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Research Article

Seed-borne infestation and management of *Alternaria Species* in mustard seed at Chitwan district, Nepal

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ABSTRACT

Alternaria blight in mustard cause heavy damage to the foliar parts resulting into poor growth and development of plants and thereby reduces seed yield. Inorder to manage *Alternaria* infestation in mustard seed for yield maximization, an experiment was conducted in the plant protection laboratory in Nepal Polytechnic Institute, Bharatpur, Chitwan in 2018, using Completely Randomized Design (CRD) with four replicates. Five treatments i.e.Uthane M-45 (2g/kg), Neem powder (3 g/kg), Bojho powder (4g/kg), *Trichoderma harzianum*, (10^7 Conidia/mL) and control (without treatment) were used for seed treatment of local variety of Mustard. Four hundred seeds for each treatment (25 seeds in each petriplate) were plated using triple layers of moistened blotter paper in petriplate and incubated at (27 ± 2^0C) for 2 days and followed deep freezing for 24 hrs. Data of disease incidence and seed germination were recorded in 3, 7 and 10 days after incubation (DAI). Seedling vigor and seedling weight were also recorded at 5 DAI. The percentage of *Alternaria spp* incidence on seeds at different DAI showed highly significant with respect to different treatments. Application of Uthane M-45 and *T. harzianum* significantly reduced the seed-borne infection of *Alternaria spp*. as compared to control. At 3 DAI Uthane M-45 completely checked the pathogen however, only 4% and 5% disease incidence was observed in 7 DAI and 10 DAI respectively. Bio control agent *Trichoderma harzianum* was found next best alternative after Uthane M-45 to control disease. Plant extract Bojho powder and Neem powder were found better than control to check the disease. Furthermore, highest germination (76%) was observed in *Trichoderma* treatment whereas, lowest germination was found in control.

Keywords: *Alternaria brassicae*, Seed borne infection, Seedling vigor, *Trichoderma harzianum*

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INTRODUCTION

Mustard (*Brassica juncea var. toria*), belonging to family cruciferae was introduced from China, and one of the very important oilseed crops grown under a wide range of agro-climatic conditions (Kant & Gulati, 2002). Major countries producing mustard are India, China and Pakistan in Asia; Canada, North America; Poland, France, Sweden and Germany. Oilseed is one of the important cash crops in Nepal too. The area, production and productivity of the oilseed was 224595 ha, 245867 mt and 1095 kg/ha respectively in FY 2017/18 (MoAD, 2018). Mustard is considered to be of high economic importance in local and international trade as it yields the most important edible oil ranging from 30-38%. From nutritional point of view, it contains 38-57% euric acid, 4.7-13% linolenic acid and 27% oleic and linolic acids, which are of high nutritive value, required for the human body (Saha, 1988). Mustard oil is also widely used in cooking and as a raw material for agro based industries engaged in manufacturing of soaps, paints, varnishes, hair oils, lubricants and grease. The Mustard cake is a valuable by product used as an animal feed and a good source of organic manure.

There is a wide gap between potential yield and farm yield in many cereal and oilseed crops in Nepal due to many biotic and abiotic constraints (Subedi *et al.*, 2019). The productivity of the oilseed crop, in the region is reduced due to a number of foliar diseases, viz., *Alternaria* blight, white rust, downy mildew and powdery mildew which caused both qualitative and quantitative losses (Kolte, 1985). Out of several diseases, *Alternaria* blight disease is the most important and destructing disease causing heavy losses all over the world. The yield loss up to 70% with no proven source of resistance against the disease reported till date in India (Meena *et al.*, 2012; Meena *et al.*, 2016). All the three species of *Alternaria* are reported to infect seedling stage on cotyledons and in the adult stage on leaves, petioles, stem, inflorescence, siliquae and seeds. The variation in shape, size, color and intensity of lesions are found on different host plants under different environmental condition. The initial infection on the lower leaves starts as minute brown to blackish lesions which multiply rapidly and later spread to the upper leaves, stem and siliquae. On the leaves, formation of concentric rings in the lesions and a zone of yellow halo around the lesion is very prominent (Kadian & Saharan, 1983). *Alternaria* blight causes substantial yield losses as a result of several factor including reduced photosynthetic potential, early defoliation, flower bud abortion, premature ripening, seed shriveling (Seidle *et al.,* 1995) and reduced seed size and impairs seed color and oil content (Kaushik *et al.,*1984). In the absence of resistant cultivars, chemical fungicides provide the most reliable means of disease control (Vyas, 1993). It may cause heavy damage to the foliar parts resulting into poor growth and development of plants and thereby reduces seed yield under congenial conditions. Moreover, literature reviewed revealed the need to generate the scientific information on various aspects including the intensities of pathogen attack to the seed and their management practices**.** The main objective of this study was to manage *Alternaria* infestation in mustard seed for yield maximization.

MATERIALS AND METHODS

Experimental site and mustard variety

The experiment was conducted in plant pathology laboratory of the Nepal Polytechnic Institute (NPI), Bharatpur, Chitwan, Nepal during 2018. The site is located at Bharatpur, the

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headquarter of Chitwan district, Mustard local variety (Kalo tori) was collected from Rapti Municipality, Bhandara, Chitwan district.

Dry inspection and sterilization

The mustard sample was physically inspected with unaided eye. Pure seeds were separated from abnormal seeds and inert matter such as soil, sand, stones and plant debris. Seeds with physical abnormalities like shrivelling of the seed coat, reduction or increase in seed size, discoloration or spots in the seed coat were (throughout the sample) classified under abnormal seeds. Glassware's were cleaned by washing with detergent and finally rinsed with tap water. After drying Glassware's were sterilized in hot air oven at 180ºC for 2 hours. The metallic equipments like forceps, needle and Cork borer were sterilized by dipping in alcohol and heating to red hot over flame of a spirit lamp.

Blotter method

Modified blotter technique (deep freezing) recommended by Limonard was followed to detect the presence of *A. brassicae* in mustard seeds throughout the study. Four hundred seeds were used for each treatment.

Agar method

The seed was grown on Agar medium to observe the plant vigor on 5 days after seeding (DAS). The contents of medium were:

Agar-Agar	: 15 g
Distilled water	: 1000 mL

For the preparation of Agar medium, fifteen gram of agar-agar powder was added in 1000 ml. of water. The media was sterilized in an autoclave at 121.6°C temperature, 15 lbs pressure per square inch for the period of 20 minutes.

Seed Testing in the Laboratory

Seed treatment with chemicals

Ten grams of seeds from each sample were taken for treatment. Required amount of Uthane M-45 i.e. 20 mg each (2 g/kg seed) for 10 gram of sample was taken in small plastic bottles. The content was shaken mechanically for 10 minutes for proper coating of fungicides.

Seed treatment with plant extracts

Already prepared Plant extract (powder) of Neem and Bojho were taken from Plant Pathology Laboratory, AFU. Ten gram of seeds was taken for treatment. For seed treatment Neem powder and Bojho powder were used at the rate of 3 g per kg of seed and 4 gm per kg of seed respectively.

Seed treatment with Trichoderma harzianum

Fungus (*Trichoderma harzianum*) one fully grown Petri dish (1×10^7 conidia/mL) was used to make the suspension by adding 10 mL distilled water. The suspension so prepared was used to treat the seeds @1 mL/g seed. The sample was shaken mechanically in sterilized conical flask for 10 minutes for proper coating of *Trichoderma harzianum*.

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Plating of Seed

Four hundred seeds from the sample for each treatment were tested using 16 sterilized petriplates (90 mm diameter) maintaining four replications. Treated seeds were placed at the rate of 25 seeds per petri dish at equal distance having three layers of moistened filter papers (12.5 cm diameter) with distilled water. One seed placed at center which was followed by two circular patterns at equi-distance containing 9 and 15 respectively with the help of forceps. The petriplates with the seeds were incubated at room temperature at (27 ± 2ºC) for 3 days. Frequent application of distilled water was done whenever felt necessary to keep the filter paper moist with the help of dropper.

Experiment design

Research was carried out in completely randomized design with five treatments and four replicates. The treatments were, Uthane M-45 (2g/kg), Neem powder (3g/kg), Bojho powder (4g/kg), *Trichoderma harzianum*, (10^7 conidia/mL) and control (without treatment).

Observation

By fourth day, the incubated seeds were observed under stereoscopic microscope. Each incubated seeds were examined for occurrence of *Alternaria brassicae* at 3, 7 and 10 DAI under stereomicroscope at 16x and 25x magnification. Identification of the fungi was done on the basis of spore morphology and habitat characteristics as described by Marthur and Kongsdal (2003). After identification of conidiophores, conidia and other part of the fungi were separated from the seeds on a clean slide.Plant vigor such as plant height and root length were measure in % DAS on seeds grown on agar medium. Seeds that produce both plumule and radical after incubation were considered as germinated seeds. Germination percentage and Increased in Germination was calculated by applying formula developed by Al- Mudrias, 1998,

$$\text{Germination (\%)} = \frac{\text{No. of seeds germinated}}{\text{Total no. of seeds planted}} \times 100$$

$$\text{Increased in Germination (\%)} = \frac{\text{Germination in treatment} - \text{Germination in control}}{\text{Germination in control}} \times 100$$

Percentage frequency (Percentage incidence) is the number of seeds (out of one hundred) on which at least a fungus appears and was noted along with germination (Simko & Piepho, 2012).

$$\text{Percentage Frequency (PF)} = \frac{\text{No. of seeds infected by } \textit{Alternaria sp.}}{\text{Total no. of seeds observed}} \times 100$$

Data collection and Analysis

Data were collected and analyzed by analysis of variance (ANOVA). Means were compared by least significance difference (LSD) for treatment difference (Gomez & Gomez, 1984; Shrestha, 2019). Statistical software used for data analysis was Microsoft Excel 2010 and Genestat 13.2.

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RESULTS AND DISCUSSION

The percentage of *Alternaria species* incidence on the seeds at different days after incubation showed highly significant variation with respect to different treatments (Table 1). At 3 days after incubation (DAI), seed of mustard without any treatment (control) showed highest (38%) pathogen incidence which was followed by seed treated with bojho powder (14%). However, Uthane M-45 almost completely checked the pathogen infestation where only 1.75% of seeds shown *Alternaria* infection followed by *Trichoderma* treated seeds where 6% seeds were found infected with the *Alternaria*. At 7 DAI, maximum *Alternaria species* infestation were seen in seed without any treatment i.e. (42.50%) which was followed by seed treated with bojho powder (21%). The lowest pathogen infestation (3.75%) was noticed in seed treated with Uthane M-45 followed by the seed treated with *Trichoderma harzianum* (9%). At 10 DAI, only 5% seeds were found infested with *Alternaria* treated by Uthane M-45 where as almost 45% seeds were infested with the pathogen in control. The similar finding was observed by Meena *et. al.* (2004) who reported that mancozeb as the best among all the treatments, resulting in the lowest disease severity on leaves of Mustard. Seed treatment with Dithane M-45 or Thiram (2-3 g/kg seed) is recommended for the control of *Alternaria* leaf spot of crucifers (Mathur *et. al.*, 1992). Uthane M-45 seems to be highly effective chemical for controlling the *Alternaria* leaf spot disease of crucifers.

The mustard seed treated with *Trichoderma harzianum* was found next best to Uthane M-45 in checking the *Alternaria species* seed infestation. The antagonistic *Trichoderma* species causes the inhibition of the spore germination percentage of *A. brassicicola* (Dennis and Webster, 1971). The antagonism of *T. harzianum* observed in the present studies is in tune with the findings of various workers (Subedi *et. al.*, 2019; Biles and Hill, 1988).The mechanism of *Trichoderma* to control pathogens may be by attacking and binding the pathogenic organisms by sugar linkage and begins to secrete extracellular protease and lipase (Cal *et. al.*, 2004). *Trichoderma sp.* grown over the pathogenic fungal hyphae, coils around them and degrades the cell walls. This action of parasitism restricts the development and activity of pathogenic fungi. Additionally, or together with mycoparasitism, some *Trichoderma* species release antibiotics (Harman, 2006). According to Rosado *et. al.* (2007), the main factor for ecological success of this genus is a combination of very active mycoparasitism mechanisms and an effective defensive strategy, induced in the plants.

Table 1: Effect of seed treatment on the incidence of Alternaria species at different days after incubation (DAI) at room temperature (27±2°C)

Treatments	Disease incidence (%)		
	3 DAI	7 DAI	10 DAI
Uthane-M 45 (2g/kg)	†1.75±0.51^{e}	3.75±0.68^{e}	5.00±0.93^{e}
Trichoderma harzianum (1× 10^7 conidia/ml)	6.75±0.79^{d}	9.50±0.95^{d}	13.25±1.20^{d}
Azadirachta indica (3 g/kg)	10.00±0.81^{c}	17.00±1^{c}	27.00±1.57^{c}
Acorus calamus (4 g/kg)	14.00±1.03^{b}	21.00±1.24^{b}	32.50±1.20^{b}
Control	38.75±2.24^{a}	42.50±2.24^{a}	44.75±1.97^{a}
F-Value	135.69	123.12	122.07
$LSD_{0.05}$	3.486	3.772	4.006
CV (%)	34.7	28.6	23.2

† means of four replications, Means in column with same superscript are not significantly different by LSD (P<0.05). g- gram, kg- kilogram, ml- millileter

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The seed germination percentage of mustard after seed treatment in 3 DAI in blotter germination method on laboratory condition is present in Table 2. The highest percentage of germination was observed in seeds treated by *Trichoderma harzianum* (78%). However, remaining all three treatments fungicides viz. Uthane M-45, Neem powder, Bojho powder showed almost same effect in germination (66%-70%). The least germination percentage was observed in control (63%) because of the high infection of *Alternaria species in* the blotted seeds. Highest germination found on *Trichoderma* treated seeds is 18% more than control which is followed by Uthane-M45 which only shows 7.7% increased in germination then seed without treatment. Seed treated with Neem powder and Bojho powder has only 4.61% and 1.53% of increased germination respectively. The highest seed germination that observed in *Trichoderma* treated seeds could be due to antagonist effects (competition, antibiosis, mycoparasitism, hyphal interactions, and enzyme secretion) of *Trichoderma harzianum* on the *Alternaria species* (Subedi *et. al*., 2019). In the present experiment seed treatment with *Trichoderma harzianum* not only suppressed the incidence of *Alternaria species* on Mustard seeds but also stimulated for the germination of the seeds.

Table 2: Effect of seed treatment on the germination of mustard after three days of incubation (DAI) at room temperature (27±2°C)

Treatments	Germination percentage (%) at 3DAI	Increased in Germination (%)
Uthane-M 45 (2g/kg)	†70.00±2.33^{b}	7.7
Trichoderma harzianum (1× 10^7 conidia/ml)	76.75±2.17^{a}	18.07
Azadirachta indica (3 g/kg)	68.00±2.17^{a}	4.61
Acorus calamus (4 g/kg)	66.00±1.55^{b}	1.53
Control	65.00±2.54^{b}	
F-Value	6.75	
$LSD_{0.05}$	6.137	
CV (%)	12.3	

† means of four replications, Means in column with same superscript are not significantly different by LSD (P<0.05). g- gram, kg- kilogram, ml- millileter

Table 3: Effect of seed treatment on height and weight of Mustard on 5 days after seeding (DAS) at room temperature (27±2°C) on Agar medium

Treatments	Seedlings Height (cm)	Seedlings weight (g)
Uthane-M 45 (2g/kg)	†3.211±0.113^{ab}	0.418^{ab}
Trichoderma harzianum (1× 10^7 conidia/ml)	3.652±0.968^{a}	0.423^{ab}
Azadirachta indica (3 g/kg)	3.503±0.122^{ab}	0.523^{a}
Acorus calamus (4 g/kg)	2.930±0.095^{b}	0.373^{b}
Control	3.602±0.102^{a}	0.528^{a}
F-value	0.064	0.022
$LSD_{0.05}$	0.548	0.104
CV (%)	10.8	15.3

† means of four replications, Means in column with same superscript are not significantly different by LSD (P< 0.05). cm- centimeter, g-gram, kg- kilogram, ml- millileter

Seedling vigor of Mustard was observed after seed treatment and incubated for 5DAS in Agar medium under lab condition. The higher plant height was observed on seeds treated with *Trichoderma* (3.652cm) which is followed by untreated seed (3.602 cm.) and both were statistically at par (Table 3). In contrast, Bojho powder treated seeds showed least plant height (2.930cm) as compared to other treatments. The highest seedlings weight was observed

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in untreated seeds having 0.528g and similar results obtained in seed treated with Neem powder (0.523g). Seed treated with Uthane-M45, *Trichoderma harzianum* was at par with Neem treated and untreated (Control) seeds. Bojho Powder treated seeds showed least plant weight (0.373 g) compared to other seed treatments.

CONCLUSION

This study confirms that seed borne infection in Mustard by *Alternaria species* could be managed successfully by using chemical fungicides and bio-control agents. The fungicide Uthane M-45 showed excellent performance (> 95% disease inhibition) at 3, 7 and 10 DAI under in vitro. Seeds treated with *Trichoderma harzianum* was also found significantly effective in suppressing the *Alternaria species* next to Uthane M-45. Seeds treated with Bojho powder and Neem powder had comparatively same result but better than control. *Trichoderma harzianum* treated seeds showed higher germination (76%). The finding of present study clearly indicated that seed treatment is very important to reduce the infesttation of seed-borne pathogen and improve the seed germination and vigor in Mustard.

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Authors contributions

P. Rijal and C. Adhikari designed and performed the experiments, analyzed data and wrote the paper. S. Subedi, S.M. Shrestha and J. Shrestha supervised the experiments and revised the manuscripts.

Conflict of interest

The authors declare no conflicts of interest regarding publication of this manuscript.

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