

Original Research Article

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## Eco-Friendly Measures for Control of *Phomopsis vexans* and Other Mycoflora of Brinjal

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### ABSTRACT

The brinjal variety Hisar Shyamal (H-8) is popular among farmers in the state of Haryana and neighbouring states of India. It experiences huge losses in fruit yield as it is not resistant to devastating seed borne diseases viz. damping off and fruit rot caused by *Phomopsis vexans* in addition to other mycoflora. Seed treatment with chemical fungicides like carbendazim is a common practice to control these seed borne diseases of brinjal. However, continuous use of these chemical fungicides are not safe both environmentally and human health point of view. Therefore, we have evaluated different plant oils and bio-fungicides as seed treatment for control of *Phomopsis vexans* and other mycoflora as an alternative eco-friendly measure. The results revealed that the healthy seed not only affected by *P. vexans* but also by other mycoflora viz. *Aspergillus*, *Alternaria*, *Penicillium* and *Cercospora*. Seed treated with neem oil (5 ml kg<sup>-1</sup> seed) and *Trichoderma viride* (10g kg<sup>-1</sup> seed) found effective in controlling the infection caused by *P.vexans* comparable to that of carbendazim with an improved shoot growth. Hence, seed treatment with neem oil (5 ml kg<sup>-1</sup> seed) and *Trichoderma viride* (10 g kg<sup>-1</sup> seed) are effective alternatives to the chemical fungicide, carbendazim in view of safe environment.

#### Keywords

Mycoflora, Plant oils, Bio-fungicides, Carbendazim

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### Introduction

Brinjal is an important vegetable crop in India and occupies 0.727 m.ha with a production of 12.3 m.t (NHB, 2017). One of the production constraints is fungal diseases like seedling blight and fruit rot caused by *Phomopsis vexans* in addition to several other seed born fungi (Udaykumar *et al.*, 1981) leading to yield loss up to 50 per cent (Beura *et al.*, 2008) and therefore, seed treatment is indeed essential. For control of such, fungal diseases, many chemical fungicides in particular the

carbendazim (0.2 %) are commonly used in brinjal (Habib *et al.*, 2007; Phansawan *et al.*, 2015). However, indiscriminate use in a long run lead to residual toxicity, induced resistance to pathogen, environmental pollution and side effects on human and animal health (Rajavel, 2000; Avinash and Hosmani, 2012). Hence, identification of plant-based compounds and or bio-fungicides would be better alternatives in the changing climate scenario. Therefore, two studies were conducted to evaluate the plant oils and bio-fungicides in comparison with carbendazim.

## Materials and Methods

Two experiments namely, effect of plant oils on seed mycoflora and; effect of bio-fungicides on seed mycoflora incidence of brinjal were conducted during 2016-17 at the Department of Seed Science and Technology, CCS HAU, Hisar, Haryana. The high yielding variety, Hisar Shyamal (H-8) was harvested during March-April 2016, stored for six months and used in the present experiments. Such seeds were surface sterilized for 2 minutes using 1.0 % (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<sup>-1</sup> seed and shade dried overnight. In the second experiment, the seeds were dressed with bio-fungicides (*Trichoderma viridae*, *Trichoderma asperellum* and *Pseudomonas fluorescens*) @ 10 g kg<sup>-1</sup> seed as 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 % w/w) and untreated healthy seed.

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 incubator at 25 + 1°C. Each treatment had three replications and in each replication, 25 seeds were placed in petri-dish equidistantly. The petri-dishes were watered using sterilized distilled water as when required. After seven days of incubation, observations were made on different mycoflora around the seeds based on morphological features namely, colony colour and pattern of growth. The identification is as follows, (i) *C. capsici* - white to light grey circular colony with fluffy mycelium, (ii) *Alternaria* – greenish white colour colony with dark brown hyphae, (iii) *Aspergillus* – black cottony mycelia, (iv)

*Penicillium* - green colony and (v) *Cercospora* – small, irregular shape, charcoal black colonies) and confirmed under microscope. Each species of mycoflora incidence was enumerated as (i) 0 (no mycoflora), (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

Seed is a primary source of inoculum carrier for the plant disease from one generation to the next. Seed borne microorganisms play a vital role in affecting the seed germination, seed quality parameters and subsequent crop yield. In the present studies, brinjal seeds harvested and stored for six months found infected not only by the *Phomopsis vexans* but also other mycoflora like *Cercospora* sp., *Penicillium* sp., *Aspergillum* sp. and *Alternaria* sp. (Table 1 and 2). In this line, in blotter method, Udaykumar *et al.*, (1981) have reported 32 species of fungi and among them; the prominent were *Alternaria*, *Aspergillus*, *Cercospora*, *Penicillium* and *Phoma*. These mycoflora produce toxins and thus inhibits seed germination leading to unhealthy stunted seedlings (Udaykumar *et al.*, 1981). Therefore, control of these fungi is very essential to decrease the disease incidence to enhance the fruit yield.

Several chemical fungicides viz., thiram, captan, carbendazim etc. found effective in controlling the fungal pathogens including *Colletotrichum capsici* and *Phomopsis vexans* by inhibiting the mycelia and colony growth (Mesta *et al.*, 2007; Singh *et al.*, 2012; Hossain *et al.*, 2013; Bhojar *et al.*, 2014). However, the continuous or indiscriminate use lead to residual toxicity, induced resistance to pathogen, environmental pollution and side effects on human and animal health (Rajavel,

2000; Avinash and Hosmani, 2012) and hence, it would be more pertinent to use bio-agents.

In the present study, the cumulative seed mycoflora across the plant oil treatments revealed that, the cumulative *Aspergillum* sp. incidence was moderate (17 No.) followed by *Phomopsis vexans* and *Alternaria* (low incidence, 10 No.) (Table 1). Similarly, Aidoo *et al.*, (2011) reported that the *Aspergillus* is a predominant fungus (21.5 %) followed by *Penicillium* (17.0%) and similar pattern of incidence was reported by Bhajbhujje (2013), Singh and Sharma (2013) and Ashiq *et al.*, (2017). Such dominance by *Aspergillus* could be due to competitive inhibition of *P. vexans*, which could be due to suppression of germination and growth of *C. capsici* as reported in chilli (Gupta, 2016).

Among the plant oils, the neem oil found effective in controlling the cumulative mycoflora on par to that of carbendazim especially in inhibition of *P. vexans* and *Cercospora* sp. (Table 1). Whereas, healthy seed (un treated) has higher incidence by three times and hence treatment with fungicides is essential and; would be apt to use plant oils such a neem oil @ 5 g kg<sup>-1</sup> seed to control the seed mycoflora particularly the *Phomopsis vexans*. Chethana *et al.*, (2012) also observed that, the plant oils like neem and pongamia (@ 20%) recorded 76.9 and 69.9 per cent inhibition of mycelia growth respectively. Bhardwaj *et al.*, (2013) reported that the neem was most effective in reducing the *Aspergillus* in peas. In addition, neem oil is effective in controlling the shoot and fruit borer also (Yadav *et al.*, 2015).

**Table.1** Effect of plant oils on seed mycoflora incidence in brinjal seeds

Seed treatment	Mycoflora incidence					
	<i>Phomopsis vexans</i>	<i>Cercospora sp.</i>	<i>Alternaria sp.</i>	<i>Penicillium sp.</i>	<i>Aspergillus sp.</i>	Cumulative incidence
Castor	++	+	+	+	++	7
Neem	0	0	+	+	+	3
Aonla	+	+	+	+	++	6
Sesame	+	+	0	0	++	4
Linseed	+	+	+	+	+	5
Pongamia	+	+	+	0	++	5
Walnut	+	+	++	0	+	5
Ajwain	+	+	0	+	++	5
Healthy seed	++	+	++	+	+++	9
Carbendazim	0	0	+	+	+	3
<b>Cumulative incidence</b>	<b>10</b>	<b>7</b>	<b>10</b>	<b>8</b>	<b>17</b>	<b>52</b>

Note:

0: No disease incidence

+: Low disease incidence

++: Moderate disease incidence

+++ : High incidence disease

**Table.2** Effect of bio-fungicides on seed mycoflora incidence in brinjal seeds

Treatments	Mycoflora incidence					Cumulative incidence
	<i>Phomopsis vexans</i>	<i>Cercosporas p</i>	<i>Alternaria sp</i>	<i>Penicillium sp</i>	<i>Aspergillus sp</i>	
<i>Trichoderma viride</i>	0	0	+	+	0	2
<i>Trichoderma asperellum</i>	+	+	0	0	++	4
<i>Pseudomonas fluorescens</i>	+	+	+	++	0	5
<i>Trichoderma viride</i> + <i>Trichoderma asperellum</i>	0	+	0	+	++	4
<i>Trichoderma viride</i> + <i>Pseudomonas fluorescens</i>	0	+	+	+	0	3
<i>Trichoderma asperellum</i> + <i>Pseudomonas fluorescens</i>	+	+	0	+	+	4
Healthy seed	++	+	++	+	+++	9
Carbendazim	0	0	+	+	+	3
<b>Cumulative incidence</b>	<b>5</b>	<b>6</b>	<b>6</b>	<b>8</b>	<b>9</b>	<b>34</b>

Note:

0: No disease incidence

+: Low disease incidence

++: Moderate disease incidence

+++ : High incidence disease

**Plate.1** Comparison of seedling health and seedling growth of brinjal among, Seed infected with *Phomopsis vexans*, (b) Healthy uninfected seed, (c) Infected seed treated with carbendazim and (d) Infected seed with neem oil







The other approach for use of bio-agents to control the mycoflora could be the use of bio-fungicides. The cumulative mycoflora across different bio-fungicides was highest by *Aspergillus* sp. (9 No.) followed by *Penicillium* (8 No.) and with a least by *P. vexans* (5 No.) (Table 2). Such a lower infestation by *P. vexans* could be due to competitive inhibition by *Aspergillus* (Gupta, 2016). Among the bio-fungicides, cumulative incidence of mycoflora was least with *Trichoderma viridae* treatment (2 No.) as against carbendazim (3 No.) and untreated seed (9 No.) (Table 2). Similar to our results, Bhojar *et al.*, (2014) reported 100 % control of mycoflora by chemical fungicide followed by followed by *T. harzanium* (75 %) in case of cowpea. Further, Mogle and Maske (2012) reported an improvement in efficiency of bio-fungicide through addition of plant extracts, wherein, *Trichoderma viridae* along with plant extracts found to decrease the incidence of *Aspergillus*, *Penicillium* by 93 % and 75 % respectively, compared to the control. These treatments were on par to the bavistin with additional benefit of increase in seed germination, root length, shoot length and seedling vigour index. In the present study, the *Trichoderma viridae* found effective in

controlling the mycoflora especially the *P. vexans* (Table 2) and therefore, the bio-fungicides can be effectively used to control seed mycoflora of brinjal seed.

The cumulative incidence of mycoflora across plant oils as well as bio-fungicides showed that the cumulative mycoflora incidence was 52 No. and 34 No. respectively (Table 1 and Table 2). Among plant oils, the neem oil showed least mycoflora incidence especially the *P. vexans*. Among the bio-fungicides *Trichoderma viridae* and its combinations was able to achieve 100 per cent control of *P. vexans*. These results suggest that the *Phomopsis* seedling blight and fruit rot can be effectively controlled by seed treatment with neem oil comparable to that of carbendazim (0.2 %) treatment. Further, the data show that the seed treatment with neem oil found effective in maintaining normal healthy seedling and comparable to that of carbendazim (Plate 1). The seedling health is in these two treatments were similar to healthy seedlings and superior to infected seed with *P. vexans* (Plate 1). Interestingly the shoot length was higher in neem oil treatment and vice-versa with the carbendazim treatment (Plate 1).

The experimental results in overall infer that, the neem oil (5 ml kg<sup>-1</sup> seed) or *Trichoderma viridae* (10 g kg<sup>-1</sup> seed) would be effective in controlling the seed borne mycoflora as comparable to that of carbendazim (0.2%), a chemical fungicide.

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