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CHARACTERIZATION OF *ALTERNARIA BRASSICAE* CAUSING *ALTERNARIA* BLIGHT OF RAPESEED- MUSTARD (*BRASSICA JUNCEA* L.) AND IT'S MANAGEMENT

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ABSTRACT
Ten fungicides, bio agent and nutrients $ZnSO_4$ + borex + sulphur were tested for their efficacy against *Alternaria* blight of rapeseed- mustard and was found to be effective in reducing disease intensity (19.06 and 20.7%) on leaves and disease intensity (5.83 and 15.1%) on pod and increase the yield (2.6tonne /ha and 1.1tonne /ha) during 2010-2011 and 2011-2012 respectively. The tested treatment spray of *Pseudomonas fluorescens*, mancozeb (0.2%) used as fungicidal check was the most effective to reduce the disease intensity (36.93 and 12.9%) on leaves, 14.93 and 12.2% on pod and increase the yield during 2010-2011 and 2011-2012, respectively. So $ZnSO_4$ + borex + sulphur and bio agents of *pseudomonas fluorescence* were recommended for management of *Alternaria* blight of rapeseed- mustard disease.

INTRODUCTION

Alternaria blight is common and destructive disease of rapeseed-mustard crop in India S.J.Kolte, (1985). *Alternaria* blight caused by *Alternaria brassicae* is a serious threat to mustard (*Brassica juncea*) cultivation (P.Chomoczynski, and N. Sacchi, 1987). The estimated area, production and productivity during 2011-2012 of rapeseed-mustard in the world were 33.1m ha, 60.7 million tones and 1832 kg/ha, respectively (Agricultural Statistics Division, GOI, (2012). Globally, India accounts for 20.2 per cent and 10.7 per cent of the total acreage and production USDA, (2012). *Alternaria brassicae* infects host species at all growth stages and affects seed germination and both quality and quantity of oil Meena *et al*. (2010). Generally, disease is appears on 45 days old plants and severe on 75 days old plants Meena *et al*. (2004). Deep lesions on siliquae increase the percentage of seed infection and decrease pod length, seeds per pod, thousand seed weight, percent seed germination and oil content S. J. kolte, (1988). Estimates of yield losses due to this disease vary between 35 and 70% in different species of oilseed *Brassicas* grown in different parts of the world. Oil yield losses due to infected seeds have been reported to be in the range of 15-36% A.N. Ansari (1988). Considering the importance of the disease the present investigations were undertaken for characterization of *Alternaria brassicae* and its management.

MATERIALS AND METHODS

Survey and collection of disease samples from different locations

The CSA University and other adjoining areas of Kanpur Nagar such as Bhaga, Chaubepur, Sarsaul were surveyed during the month of December to March, 2011-2012. The affected leaves of mustard show characteristics symptoms of *Alternaria* blight were brought into the laboratory for detection and isolation of the pathogen responsible for the disease.

Isolation and purification of the pathogen

The leaf spot and lesions, showing the initial and conspicuous characteristic symptoms of *Alternaria* blight were selected for isolation of the pathogen.These selected infected spots were washed 3-4 times in sterilized distilled water and then surface sterilized by dipping in 4% NaOCl solution for 1 min, followed by washing with sterilized water 3-4 times. Excess of moisture was removed by putting these pieces in between two folds of sterilized blotting paper under aseptic conditions in the inoculation chamber. Surface sterilized leaf spot pieces were then aseptically transferred into 9 cm Petri dishes containing Potato Dextrose Agar (PDA) and incubated at 25 ± 2ºC for seven days. Thereafter, growing mycelia from margin of apparently distinct colonies of the leaf spot pieces on the medium were aseptically transferred into another petri plate containing PDA medium, where it was grown for 15 days at 23 ± 2ºC in the BOD incubator. On the basis of their conidiophore and conidial morphology as described by Simmons, (2007), the pathogen was identified as *Alternaria*

brassicae (Berk) Sacc and purified by single spore isolation method.

Morphological characters and identification of the pathogen:

Ocular micrometer was calibrated and by use of micrometry Meena *et al*. (2005), Genus *Alternaria* was described by Nees in, (1816) with *A. tenuis* as the type and only member of the genus, which later was renamed as *A. alternata* as type species. Elliot, (1917) suggested that the genus could be organized into six groups based upon common characteristics of conidia length, width and septation, with each group designated by a typical species. Neergaard, (1945) proposed three sections for the genus based upon the formation of conidia in long chains (Longicatenatae), short chains (Brevicatenatae), or singly (Noncatenatae). According to Ellis, (1971), it contains 44 species. *Alternaria* species are either parasites on living plants or saprophytes on organic substrate. The host range of pathogenic *Alternaria* is very broad. It is easy to recognize *Alternaria* species by the morphology of their large conidia. They are catenate, formed in chains or solitary, typically ovoid to obclavate, often beaked, pale brown to brown, multi-celled and muriform Ellis, (1971). Simmons (1995) expanded concepts from both Elliot and Neergaard in loosely organizing the genus into 14 species-groups based upon characteristics of conidia and catenulation. Additional species groups discussed in other work include the *A. arborescens, A. brassicicola, A. porri,* and *A. radicina* groups (Roberts *et al.*, 2000; Pryor and Gilbertson, 2000, 2002). Although the use of species-group designation does not resolve definitive species boundaries within *Alternaria,* advantages of its use are that it organizes at the sub-generic level the morphologically diverse assemblage of *Alternaria* species and permits the generalized discussion of morphologically similar species without becoming overly restricted due to nomenclatural uncertainty.

Morphological characters

Based on morphological characteristics, the causal fungus was identified as *Alternaria brassicae* (Berk) Sacc. Colonies of *Alternaria* brassicae were amphigenous effused rather pale olive, hairy and immersed mycelium. Conidia were produced in chains of up to 4 with average 3.8 transverse septa and 1.8 longitudinal septa, pale olive and the beak about 1/3 to ½ the length of the conidia which confirmed with earlier studies (Ellis, 1971).

(a) Colony character- Colour of colony and growth of colony or type of growth

(b) Mycelium character - Colour of the hyphae, branching of the hyphae, septation of the hyphae and width of the hyphae

(c) Conidiophore character -Colour size and septation

(d) Conidial character-Colour, shape, arrangement, size (length and width), septation, beak and conidial chain

Screening of mustard germplasm for disease resistance

Forty two cultivars /genotypes of rapeseed-mustard group (*Brassica juncea, Brassica campestris, Brassica carinata, Brassica napus, Eruca sativa* and *B. rapa*) were screened during the Rabi 2011-12 under artificial conditions. Against *Alternaria Brassicae* the planting of single line of highly susceptible varieties Varuna was incorporated after each five rows. The genotypes were sown in two rows each of 3 m length with spacing of 40 × 10 cm in R.B.D. The recommended agronomic practices were adopted for raising the crop. To maintain high humidity level in micro climate of the field, irrigation was applied for favouring the development of disease.

The inoculums of *A. brassicae* was prepared by mycelia mat grown on Czapek's nutrient solution for 10 days at 24 ± 1ºC. It was homogenized in warring blender for 3 minutes in sterilized water and sprayed at branching and siliqua formation stages. After 15 days of inoculation the number of affected leaves was counted and the in 0-5 point grade of the recommended by Hussain and Thakur (1963) of the varietal infection as follows

Different combinations of fungicides and bio-agents for integrated disease management

The field trial was conducted with 10 treatments of different combination of fungicides, micro nutrients, and bio-agents as seed treatment and soil application.

The experiment was conducted in a Randomized Block Design (R.B.D.) with three replication at oilseed farm, Kalyanpur of the University during 2011-2012. The susceptible variety Varuna was sown at spacing of 40X10 cm between row and column with 3m X 5m plot size. Bio-agents were collected from Govind Ballabh Pant University of Agriculture and Technology, Pantnagar. Moistened seeds of variety (Varuna) were treated with the bio-agents @10g/kg seed and treated seeds were shade dried before sowing.

Different combination of micronutrients and fungicides for

Table 1: Details of different samples of cruciferous plants

S. No.	Name of host	Botanical Name	Locality
1.	Yellow sarson	*Brassica campestris* var. *dichotoma*	Oilseed Research Farm, Kalyanpur, C.S.A.U.A.T., Kanpur
2.	Black toria	*B. campestris* var. toria	Students Instrumental Farm, C.S.A.U.A.T., Kanpur
3.	Rai	*B. juncea*	Nawabganj Farm, C.S.A.U.A.T., Kanpur
4.	Cabbage	*B. oleracea* var. *capitata*	Farmer's field Shobhan, Kanpur
5.	Radish	*Raphanus sativum*	Farmer's field Chaubepur, Kanpur
6.	Taramira	*Eruca sativa*	Farmer's field Sarsaul, Kanpur

Table 2: Gradation for disease reaction

Grade	% infection	Reaction
0	Nil	Immune (I)
1	Upto 5% infection	Resistant (R)
2	Upto 10% infection	Moderately resistant (MR)
3	Upto 20% infection	Moderately susceptible (MS)
4	Upto 30% infection	Susceptible (S)
5	40% or more	Highly susceptible (HS)

Table 3: Fungicides and bio agent used for the management of *Alternaria Brassicae*

Sr. No	Treatment	Dose	Active ingredient	Mode of application
1	T1	10g/kg seed	*Trichoderma harzianum* + *pseudomonas florescence*	Seed treatment
2	T2	15kg/h	Zink sulphate + Borax + sulphur	Soil application
3	T3	Removal of three lower leaves	-	-
4	T4	2g/kg seed	Iprodine + carbendazim, carbendazim + manozeb	Seed treatment, spray
5	T5	0.2%	Zink sulphate + Borax, carbendazim + manozeb	Basal application, spray
6	T6	Basal application	Zink sulphate + Borax + sulphur,*pseudomonas florescence*	Basal application, foliar spray
7	T7	0.2%	Ridomil MZ-72	foliar spray
8	T8	2g/kg seed	Iprodine + Carbendazim	seed treatment
9	T9	0.1%	Propioconazole	foliar spray
10	T10 (control)	-	-	-

Table 4: micronutrients and fungicides used for the management of *Alternaria Brassicae*

Sr. No	Treatment	Dose	Active ingredient	Mode of application
1	T1	15kg/ha	Znso4	Soil application
2	T2	10kg/ha	borax	Soil application
3	T3	-	sulphur	As per local recommendation
4	T4	15kg/ha + 10kg/ha	Znso4 + borax	Basal application
5	T5	15kg/ha	Znso4 + sulphur as per recommendation	Soil application
6	T6	10kg/ha	Borax + sulphur as per recommendation	Soil application
7	T7	15kg/ha	Znso4 + borax + sulphur	Basal application
8	T8	1%w/v	Slaked lime	Spray (50 days of after sowing)
9	T9	0.2%	mancozeb	spray
10	T10 (control)	-	-	-

the management of major diseases of Indian mustard:

The experiment was conducted in a Randomized Block Design with three replication at oilseed farm, Kalyanpur of the university during Rabi 2011-2012. The susceptible variety Varuna was sown at spacing of 40X10 cm between row and column with 3m X 5m plot size. Evaluation of different fungicides and micro nutrients with different combination

RESULTS AND DISCUSSION

Morphological characters and identification of the pathogen

The morphological studies of *Alternaria brassicae* were made on host and in medium (PDA) by using compound microscope. The taxonomy of *Alternaria* on brassicas has been based principally on morphology and sometimes host plant association of each of the species occurring (*Alternaria brassicicola , Alternaria brassicae and Alternaria raphani*) has a distinct morphology considering the diversity of conidium shapes and sizes among *Alternaria* spp. All commercial cultivars of *brassicae* are susceptible to this pathogen (Tewari, 1991). All the isolates were microscopically identified based on their morphology on PDA using light microscope (Carl Zeiss, Germany) and available literature (Ellis, 1971).

Colony

Colony was fast growing, amphigenous usually in the beginning ashey grey, fluffy, circular and later turning into dark greenish olive with abundant sporulation.

Mycelium

The mycelium was septate and branched. In initial stags it is light brown in colour which becomes darker with advance in age of mycelial growth, cylender, radiating branched, and filaments of hyphae measure 3.0-5.9 μm in width.

Conidiophores

Conidiophores arising in group of 2-10, emerging from stomata usually simple, septate (0-8), amphigenous with slightly swollen base and rendered apices unbranched, even conidiophores 3-5 geniculate with prominent scans, 34.5-184.5 μm in length, olive brown, formed single, either as side branches or terminally on the hyphae 4.7 micron diameter.

Conidia

Conidia usually produced singly at the apex of the conidiophores but sometimes in short acropetal chain (2-4). They were obelavate to muriform, ovate elongated. Conidia measure 86.4-240.5 × 15.5-30.0 μm in size with 5-16 transverse septa and 0-8 longitudinal or oblique septa.

Beak

Beak was usually pale brown, short, cylindrical, 10-130 μm in length and 3-8 micron in width.

Effect of different combinations of fungicides, micronutrients and bio agents for integrated disease management

Evaluation of different fungicides, chemicals and bio-agents with ten different combination viz T_1- Seed Treatment (ST), *Trichoderma harzianum* @10g/kg seed + *Psudomonas floreseance* T_2- Zink sulphate (soil application) @15kg + borax@10kg + sulphur@15kg/h T_3- Removal of three lower leaves T_4-(ST), Iprodione + carbendazim (1:1)@2g/kg followed by two spray of carbendazim + mancozeb@0.2% T_5- Zink sulphate + Borax + Sulphur(basal application) followed by

Table 5: Effect of different combination of fungicides, nutrients and bio-agents for the integrated disease management of *Alternaria* blight.

S.No.	Treatment	Disease intensity on leaves (%)			Disease intensity on pod (%)		
		2010-11	2011-12	Mean	2010-11	2011-12	Mean
1	T-1	46.4(42.91)	57.4(49.2)	51.9(46.05)	36.1(36.92)	23.8(29.20)	29.95(33.06)
2	T-2	45.03(42.09)	50.3(45.2)	47.66(43.64)	35.06(36.29)	21.5(27.6)	28.28(31.94)
3	T-3	47.73(43.67)	22.2(28.1)	34.96(35.88)	37.63(37.83)	16.3(23.8)	26.96(30.81)
4	T-4	48.63(44.21)	40.7(39.6)	44.66(41.90)	90.03(39.22)	26.4(30.9)	58.21(35.06)
5	T-5	28.9(32.44)	50.3(45.1)	39.6(38.77)	23.66(29.01)	22.4(28.2)	23.03(28.60)
6	T-6	19.06(25.64)	20.7(27.00)	19.88(26.32)	5.83(13.70)	15.1(22.80)	10.46(18.25)
7	T-7	52.16(46.24)	27.6(31.7)	39.88(38.97)	41.23(39.87)	17.9(25.00)	29.56(32.43)
8	T-8	42.3(40.51)	18.6(25.5)	30.45(33.00)	36.16(36.96)	13.2(21.3)	24.68(29.13)
9	T-9	48.43(44.10)	61.3(51.5)	54.86(47.80)	40.8(39.64)	31.8(34.3)	36.(36.97)
10	T10 (control)	72.36(58.32)	70.9(57.3)	71.63(57.81)	37.3(37.62)	54.4(47.6)	45.85(42.61)
	CV	11.16	2.515		10.83	2.914	
	CD	8.04	1.732		6.44	1.732	

Table 5: Cont.....

S.No.	Treatment	Yield (kg/ha)			Test seed weight (gm)		
		2010-11	2011-12	Mean	2010-11	2011-12	Mean
1	T-1	2600	1008	1804	4.27	4.25	4.26
2	T-2	2623	1039	1831	4.11	4.1	4.1
3	T-3	1956	1275	1615.5	4.2	4.21	4.2
4	T-4	2489	1099	1794	4.09	4.3	4.19
5	T-5	2178	1039	1608.5	4.22	4.13	4.17
6	T-6	2645	1193	1919	5.08	4.2	4.64
7	T-7	1955	1147	1551	4.4	4.08	4.24
8	T-8	1622	1284	1453	5.02	4.79	4.9
9	T-9	2644	1005	1824.5	4.45	4.16	4.3
10	T10 (control)	2355	998	1676.5	4.19	4.09	4.14
	CV	6.119	6.863				
	CD	2.421	130.608				

Table 6: Effect of different combination of nutrients and fungicides for management of major disease of Indian mustard

S.No.	Treatment	Disease intensity on leaves (%)			Disease intensity on pod (%)		
		2010-11	2011-12	Mean	2010-11	2011-12	Mean
1	T-1	46.1(42.74)	20.1(26.60)	33.1(34.67)	34.83(36.1)	18.70(25.)	26.76(30.8)
2	T-2	35.03(36.2)	42.4(40.6)	38.71(38.4)	24.6(29.67)	35.4(36.5)	30.00(33.0)
3	T-3	42.73(40.7)	55.5(48.1)	49.11(44.4)	33.00(35.0)	46.6(43.0)	39.80(39.0)
4	T-4	40.06(39.1)	49.5(44.70)	44.78(41.9)	37.16(37.6)	42.5(40.7)	39.83(39.1)
5	T-5	35.96(36.6)	23.7(29.1)	29.83(32.8)	27.5(31.62)	23.2(28.8)	25.35(30.2)
6	T-6	45.56(42.4)	15.7(23.3)	30.63(32.8)	42.76(40.8)	14.9(22.6)	28.83(31.7)
7	T-7	29.9(33.12)	29.3(32.7)	29.6(32.91)	20.5(26.81)	28.3(32.1)	24.4(29.45)
8	T-8	49.4(44.65)	37.4(37.6)	43.4(41.12)	35.33(36.4)	31.4(34.0)	33.36(35.2)
9	T-9	36.93(37.4)	12.9(21.0)	24.91(29.2)	14.93(22.6)	12.1(20.1)	13.51(21.3)
10	T10(control)	63.3(63.49)	60.2(50.9)	61.75(57.1)	47.2(43.39)	52.5(46.4)	49.85(44.8)
	CV	9.03	2.783		6.66	1.759	
	CD5%	6.1	1.699		3.68	0.998	

Table 6: Cont.....

S.No.	Treatment	Yield (kg/ha)			Test seed weight (gm)		
		2010-11	2011-12	Mean	2010-11	2011-12	Mean
1	T-1	2245	1375	1810	3.75	4.23	3.99
2	T-2	1912	1287	1599.5	3.56	4.19	3.87
3	T-3	1956	1314	1635	4.47	4.15	4.31
4	T-4	2400	1201	1800.5	4.35	4.21	4.28
5	T-5	2422	1411	1916.5	4.74	4.25	4.49
6	T-6	2000	1399	1699.5	3.82	4.13	3.97
7	T-7	1956	1238	1597	3.75	4.18	3.96
8	T-8	2178	1312	1745	4.43	4.27	4.35
9	T-9	2222	1455	1838.5	4.4	4.82	4.61
10	T10(control)	1711	1148	1429	4.12	4.12	4.12
	CV	5.65	3.43				
	CD5%	2.037	76.98				

Angular transformed values are given in Parentheses

tow spray of carbendazim + mancozeb@0.2% T_6- Zink sulphate + borax + sulphar (basal application) followed by Foliar Spray of *P. fluorescens* T_7-Removal of three leaves followed by foliar spray of Ridomil MZ-72@0.2%, T_8- (ST), Iprodione +carbendazim (1:1)@2g/kg seed followed by removal of three lower leaves,T_9-(ST) Propioconazole (Tilt) @ 0.1% followed by foliar spray @ 0.1% T_{10}- Control find out the efficacy of different combination against *alternaria* blight. Data presented in Table 7 revealed that all the treatments were found significantly effective in reducing the disease intensity and increasing the yield over the control during both the years (2010-2012).

The data presented in table 3 clearly indicated that amongst the tested treatments $ZnSO_4$+borex+sulphur followed by spray of *Pseudomonas fluorescens* (T_6) were found most effective to reduce the disease intensity (19.06 and 20.7%) on leaves and disease intensity (5.83 and 15.1%) on pod respectively. However, the highest thousand grain weight 5.08 g and 4.20 g during both the years. Results were conformity with other researchers Lal *et al.*, (2000), Kushwaha and Narain (2001) Singh (2001). The problem is being addressed by application of sulphur, borax and zinc. However, this seems to be the possible first report of use of such chemicals viz., sulphur, borax, zinc, etc. for effective management of blight of mustard. The results obtained revealed that disease intensity was lowest in $ZnSO_4$+borex+sulphur sprayed plot followed by *Pseudomonas flurosence* similarly, Patni *et al.* (2005) and Prasad and Lallu (2006) reduction in *Alternaria* blight intensity and also increased the test weight and seed yield.

Effect of different combination of micronutrients and fungicides for management of major diseases of Indian mustard

Evaluation of different fungicides and micro nutrients with different combination *viz,* (T_1) $ZnSO_4$@15 kg/h, (T_2) borax@10kg/h, (T_3) sulphur as per local recommendation, (T_4) $ZnSO_4$ @15kg/h+Borax@10kg/h, (T_5) $ZnSO_4$@15kg/h +sulphur as per recommendation, (T_6) borax@10kg/h + sulphur as per recommendation, (T_7) $ZnSO_4$+borax+sulphur , (T_8) spray of slaked lime @1% w/v at 50 days of after sowing ,(T_9) spray of mancozeb @0.2% and 50 days after sowing , (T_{10})control to find out the efficacy of different combination against *Alternaria* blight. Data presented in Table 4 revealed that all the treatments were found significantly effective in reducing the disease intensity and increasing the yield over the control during both the years (2010-2012).

The data presented in table 4 clearly indicated that amongst the tested treatment spray of mancozeb 50 day after sowing (T_9) were found most effective to reduce the disease intensity (36.93 and 12.9%) on leaves, disease intensity (14.93 and 12.2%)on pod and increase the yield (2222 kg/ha and 1455 kg /ha) during 2010-2011 and 2011-2012, respectively, However, the highest thousand grain weight 4.40g and 4.82 g was also recorded with the treatment of spray of mancozeb 50 day after sowing during both the years. This is in line with the observation made by other researchers Kolte *et al.,* Prasad and Naik (2003) and Singh and Singh (2006).

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