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Field Response of Brassica Germplasm against Alternaria Leaf Spot Disease and its Management

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ABSTRACT

Brassica is an edible oilseed crop and world's third most important oilseed source. *Alternaria* leaf spot disease of *Brassica,* caused by *Alternaria brassicae* (Berk) is major limiting factor for its yield loss. In current study forty-two *Brassica* germplasm (*B. napus* = 17, *B. juncea* = 25) were evaluated against *Alternaria* leaf spot disease at research area of Plant Pathology Research Institute (PPRI), Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. These germplasm were sown in augmented design with 10 cm plant x plant and 60 cm row x row distance in single replication. A susceptible check KJ-159 was sown after every two entries. Data was recorded on 0-9 disease severity rating scale. Out of 17 germplasm of *B. napus* no germplasm was found immune. Two germplasm exhibited highly resistant while eight germplasm showed resistance response against the disease. Six germplasm were moderately resistant and only one showed moderately susceptible response. Similarly, out of 25 germplasm of *B. juncea* seven were found moderately resistant and ten showed moderately susceptible response. Seven were susceptible while only one germplasm was highly susceptible. Then *in-vitro* efficacy of different fungicides was evaluated against *A. brassicae*. For evaluation of fungicides Potato Dextrose Agar (PDA) amended with five different fungicides at different concentrations (10, 50, 100 and 200 ppm) was used. Experiment was conducted in completely randomized design with ten replications. Data was recorded on mycelial growth (mm) of fungus. Mancozeb (Ethylene bisdithiocarbamate) and Nativo (Tebuconazole) significantly inhibited the growth at all concentrations while Antracol (Propineb) was significantly least effective against *A. brassicae.* The current research provided the new resistance source of *Brassica* germplasm to the breeders against *Alternaria* leaf spot disease and its management.

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INTRODUCTION

Brassica is one of the major oilseed crops of Pakistan. In Pakistan, five species are cultivated named *Brassica nigra*, *Brassica juncea*, *Brassica napus*, *Brassica compestris* and *Brassica carinate*. Out of total cultivated area, rapeseed and mustard are being cultivated on 222 thousand hectares in Pakistan. The yield of rapeseed and mustard in the country is being increased by advanced technologies and farmers awareness, and yield in 2020-21 was 488 thousand tonnes (Anonymous, 2021).

Alternaria leaf spot of *Brassica* is caused by *Alternaria brassicae* (Berk) that decreased yield from 17-50% but severe outbreak of disease decreased about 70 % yield (Singh and Singh, 2005; Singh and Singh, 2006; Kolte and Awasthi, 1987; Saharan, 1992; Jyoti *et al.,* 2021). For disease development, the vapor pressure, evaporation, air speed and sunshine are directly corelated with severity (Punia *et al.,* 2021). Also, the optimal relative humidity ranges from 60-96% with the daily maximum temperature range from 18-28 ºC and daily range of minimum temperature from 10-14 ºC play rule in disease development and severity (Harper and Berkenkamp, 1975; Sarkar and Sengupta, 1978). The *A. brassicae* isolated from horeseradis (*Armoracia rusticana*) leaf spots in Serbia showed optimum quadratic growth and sporulation at 15-20 ºC (Blagojević *et al.,* 2020) and in Australia showed higher percent disease index at 17-22 ºC on mustard and canola (Al-Lami *et al.,* 2020).

In Pakistan 11-20 percent yield losses were noted in *B. napus* and 11-23 percent in *B. juncea* (Anwar, 2006). It also infects many other plants like *Orychophragmus violaceus*, an ornamental plant/ annual green grass in China. The incidence of leaf blight disease was reached 50-80% in the month December (Qian *et al.,* 2021). The forage oat (*Avena sativa*) was also found under attack of *A. brassicae* in northern China and disease incidence was very high (93%) (Zhang *et al.,* 2021). Infected pods develop abnormally, shrink dry and split prematurely that leads to fall down and become secondary source of disease spread (Schwartz and Gent, 2004). In favorable conditions, *A. brassicae* is more severe on leaves than pods and stems in older canola and mustard plants (Al-Lami *et al.,* 2019).

At present, there is no adequate available source of resistance against *A. Brassicae* due to the lack of identification of resistant genes in oilseed *Brassica* (Jyoti *et al.,* 2021). Under natural conditions, *Brassica campestris* 15 lines/ varieties out of 149 were found moderately resistant against leaf spot disease. But, during in-vitro screening only 2 varieties were among moderately resistant category (Singh *et al.,* 2008). It was studied that resistant and moderately resistant varieties of rapeseed and mustard (Kalyan and Bhagirathi) showed good results against *Alternaria* stress as compared to the susceptible varieties under different epidemiological factors (Mamgain *et al.,* 2017). However, different fungicides are being used successfully for the management of this fungal disease (Meah *et al.*, 1988; Howlider *et al.*, 1988).

The crop yield and 1000 seed weight were found highest with decreased in *Alternaria* leaf spot by two sprays of Mancozeb 75 WP (0.2%) followed by one spray of Ridomil MZ 72 (0.25%) (Singh and Singh, 2006). The malt extract agar was used to isolate *Alternaria* pathogen from diseased samples collected from different farms of Punjab, Pakistan. Furthermore, efficacy of chemicals available commercially were evaluated in-vitro. Three chemicals Tebucanazole, Wisdom and Benedict showed about 90% efficacy against leaf spot pathogen in Canola (Akhtar *et. al.,* 2014). In another study, 44% disease reduction with 37.6% yield increase when Metalaxyl and Mancozeb used together. When Carbendazim was combined with Ridomil MZ, there were 41.2% disease control and 33.5% yield increase. Also, the biocontrol agent *T. harzianum* was found in 28% disease reduction and 12% yield increase (Rai *et al.,* 2014). Some plant extracts, biocontrol agents and chemicals were studied against *Alternaria* blight pathogen. The Wisdom and Proctor were found best in all experiments, and use of fungicides were found best for said disease management (Ahmad and Ashraf, 2016).

Hence, the present study was planned to find the source of resistance in *Brassica* germplasm against *Alternaria* leaf spot disease and to determine the effective dosage of fungicides against pathogen *in-vitro*.

MATERIALS AND METHODS

Isolation, Purification and Multiplication of the Pathogen

Diseased plant leaves were collected from Oilseed Research Institute (ORI), Faisalabad. The infected leaves were washed with tap water, dried and cut into 4-5cm pieces. The pieces were surface sterilized by sodium hypochlorite (1%) for 1 min and then washed three times in distilled sterilized and air-dried on sterilized filter paper. The pieces were transferred on Potato Dextrose Agar (PDA) media supplemented with 1 g/L streptomycin. The petri plates were wrapped and incubated at 25±1°C for 7 days. The pathogen was purified and identified based on spore structure, size and shape (Wechter *et al*., 2007). The mass multiplication of *A. Brassicae* was done on mustard seeds as described by Shahbaz et al. (2014).

Evaluation of *Brassica* Germplasm against *Alternaria* Leaf Spot Disease

The field trials were conducted at the research area of Plant Pathology Research Institute, Faisalabad. Seeds of seventeen varieties/ advance lines of *B. napus* (RBN-09038, RBN-11049, RBN-13017, RBN-13019, RBN-13022, RBN-13028, RBN-13029, 13CBN005, 13CNB006, 14CBN001, 14CBN009, KN-263, KN-277, KN-279, KN-294, Faisal Canola, Rohi Sarson) and 25 genotypes of *B. juncea* (13CBJ004, 13CBJ006, 14CBJ002, 14CBJ004, BRJ-1103, BRJ-1104, BRJ-1304, BRJ-1305, RBJ-08015, RBJ-12019, RBJ-13029, RBJ-13030, RBJ-13033, RBJ-13046, RBJ-14011, ZBJ-06012, ZBJ-08051, ZBJ-12012, KJ-221, KJ-230, KJ-238, KJ-251, Super Raya, and Khanpur Raya) were taken from Oilseed Research Institute, Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. Seeds were sown with dibbler and plant-to-plant and row to row were maintained 10 cm and 60 cm respectively. To enhance the inoculum pressure, a susceptible check line KJ-159 was used after every two test entries. All the standard agronomic practices were adopted. The experiment was conducted in augmented design. Aqueous fungal suspension having the spore concentration 1x10^7 spores/ml was sprayed on the plants for disease initiation and development. The disease data recording was started after the appearance of the disease symptoms and continued at 7 days intervals till the maturity of the crop. The disease was quantified according to the modified disease rating scale of Mayee and Datar (1986) as described below (Table 1).

Table 1. Disease rating scale for the evaluation of brassica germplasm against the *Alternaria* leaf spot.

Ratings	Symptoms on plants	Reaction
0	No disease	Immune
1	A few scattered plants blighted with 1-2 spots/plant	Very Highly Resistant (VHR)
2	A few scattered plants blighted with5-10 spots/plant	Highly Resistant (HR)
3	A few scattered plants blighted with 11-25 spots/plant	Resistant (R)
4	A few scattered plants blighted with 26-50 spots/plant	Moderately Resistant (MR)
5	Blighted plant more common, nearly every leaf, stem and branch infected	Moderately Susceptible (MS)
6	Every plant infected with about 50% of leaf area and stem	Susceptible (S)
7	Every plant severely infected with about 75% of leaf area and stem	Highly Susceptible (HR)
8	Every severely infected defoliation is common and 95% of stem surface affected	Very Highly Susceptible (VHS)
9	Defoliation severe and 100% leaf and stem area affected and destroyed	Completely Susceptible (CS)

In vitro* Evaluation of Chemicals against *A. Brassicae

Five different fungicides *viz.,* Score (Difenoconazole), Topsin-M (Thiophanate Methyl), Mancozeb (Ethylene Bisdithiocarbamate), Antracol (Propineb) and Nativo (Tebuconazole) were evaluated at 10, 50, 100 and 200 ppm against *A. Brassicae* under laboratory conditions by poisoned food technique. Potato Dextrose Agar (PDA) was prepared and the required number of fungicides was added in a lukewarm agar medium. The medium was poured into sterilized Petri plates separately. The discs from artificially grown *A. Brassicae* were cut by a sterilized cork borer and transferred in the center of the fungicide amended plates. The plates were wrapped and incubated at 25 ± 2ºC for seven days. The data was recorded by measuring the colony growth of the fungus. The experiment was conducted in Complete Randomized Design with five repeats.

Statistical Analysis

The data was statistically analyzed using Analysis of Variance (ANOVA) and the treatment means were compared using Fisher's Least Significant Difference test (Steel and Torrie, 1986) using the "R" statistical analysis software (Chambers, 2008). The data were visually presented by "Microsoft Office (ver.2013) software packages.

RESULTS

Evaluation of *Brassica* germplasm against *Alternaria* leaf spot disease

Among the 17 genotypes of *B. napus*, only two (RBN-13017 and 14CBN001) were found highly resistant. Eight lines/ varieties (KN-279, RBN-13022, KN-294, 14CBN009, RBN-11049, RBN-13028, RBN-13019 and KN-263) were resistant and six (RBN-13029, Faisal Canola, Rohi Sarson, KN-277, 13CBN005 and RBN-09038) were moderately resistant *Alternaria* leaf spot disease. One advance line 13CNB006 was categorized as moderately susceptible, but no one showed a susceptible response (Table 2).

Within 25 different genotypes of *B. juncea* screened out against *Alternaria* leaf spot disease. No line/ variety was found highly resistant and resistant. Only, seven lines/ varieties (14CBJ002, BRJ-1305, RBJ-14011, ZBJ-06012, KJ-221, 13CBJ006 and KJ-230) were found moderately resistant against the disease. Ten lines/ varieties viz 14CBJ004, KJ-238, RBJ-13033, ZBJ-12012, RBJ-13029, BRJ-1304, BRJ-1103, RBJ-08015, BRJ-1104 and 13CBJ004 exhibited moderately susceptible response. Seven lines/varieties (ZBJ-08051, KJ-251, Super Raya, ZBJ-08051, RBJ-12019, RBJ-13046, Khanpur Raya) were susceptible, and RBJ-13030 was highly susceptible to the disease (Table 2).

Table 2. The reaction of *B. napus and B. juncea* germplasm against *Alternaria* leaf spot disease.

Rating	Genotype's response*	*B. napus*	*B. juncea*
0	Immune	-	-
1	Very Highly Resistant	-	-
2	Highly Resistant	RBN-13017 and 14CBN001	-
3	Resistant	KN-279, RBN-13022, KN-294, 14CBN009, RBN-11049, RBN-13028, RBN-13019 and KN-263	-
4	Moderately Resistant	RBN-13029, Faisal Canola, Rohi Sarson, KN-277, RBN-09038 and KN-309	14CBJ002, BRJ-1305, RBJ-14011, ZBJ-06012, KJ-221, 13CBJ006 and KJ-230
5	Moderately Susceptible	13CNB006	14CBJ004, KJ-238, RBJ-13033, ZBJ-12012, RBJ-13029, BRJ-1304, BRJ-1103, RBJ-08015, BRJ-1104 and 13CBJ004
6	Susceptible	-	ZBJ-08051, KJ-251, Super Raya, ZBJ-08051, RBJ-12019, RBJ-13046 and Khanpur Raya
7	Highly Susceptible	-	RBJ-13030
8	Very Highly Susceptible	-	-
9	Completely Susceptible	-	-
	Total	17	25

*Reaction based on disease rating scale

In-vitro **Evaluation of Different Fungicide Against *A. Brassicae***

Significant difference in inhibition of fungal growth was recorded by the fungicides with respect to the concentration. With the increase of the fungicide, the decrease in fungal growth was observed. At 10 ppm concentration, Mancozeb was the most effective to inhibit the fungal growth (11.6 mm) as compared to the other fungicides. The Antracol was found least effective (81 mm) to inhibit fungal growth. At 50 ppm concentration, significant decrease in fungal growth was noticed by all fungicide. No fungal growth (0.0 mm) was observed where Mancozeb was applied. Nativo was found less effective (11.4 mm) as compared to Mancozeb but was found more effective as compared to the other fungicides. Antracol was the least effective (64.2 mm) to retard the fungal growth, however, was effective as compared to control. At 100 ppm concentration, similar trend was seen as was observed in 10 and 50 ppm concentrations. Mancozeb completely retarded (0.0 mm) the mycelia growth of *A. Brassicae*. Nativo was the second most effective (4.8 mm) fungicide to inhibit the mycelial growth of the fungus. At 200 ppm concentration, significant increase in fungicides efficacy was seen as compared to 10, 50, 100 ppm concentrations. No fungal growth (0.0 mm) was seen where native and mancozeb applied. Antracol was found least effective (40.8 mm) to inhibit the growth of the fungi as compared to the other fungicides. Topsin-M was more effective as compared to score and Antracol but less efficient to Nativo and Mancozeb (Table 3).

Table 3. *In-vitro* efficacy of fungicides at different concentrations to inhibit fungal mycelial growth.

Treatment	Mycelial growth (mm)			
	10 ppm	*50 ppm*	*100 ppm*	*200 ppm*
Topsin-M	61.2 E*	40.6 I	25.2 K	11.8 M
Nativo	20.6 L	11.4 M	4.8 N	0.0 O
Score	71.4 C	57.6 F	44.0 H	30.6 J
Antracol	81.0 B	64.2 D	52.4 G	40.8 I
Mancozeb	11.6 M	0.0 O	0.0 O	0.0 O
Control	90.0 A	90.0 A	90.0 A	90.0 A
				LSD = 1.21

*Mean values sharing similar letters do not differ significantly α = 0.05

DISCUSSION

Alternaria leaf spot has become a serious disease significantly lowers the Brassica yield every year in Pakistan. The use of resistant varieties is cost effective and environmentally safe approach for the plant disease management (Nolte *et al*., 2020). The resistant genotypes have the resistant genes which codes the receptor (Yadav *et al*., 2014) for the recognition of pathogens' effectors to initiate the defense response in the plant (Couto and Zipfel, 2016). Furthermore, the resistant genes encode several antimicrobial compounds and pathogen related proteins that restricts pathogen invasion and colonization in the host plant. The present study revealed that the genotypes of *B. napus* and *B. juncea* exhibited significant variation in response against the disease. Among 17 genotypes of *B. napus,* two varieties/lines RBN-13017 and 14CBN001 were found highly resistant and eight varieties/lines (KN-279, RBN-13022, KN-294, 14CBN009, RBN-11049, RBN-13028, RBN-13019 and KN-263) were resistant to the disease. In case of *B. juncea,* among 25 varieties/lines, no one was found resistant against the disease and 14CBJ002, BRJ-1305, RBJ-14011, ZBJ-06012, KJ-221, 13CBJ006 and KJ-230 were moderately resistant. This reflects that *B. napus* has more genomic resistance potential against the disease as compared to *B. juncea.* Similar experiments were carried out by Yadav *et al*. (2014) who screened 31 varieties/lines against Alternaria blight of the rapeseed-mustard and found one genotype resistant and five varieties/lines moderately resistant. Das (2016) screened 134 varieties/lines against the *Alternaria* blight disease and found only five varieties (RSPN-29, CNH-13-2, AKGS-1, RSPN-28 and CNH-13-1) moderately resistant to the disease.

The use of chemicals for the plant disease management is an efficient approach, however, have some hazardous effects on the environment. So, the selection of environmentally safe and effective chemical for the disease management is mandatory. From the present study, Mancozeb was found the most effective chemical to inhibit the fungal growth in lab conditions. The Mancozeb is a multisided fungicide and interferes with enzymes containing sulphydryl groups which results into disruption of six biochemical process in the fungal cell wall and mitochondria (Gullino *et al*., 2010; Kaars, 1984). The efficacy of the fungicides directly depends on the a-biotics environmental conditions (Balardin *et al*., 2010). These conditions may vary with the type of fungicide used. In present *in-vitro* experiment was conducted at 25 ^{o}C which is ideal for the chemical's fungicidal activity. The Nativo was the second most effective fungicide. The fungicide has Tebuconazole and Trifloxystrobin as an active ingredient belongs to trialzole and strobilurin group respectively. The Tebuconazole disrupts the fungal cell wall by inhibiting the ergosterol biosynthesis and Trifloxystrobin binds to the quinol oxidation (Qo) site of cytochrome b and inhibit mitochondrial respiration. Saha (1989) tested ten random fungicides against *Alternaria* leaf spot disease of rapeseed mustard in lab conditions and found Ziram and Ceresan were most effective to completely inhibited the growth of *A. brassicae*. Harde and Atar (2014) evaluated ten fungicides against the Alternaria leaf blight of mustard in lab conditions. Significant increase in efficacy was observed with increase of the concentration. Iprodione 50 WP was found most effective to inhibit the fungal growth.

CONCLUSION

From the present study, it was revealed that two genotypes of *B. napus* "RBN-13017 and 14CBN001" and seven genotypes (14CBJ002, BRJ-1305, RBJ-14011, ZBJ-06012, KJ-221, 13CBJ006 and KJ-230) of *B. juncia* or may be used for the future breeding program. For the disease management, Mancozeb and Nativo may be used.

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