ISSN 0258-7122 (Print), 2408-8293 (Online)
Bangladesh J. Agril. Res. 46(4): 407-416, December 2021

MANAGEMENT OF SEED ASSOCIATED FUNGI AND *ALTERNARIA* LEAF SPOT DISEASE OF BLACK MUSTARD (*BRASSICA NIGRA* L.) USING BOTANICALS

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Abstract

The efficacy of five native botanicals viz., neem, castor, akondo, basok and ata against the seed associated fungi and in controlling the leaf spot disease of black mustard was evaluated. In blotter method, maximum reduction (85.91%) of seed associated fungi was observed in the seeds treated with ata (1:1) while neem (1:1), ata (1:2) and castor (1:1) gave 83.52, 82.59 and 82.39%, respectively reduction of fungi over control. Maximum seed germination (93%) was observed in seeds treated with akondo (1:1), where neem (1:1), castor (1:1) and akondo (1:2) supported 92, 92 and 90% seed germination, respectively. Maximum reduction of percent disease incidence (29.22%) and percent diseases severity (24.67%) were recorded with the application of neem (1:1) at 28 days after sowing with highest shoot length (16.09cm), root length (4.69cm), vigor index (1842.56%) and seed yield (33.70%) comparison to control in the net house. The findings neem (1:1) was found as an effective botanical for the eco-friendly management of seed associated fungi and *Alternaria* leaf spot diseases of black mustard.

Keywords: *Alternaria* leaf spot, seed associated fungi, botanicals, mustard.

Introduction

Black mustard (*Brassica nigra* L.) is a widely cultivated annual herbaceous oilseed crop which belongs to the family Brassicaceae. In Bangladesh, the annual production of mustard was 311739.82 MT from 270138.5 ha area of land with an average yield of 1.154 ton/ha (BBS, 2019). So far, 14 diseases of mustard were identified in Bangladesh where *Alternaria* leaf spot caused by *Alternaria* spp. is deliberated as the major one for low yield (Al-Lami *et al.*, 2020; Ghosh *et al*., 2020; BARI, 2007). *Alternaria* spp. perpetuates in seed and may pass on to the growing plant and hence, play the most devastating role in the reduction of crop yield and quality (Kumar *et al*., 2014; Anju *et al*., 2013; Latif *et al.*, 2006; Sivapalan and Browning 1992). The seed borne fungus also has a direct or indirect role in reducing seed germination, seed size, seed color, seed oil content etc. (Ismail *et al*., 2012; Meena *et al*. 2010; Rajendra and Lailu, 2006). Seed borne diseases of mustard could be minimized by using different chemicals as seed treating agents. However, the seed treating chemicals also lead to

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developing resistance in pathogen, causing human health hazards, and polluting environment (Ahmad and Ashraf, 2016). Botanicals including various medicinal plants are the major harbor of different antimicrobial compounds like alkaloids, flavonoids, glycosides, phenolic compounds, saponins, tannins and terpenoids etc. (Sen and Batra, 2012; Das *et al*., 2010; Shihabudeen *et al*., 2010). Because of the presence of the versatile antimicrobial compounds in the plant body, native medicinal plants namely neem, basok, castor, ata, akondo etc. may offer a broad range of protection in plants against a wide range of diseases (Kumar *et al*. 2017; Kavita and Dalbeer, 2015; Aboellil 2007; Hosna *et al*., 2003). Hence, using different native botanicals for the management of seed borne disease of mustard might open a new horizon for eco-friendly and cheap crop production technology (Ghosh *et al*., 2020; Meena *et al.*, 2013). So far, no or a few research works have been conducted for the eco-friendly management of seed borne fungi and *Alternaria* leaf spot disease of black mustard in the northern region of Bangladesh by using native botanicals. Therefore, the present study was designed to develop an eco-friendly and sustainable management technology for different seed borne fungi and *Alternaria* leaf spot disease of black mustard.

Materials and Methods

An experiment was carried out to control *Alternaria* leaf spot disease of black mustard by using different native botanicals during the cropping seasons of 2018-2019 in the Department of Plant Pathology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur.

Bio-efficacy of the selected botanicals to reduce seed associated fungi of black mustard in *in vitro*

Native botanicals viz. neem (*Azadirachta indica*), akondo (*Calotropis gigantea*), basok (*Justicia adhatoda*), castor (*Ricinus communis*) and ata (*Annona squamosa*) were collected from the HSTU campus, Dinajpur. The fresh leaves of each plants were washed in running tap water followed by drying in the shed. In 100 ml sterilized distilled water, 100 g leaves were added and blended by using an electric blender to prepare a 1:1 concentration solution followed by filtering through a double layer thin muslin cloth. The prepared aqueous extract was diluted further to prepare 1:2, 1:3 and 1:4 concentrations and was kept at 4°C for further use (Ul-Haq *et al*. 2014).

Mustard seeds were collected from local market from where, 400 seeds were randomly selected and dipped separately in each of the different concentrations (1:1, 1:2, 1:3 and 1:4) of aqueous extracts for 30 minutes. Twenty five (25) treated seeds were placed in each Petri plates (90 mm) following the standard blotter method (ISTA, 1996) and the plates were arranged in completely randomized design (CRD) with 3 replications. Seed soaked with sterilized distilled water and Provax 200 WP @ 25% of seed wt. were used as the negative and positive control,

respectively. Petri plates were then incubated at 25±2°C for 7 days. After that, each seed was examined individually to observe the fungal association according to the laboratory seed health testing methods (Mathur and Kongsdal, 2003; Barnett and Hunter, 1998) by using a stereomicroscope. For each of the treatments, data on seed germination (%), seed infection (%) and normal seedling (%) were also recorded.

Net house evaluation of selected botanicals on disease reduction and yield contributing characters of black mustard

The concentrations that showed better performance in *in-vitro* evaluation against the seed associated fungi namely neem (1:1), castor (1:1), akondo (1:1) and akondo (1:2) were selected for net house assessment in controlling *Alternaria* leaf spot disease of black mustard. Sterilized loam soil mixed with well decomposed cow dung (1:2) was used for sowing of the seeds in the pot. Seeds were treated separately with the plant extracts and Provax 200 WP. In each pot, 100 treated seeds were sown following CRD with three replications. From the 10 randomly selected plants, data were recorded on seed germination (%), shoot length (cm), root length (cm), vigor index (%) (Abdul-Baki and Anderson, 1973), percent disease incidence (%) (McKinny, 1923), percent disease severity (%) and crop yield (g).

Vigor Index (%) = (Mean shoot length + Mean root length)× Germination (%)

$$\text{Disease incidence (\%)} = \frac{\text{Number of plants infected}}{\text{Total number of plants examined}} \times 100$$

Disease severity was recorded following 0-5 scale given by Sharma and Kolte (1994). where, 0 = no visible symptoms, 1 = 1-10%, 2 = 11-25%, 3 = 26-50%, 4 = 51-75% and 5 = >75% leaf area infected.

$$\text{Disease severity (\%)} = \frac{\text{Summation of numerical ratings of observed plants}}{\text{Number of plant observed} \times \text{Maximum rating scale}} \times 100$$

All the recorded data on different parameters were statistically analyzed with MSTAT-C package program. The mean separation was computed by Duncan's Multiple Range Test (DMRT) at 5% level of significance.

Results and Discussions

Bio-efficacy of some botanicals in reducing seed associated fungi of black mustard in *in vitro*

Among the botanicals, castor resulted in lower range of seed associated fungi (0.25-0.82%) followed by basok, ata, akondo and neem in comparison to control (Table 1). However, maximum reduction of seed associated fungi (85.91%) was observed in seeds treated with ata (1:1). Provax 200 WP showed 77.45% to 94.46% reduction of seed associated fungi of black mustard.

Table 1. Efficacy of different botanicals against seed associated fungi of *Brassica nigra*

Treatments	*Fusarium* sp	*Alternaria* sp	*Aspergillus* sp	*Penicillium* sp	*Rhizopus*	*Phoma*	*Curvularia*	Mean	Reduction (%)
Neem (1:1)	3.00 bc	1.00 c	-	-	-	-	-	0.57	83.52
Neem (1:2)	4.25 bc	2.00 abc	0.25 a	-	-	-	0.25 b	0.96	72.25
Neem (1:3)	5.00 b	2.75 ab	1.25 a	-	-	-	-	1.28	63
Neem (1:4)	6.00 b	1.50 bc	1.00 a	1.00 a	-	-	0.50 ab	1.28	63
Provax 200 WP	1.25 c	0.50 c	-	-	-	-	-	0.25	92.77
Control	10.25 a	3.50 a	1.25 a	1.25 a	1.00 a	5.25a	1.75 a	3.46	0
Akondo (1:1)	3.00 c	0.50 b	-	-	-	-	-	0.5	81.06
Akondo (1:2)	3.50 c	0.50 b	0.25 a	-	-	-	-	0.6	77.27
Akondo (1:3)	6.00 b	5.75 a	1.25 a	-	-	-	-	1.85	29.92
Akondo (1:4)	7.25 b	0.50 b	1.00 a	-	-	-	-	1.25	52.65
Provax 200 WP	2.25 c	0.75 b	-	-	-	-	-	0.42	84.09
Control	12.5 a	4.50 a	1.50 a	-	-	-	-	2.64	0
Basok (1:1)	3.00 cd	0.1 a	1.75 a	0.25 a	-	-	-	0.72	47.82
Basok (1:2)	4.25 bc	0.1 a	1.25 a	-	-	-	-	0.8	42.02
Basok (1:3)	5.50 b	-	0.75 b	-	-	-	-	0.89	35.5
Basok (1:4)	5.50 b	0.1 a	0.50 b	0.25 a	-	-	-	0.9	34.78
Provax 200 WP	1.75 d	-	-	-	-	-	-	0.25	81.88
Control	9.25 a	0.1 a	0.10 a	0.25 a	-	-	-	1.38	0

Table 1. Cont'd

Treatments	*Fusarium* sp	*Alternaria* sp	*Aspergillus* sp	*Penicillium* sp	*Rhizopus*	*Phoma*	*Curvularia*	Mean	Reduction (%)
Castor (1:1)	1.75 c	-	-	-	-	-	-	0.25	82.39
Castor (1:2)	2.00 c	-	0.50 a	-	-	-	-	0.35	75.35
Castor (1:3)	2.75 bc	0.25 b	0.50 a	0.25 a	-	-	-	0.53	62.67
Castor (1:4)	4.25 b	0.25 b	0.50 a	0.75 a	-	-	-	0.82	42.25
Provax 200 WP	2.25 bc	-	-	-	-	-	-	0.32	77.46
Control	8.00 a	1.00 a	0.50 a	0.25 a	-	-	0.25 a	1.42	0
Ata (1:1)	3.50 bcd	-	0.75 d	0.75 d	-	1.25 a	-	0.89	85.91
Ata (1:2)	3.00 cd	0.50 c	1.00 d	0.75 d	1.25 b	0.25 a	1.00 bc	1.1	82.59
Ata (1:3)	5.00 bc	-	2.75 c	4.50 c	0.75 bc	1.00 a	1.25 bc	2.17	65.66
Ata (1:4)	5.75 b	2.25 b	4.50 b	6.50 b	1.00 bc	0.25 a	2.50 b	3.25	48.57
Provax 200 WP	2.00 d	-	0.25 d	0.25 d	-	-	-	0.35	94.46
Control	12.00 a	6.25 a	7.25 a	8.50 a	4.75 a	0.75 a	4.75 a	6.32	0

Each value represented the mean of number of infected seed.

In a column, means showing uncommon letters are significantly different at $p \leq 0.5$ by DMRT.

Likewise seed associated fungi, seeds treated with castor also demonstrated the highest range of seed germination (79-92%) and normal seedlings (71-84%) compared to other botanicals used in this study (Table 2).

Neem, akondo, basok and ata resulted in higher seed germination ranging from 74-92, 73-93, 79-81 and 77-82%, respectively and produced normal seedlings by 51-72, 54-75, 63-86 and 56-71%, respectively (Table 2). Based on botanical concentrations, akondo (1:1) extract showed higher seed germination followed by neem (1:1), castor (1:1) and akondo (1:2). Similarly, maximum t number of normal seedlings were also observed in basak (1:4) followed by castor (1:1) compared to control (Table 2). However, Provax 200 WP treatment was produced significantly higher seed germination as well as normal seedlings than that of other treatments.

Table 2. Effect of botanicals on seed germination and normal seedling of *Brassica nigra*

Treatments	Seed germination (%)	Number of normal seedling
Neem (1:1)	92.00 a	72.00 b
Neem (1:2)	80.00 b	60.00 c
Neem (1:3)	78.00 bc	57.00 cd
Neem (1:4)	74.00 c	51.00 d
Provax 200 WP	92.00 a	82.00 a
Control	63.00 d	44.00 e
Akondo (1:1)	93.00 a	75.00 ab
Akondo (1:2)	90.00 a	70.00 b
Akondo (1:3)	79.00 b	62.00 c
Akondo (1:4)	73.00 bc	54.00 d
Provax 200 WP	90.00 a	82.00 a
Control	67.00 c	46.00 e
Basok (1:1)	81.00 b	70.00 b
Basok (1:2)	78.00 b	63.00 b
Basok (1:3)	81.00 b	65.00 b
Basok (1:4)	79.00 b	86.00 a
Provax 200 WP	94.00 a	86.00 a
Control	52.00 c	43.00 c
Castor (1:1)	92.00 a	84.00 a
Castor (1:2)	84.00 b	76.00 bc
Castor (1:3)	80.00 b	71.00 c
Castor (1:4)	79.00 b	73.00 c
Provax 200 WP	93.00 a	82.00 ab
Control	67.00 c	55.00 d
Ata (1:1)	82.00 b	71.00 b
Ata (1:2)	77.00 bc	70.00 b
Ata (1:3)	77.00 bc	61.00 c
Ata (1:4)	78.00 bc	56.00 cd
Provax 200 WP	95.00 a	83.00 a
Control	75.00 c	49.00 d

Each value in the column was the mean of three replications.

In a column, means showing uncommon letters are significantly different at p≤0.5 by DMRT.

Net house evaluation of selected botanicals on disease reduction and yield contributing characters of black mustard

Percent Disease Incidence (PDI %) and Percent Diseases Severity (PDS %) of *Alternaria* leaf spot of black mustard were scored at 7, 14, 21 and 28 Days After Sowing (DAS). At 7 DAS, *Alternaria* leaf spot symptoms were observed only in the control plant (without applying the botanicals) having PDI 13.00% and PDS 13.46%. With time, both PDI and PDS increased in both the untreated and treated plants. At 28 days i.e., on the final count, maximum PDI (43.00%) and PDS (31.62%) were observed in the control plant where, minimum PDI (19.33%) and PDS (15.00%) were observed in Provax 200 WP treated plant followed by neem (PDI, 29.22 and PDS, 24.67%) treatment (Figure 1).

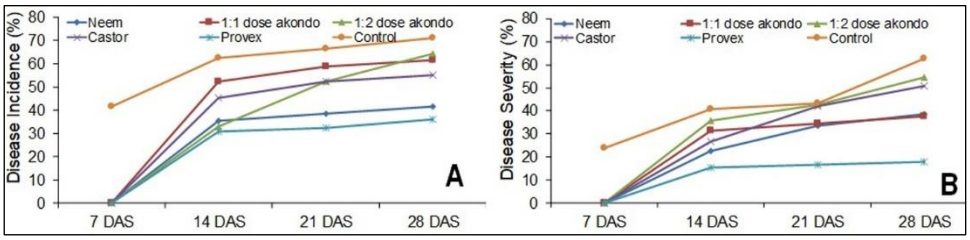

Fig. 1. A) PDI and B) PDS of *Alternaria* leaf spot disease of *Brassica nigra* treated with neem (1:1), akondo (1:1) akondo (1:2), castor (1:1) and Provax 200 WP.

Among the four best performed concentrations of used botanicals, seed treated with Provax 200 WP significantly higher seed germination than all other treatments followed by neem (1:1), akondo (1:1), castor (1:1) and akondo (1:2). Provax treatment also supported the significantly higher shoot length (17.63 cm), root length (5.59 cm) and vigor index (2175.01%). Among the botanicals, mustard seeds treated with neem (1:1) resulted in higher shoot length, root length and vigor index which also demonstrated increased seed yield by 33.70% over control (Table 3).

Among the botanicals, significant reduction of all kind of seed associated fungi (0.25-0.82%) of black mustard was achieved using castor followed by basok, ata, akondo and neem. However, considereing the concentration, maximum reduction of seed associated fungi was observed in seeds treated with ata (1:1), neem (1:1), ata (1:2) and castor (1:1). Similar to the finding of the present study, seeds treated with different botanicals were also found to reduce alternaria leaf spot disease of mustard (Ahmad and Ashraf, 2016; Kavita and Dalbeer, 2015). In addition to other botanicals, neem was reported as one of the best candidate for the reduction of seed association, inhibition of the radial growth of different plant pathogenic fungi including *Bipolaris oryzae*, Magnaporthe oryzae Pathotype triticum,

Colletotrichum lindemuthianum, *Drechslera sacchari* and *Fusarium semitectum* (Faruk et al., 2002; Fatema-Tuz-Zohura et al., 2018*;* Panna *et al.*, 2009*;* Shova et. Al., 2020). Likewise neem, methanol and aqueous Leaf extract of ata, castor and akanda were also demonstrated the inhibition of the growth of different plant pathogenic bacteria including *Ralstonia*, *Xanthomonas* etc. (Mondal *et al., 2017; Hasan et al., 2011).* In addition to the reduction of seed associated fungi, castor also resulted the higher range of seed germination as well as the production of normal seedlings compare to other botanicals. However, akondo (1:1) extract showed the maximum seed germination followed by neem (1:1), castor (1:1), and akondo (1:2). Likewise the present investigation, several reports also revealed the efficacy of different botanicals on the reduction of seed associated fungi along with the increasing of seed germination of mustard and other crops (Ghosh *et al.,* 2020; Meena *et al.,* 2013; and Rajendra and Lailu, 2006).

Table 3. Yield and yield contributing characters of *Brassica nigra* in response to the application of neem (1:1), akondo (1:1), akondo (1:2), castor (1:1) extracts and Provax 200 WP

Treatments	Germination (%)	Increased germination (%)	Shoot length (cm)	Root length (cm)	Vigor index (%)	Seed Yield (g/10	Increased yield (%)
Neem (1:1)	88.67 b	29.76	16.09 a	4.69 ab	1842.56	21.50 a	33.70
Akondo (1:1)	85.67 bc	25.37	15.63ab	3.37 bc	1627.73	18.17 c	12.99
Akondo (1:2)	75.00 d	9.76	13.11bc	2.95 c	1204.5	16.08 d	0.00
Castor (1:1)	83.00 c	21.46	15.47ab	3.42 bc	1567.87	20.67 ab	28.54
Provax 200 WP	93.67 a	37.08	17.63 a	5.59 a	2175.01	20.00 b	24.37
Control	68.33 e		11.85 c	2.58 c	986.00	16.08 d	
SE	1.07		0.74	0.43		0.42	
CV %	1.59		6.03	14.03		2.77	

Each value was the mean of three replications.

In a column, means showing uncommon letters are significantly different at $p \leq 0.5$ by DMRT.

In net house conditions, seed treated with neem (1:1) was found results higher seed germination followed by akondo (1:1), castor and akondo (1:2). Similar to the present study, increased seed germination of different crops including wheat and mustard as a response to the effect of botanicals were also reported (Shova *et al.,* 2020; Gautam *et al.,* 2018; Kumar *et al.,* 2017; Rajendra and Lailu, 2006; Hosna *et al.,* 2003). However, neem was reported as the most effective botanical to reduce the seed associated fungi, disease incidence and increased seed germination (Panna *et al.,* 2009; Ahmed *et al.,* 2002). Along with the seed germination, seeds treated with neem (1:1) also resulted increased shoot length, root length, vigor index and seed yield by 33.70% over control. Several reports showed the similar findings in regards to the increased crop growth and yield due to the application of botanicals in different crops (Gautam *et. al.,* 2018;

Ahmad and Ashraf, 2016; Kavita and Dalbeer, 2015; Hosna *et al.,* 2003). Alkaloids, oils, tannins, phenols, saponins, glycosides, flavonoids, Azardirachtin, 1-maliantriol, salannin, nimbin, nimbdin, triterpenoids, phenolic compounds etc. are present in neem, ata, castor, basok and akanda which might offer the antibacterial activity against different types of plant pathogens (Ibekwe *et. al.* 2001; Chavda *et. al.*, 2012, Gowdhami *et. al.,* 2014, Victoria *et. al*., 2014). Due to the presence of suck king of antimicrobial compounds in their body, botanicals especially medicinal plants such as neem, castor, akondo, basok, ata, etc. are used globally for controlling different plant diseases (Kakraliya *et al.,* 2018; Zohura *et al.,* 2018; Sharma and Kumar, 2016; Mathur *et al.,* 2011; Latif *et al.,* 2006;). The effect of botanicals against a pathogen can be varied based on antimicrobial compounds present on the botanical extracts (Tijjani *et al.,* 2014; Shrestha and Tiwari, 2009). Along with the control of the various fungal associations of seeds, botanicals can also increase different agronomic traits responsible for increasing the yield and quality of the crop (Gautam *et al.,* 2018; Sandeep, 2018). Phyto-compounds present in botanicals can enhance germination of seeds, robust.

Conclusion

The findings of the study revealed that the botanical extracts effectively reduced the prevalence of fungi associated with black mustard seeds, the *Alternaria* leaf spot of black mustard along with increase in seed germination, normal seedlings, seedlings vigor and seed yield. Neem (1:1) was found as the most significant candidate for the eco-friendly and sustainable management of *Alternaria* leaf spot disease of black mustard.

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