mycoflora species can survive in and on the seed surface[19] and; readily colonizes as mycelia on the seed coat and peripheral layers of the endosperm and embryo. For protection of these fungi, seed dressing is the most ancient and cost effective technique. Seed dressing with chemicals is a common practice to control the pathogens. However, indiscriminate use of chemical fungicides leads to residual toxicity, induced resistance in pathogens and environmental pollution[20]. Alternatively, the plant products or biological agents are natural, low cost and eco-friendly are the better option in the present day climate change scenario. Plant oils like neem or karanj oils are reported to provide maximum protection against mycoflora by suppressing the pathogen activity and growth[3]. In addition, these oils have growth promoting effect through increased seedling vigour. For instance, neem oil spray (15%) increased the seedling length and seedling dry weight of chilli[14]. The biological agents like, *Trichoderma viride* and *Pseudomonas fluorescens* found effective in controlling the mycoflora of chilli[22]. Seed treatment with *T. viride* colonizes entire seed surface and kills not only the pathogens present on the cuticle, but also provides protection against soil-borne pathogens. The hyphae of *Trichoderma* wrap around the pathogenic fungi and produce antibiotics and extracellular enzymes, which lyses the cell wall of these pathogens that damage them. Further, the seed treatment with *Trichoderma* sp also leads to multiplication of *Trichoderma* in soil and protects the root system against the damping off caused by the soil borne pathogens. *Trichoderma* sp. believed to have a high competition for nutrients and space with other microbes, but produce secondary metabolites that may play a role in competition against other microbes. Seed treatment with *T. viride* inhibits 69.4 per cent of mycelia growth in brinjal against the damping off disease, in addition to increased seed germination by 82.4 per cent[5].Thus, in the present day climate change scenario, the eco-friendly measures not only helps in managing seed borne diseases but also increases the seed quality, seed germination, vigour and the yield of the crops and in summary leads to healthy plant, healthy man and safe environment. In this view, the present experiments were conducted to study the effect of plant oils and bio-fungicides on mycoflora of chilli seeds to identify effective plant oil and organic fungicide comparable to that of chemical fungicide, carbendazim.

MATERIAL AND METHODS

Two experiments were conducted to study the effect of plant oils and bio-fungicides on seed mycoflora of untreated seed of chilli during the year 2016-17 at the Department of Seed Science and Technology, CCS HAU, Hisar. The variety, RCH-1 harvested during Feb-March 2016 was used for the experiment. Different plant oils used in the experiment were purchased from the local market. The bio-fungicides were used in powder formulation for seed treatment. *Trichoderma asperellum* was obtained from Bio-control laboratory, Sirsa, Government of Haryana and the rest from local market. The chemical fungicide, carbendazim (0.2 % w/w) was used as control treatment. Healthy chilli seeds were surface sterilized for two minutes using 1.0 per cent (v/v) sodium hypochlorite, washed with sterilized distilled water thoroughly and then treated with different plant oils (castor, neem, aonla, sesamum, linseed, pongamia, walnut and ajwain) for one hour @ 5ml kg^{-1} seed and shade dried overnight. In the second experiment, the seeds were dressed with bio-fungicides (*Trichoderma viride*, *Trichoderma asperellum and Pseudomonas fluorescens*) @ 10 g kg^{-1} seed individually or in combination (5+5 g) of bio-fungicides for 10 minutes and used for the experiment. The checks for these experiments were carbendazim (0.2 %) and untreated healthy seed as control. The treated seeds were placed in petri dishes lined with two layers of filter paper (Whatman No.1) and incubated for seven days in BOD at 25 $\pm$ 1^0C. Each treatment had three replications and in each replication, 25 seeds were placed equidistantly in petri-dish. The petri-dishes

were watered using sterilized distilled water as when required. Seven days after incubation, observations were made on different mycoflora around the seeds based on morphological features viz., colony colour and pattern of growth and confirmed under microscope. Each species of mycoflora was enumerated as (i) 0, (0 numbers), (ii) + (1 to 3 numbers), (iii) ++ (3 to 7 numbers) and (iv) +++ (more than 8 numbers) in a plate of 25 seeds, which represents no mycoflora incidence, low incidence, moderate incidence and high incidence respectively.

RESULTS AND DISCUSSION

The *Colletotrichum capsici* causes seedling blight or damping off in the nursery and anthracnose and fruit rot at later stages of plant growth. In the present study, not the only *C. capsici* but also four other mycoflora were observed (Table 1 and Table 2). Similarly, others also reported several mycoflora like *C. capsici*, *Alternaria*, *Cercospora*, *Penicillium* and *Aspergillus* sp[4,10,13,17,23] . The incidence of *C. capsici* was low (5 No.) compared to other mycoflora and; the *Aspergillus* sp. dominated (16 No.) the other species (Table 1). In contrast, Padaganur and Naik[17], Hemannanavar *et al*.[6] and Santhoshreddy *et al*.[23] reported a higher incidence of *C. capsici* followed by *Aspergillus* sp. However, in a recent study, Birla[4] reported that the incidence of *C. capsici* and *Aspergillus* sp. are in equal proportions. The lower *C. capsici* incidence in the present study (Table 1) could be due to antagonistic effect of other mycoflora, for instance it was showed that the *Aspergillus* inhibit the growth of *C. capsici* by 54.9 per cent[1]. Such differences in frequency of occurrence of particular mycoflora could be due to differences in variety and place of cultivation[21]. In line of our results, Jogi *et al*.[8] reported that the *Aspergillus* species incidence (45.0 %, three species together) was higher compared to the *C. capsici* (6.8 %), and the incidence of *C. capsici* profoundly reduced the seed germination (68.1 %) and seedling vigour index (71.4 %).Since, *C. capsici* and other mycoflora are seed borne fungus, seed treatment with systemic fungicide before sowing has a great role in controlling mycoflora and related diseases. In this context[13] have shown that the mycelia growth of *C. capsici* was inhibited to the extent of 79 per cent (Thiram, 0.2%), 70 per cent (Captan, 0.2%), 90 per cent (Carbendazim, 0.1 %) and 44.9 per cent (neem leaf extract, 2%) and thus reduced seedling mortality. Further, chilli seeds treated with fungicide (Thiram @ 2.5 g kg^{-1} seed) decreased the disease index of 35 days old seedlings from 41.7 per cent (in untreated control) to 24.3 per cent in treated seeds with improved seedling health[9]. However, indiscriminate use of chemical fungicides leads to residual toxicity, induced resistance in pathogens and environmental pollution[20]. Alternatively, the plant products or biological agents are natural, low cost, and eco-friendly is the better option in the present day climate change scenario. In the present study, the cumulative mycoflora incidence on the healthy seed was high as compared to plant oils and carbendazim. The mycoflora incidences due to carbendazim and neem oil treatments were similar (Table 1). In this regard, Sundararamamoorthy *et al*.[25] have shown that neem leaves extract (60 % w/v) using hot water completely inhibited the mycelia growth of *C. capsici*, which was equivalent to carbendazim (0.1 %). Further, Kulkarni *et al*.[10] showed that 25 g leaves extract of neem for 100 g seed proved the best in controlling the *C. capsici* and other mycoflora. These results indicate that the neem oil be effectively used in place of carbendazim to reduce the infection of *C. capsici* and other mycoflora.The cumulative mycoflora across the bio-fungicides was highest in un-treated healthy seeds (Table 2). The seed treatment with *Trichoderma viride* and *Trichoderma viride* + *Pseudomonas fluorescens* reduced the mycoflora incidence comparable to that of carbendazim (Table 2). Santhoshreddy *et al*.[23] have reported that seed treatment with bio-fungicides such as *Pseudomonas fluorescens* and *Trichoderma harzianum* and their combinations showed only 14 to 16 % seed mycoflora infection compared to 43.0 per cent in untreated seeds of chilli. Begum *et al*.[2] reported that, the seed treatment with *Trichoderma harzianum* found effective in inhibiting the mycelia growth of *C. capsici* by 70 per cent. The *P. fluorescens* is very

effective in controlling the fungal diseases of chilli and brinjal. It induces systemic acquired resistance (SAR) mainly through (i) the establishment of other favoured rhizosphere microorganisms, (ii) produces HCN which can check growth of phytopathogen and (iii) promotes plant growth by production of phytohormones such as auxins and gibberellins and also by phosphate solubilisation[7].

Table 1: Effect of plant oils on mycoflora incidence on healthy brinjal seeds

Treatments	Mycoflora incidence					
	C. capsici	*Cercospora* sp	*Alternaria* sp	*Penicillium* sp	*Aspergillus* sp	**Cumulative incidence**
Castor	+	+	+	+	++	**6**
Neem	0	0	+	+	+	**3**
Aonla	+	+	+	+	+	**5**
Sesamum	+	+	+	+	++	**6**
Linseed	+	+	+	++	++	**7**
Pongamia	+	+	+	+	++	**6**
Walnut	+	+	+	+	+	**5**
Ajwin	+	+	0	+	+	**4**
Healthy seed (untreated)	+	++	++	++	+++	**10**
Carbendazim treated	0	0	+	+	+	**3**
Cumulative incidence	**8**	**9**	**10**	**12**	**16**	**55**

Note: 0 : No disease incidence
+ : Low disease incidence
++: Moderate disease incidence
+++: High incidence disease

Table 2: Effect of bio-fungicides on mycoflora incidence in healthy chilli seed

Treatments	**Mycoflora incidence**					
	C. capsici	*Cercospora* sp	*Alternaria* sp	*Penicillium* sp	*Aspergillus* sp	**Cumulative incidence**
Trichoderma viride	0	0	+	0	+	**2**
Trichoderma asperellum	+	0	+	+	++	**5**
Pseudomonas fluorescens	0	+	+	+	+	**4**
Trichoderma asperellum + *Trichoderma viride*	+	0	0	+	++	**4**
Pseudomonas fluorescens + *Trichoderma viride*	0	0	+	0	+	**2**
Pseudomonas fluorescens + *Trichoderma asperellum*	+	0	+	0	+	**3**
Healthy seed	++	++	++	++	+++	**11**
Carbendazim treated	0	0	+	+	+	**3**
Cumulative incidence	**5**	**3**	**8**	**6**	**12**	**34**

Note: 0 : No disease incidence
+ : Low disease incidence
++: Moderate disease incidence
+++: High incidence disease

CONCLUSION

These results infer that seed dressing with neem oil @ 5 ml kg^{-1} seed decreases the mycoflora infection by 70 per cent compared to the untreated seed and; seed dressing with *Trichoderma viride* or *Trichoderma viride* + *Pseudomonas fluorescens* decreased the mycoflora infection by 81.8 per cent. Both these seed treatments are comparable to or better than carbendazim (0.2 %) treatment. Hence, neem oil (5ml kg^{-1} seed) or *Trichoderma viride* (10g kg^{-1} seed) can be effectively used to control seed mycoflora in place of carbendazim.

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