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Biochemical studies of different varieties of Indian mustard (*Brassica juncea* (L.) Czern & Coss) against alternaria blight (*Alternaria brassicae* (Berk) Sacc.)

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Abstract
Indian mustard (*Brassica juncea* L. Czern. & Coss.) are the world's third most important sources of vegetable edible oil. Which is adversely affected by many biotic and abiotic stresses. Among biotic stresses, diseases are the most common factors. Alternaria blight disease caused by the soil-borne necrotrophic fungal pathogen, *(Alternaria brassicae* (Berk) Sacc.*)* is a most serious problem in present scenario which damages to all growing part of Mustard including stem, leaves and pods and cruciferous crops worldwide. In present investigation was conducted at the Student's instructional Farm of N.D. University of Agriculture and Technology Kumarganj Faizabad (U.P.) during rabi season 2016-17.The 10 different hosts were screened in field conditions against Alternaria blight. Disease was appeared on leaves after 45 days of *Alternaria brassicae.* Giriraj (21.6%), Kranti (22.7%) and RGN-48 (20.5%) were found highly susceptible to Alternaria blight due to higher disease index. Varuna (15.2%) and Pusa Mustard-27 (15.5%) showed the lowest disease index, known as moderately resistant verities. The isozyme of peroxidase and catalase by native -PAGE electrophoresis also showed variability in bands.

Keywords: Rapeseed-Mustard, *Alternaria* Blight, Disease Resistance, peroxidase, catalase

Introduction
Brassica species are the largest oilseed production followed by peanut (*Arachis hypogaea* L.) and sunflower (*Helianthus annus* L.). In India, rapeseed-mustard is grown over in diverse agroclimatic conditions ranging from north-eastern/north-western hills to down south. Indian mustard was originally introduced from China into north – eastern India. These crops require cool growing season and moderate temperature throughout, hence are sown in the month of 1 November to 15 November in northern India (Das *et al.*, 2009) [4]. *Brassica juncea* (L.) Czern & Coss., also known by the name of Indian mustard, belongs to the plant family *Brassiceae* (Cruciferae) or the mustard family. *Brassica juncea* (n = 18) is an amphidiploid species derived from inter specific crosses between *B. nigra* (n = 9) and *B. rapa* (n = 10).

Among the biotic stresses, *Alternaria blight* disease of mustard caused by *brassicae* (Berk.) Sacc. Has been reported from all the continents of the world which affects most cruciferous crops. It is one among the important diseases of rapeseed-mustard causing yield losses up to 47% (Kolte, 1985) [8]. *Alternaria* is a very destructive pathogen causing a widespread destruction in vegetables and other economically important crops. The ideal and most economical mean of managing the *Alternaria blight* disease of rapeseed-mustard would be the use of resistant varieties (Mamgain *et al.*, 2013) [9].

Biochemical and physiological changes associated with induction of resistance are due to the response to inducing agents which are in the form like phytoalexins (Paxtron), lignin, callose and plant pathogenesis related proteins. Inducers also lead to formation of additional secondary xylem vessels in plant system (De Cal *et al.*, 2000) [5]. The plants peroxidases have been implicated in a variety of defense-related processes, including the hypersensitive response, lignifications, cross-linking of phenolics and glycoprotein, suberization and phytoalexin production. Catalase is frequently used by cells to rapidly catalyze the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water molecules

thus avoiding cellular disintegration (Bolwell and Wojtaszek, 1997) [2].

Peroxidase convert H_2O_2 to water provides an efficient system to prevent oxidative damage. Induction and accumulation of POX correlated with the onset of induced resistance suggest an active role for this enzyme in defense against pathogenic fungi and retard fungal growth (Jung *et al.*, 2004) [7]

Materials and Method

The experiment was conducted at the Student's instructional Farm of N.D. University of Agriculture and Technology Kumarganj Faizabad (U.P.) during rabi season 2016-17. The planting of ten Indian Mustard were done under natural conditions. The varieties were sown in two rows each of three meter length with spacing of 30x10 cm in randomized block design (RBD) with three replications.

Table 1: 0-9 Scale for scoring disease severity of Alternaria blight in rapeseed-mustard

Rating	Scale	Disease
0	0	Immune
1	<5	Highly Resistant (HR)
2	5-10	Resistant
3	11-25	Moderately Resistant
4	26-50	Susceptible
5	>50	Highly Susceptible

Observations were recorded on randomly selected five plants from each varieties/lines. Screening was rigorously carried out at modified 0-5 point scale (Table 1) as suggested in the proceeding of All India Coordinated Research Project on Rapeseed-Mustard Pathology Planning and Review Session during 2001-02 (Anonymous, 2002) [1]. Numerical rating grade was given on the basis of percentage of area covered by pathogen on the leaves. On the basis of disease intensity varieties/lines were classified into different groups viz., near immune/highly resistant, resistant, moderately resistant, moderately susceptible, susceptible, and highly susceptible.

$$\text{Per cent disease intensity (PDI)} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves examined x Highest rating}} \times 100$$

Peroxidase activity

Peroxidase activity was determined by the method of Plewa *et al.*, (1991) [13]. The conversion of guaiacol to tetraguaiacol was measured at 470 nm. The reaction mixture of 1 ml was contained 935 µl phosphate buffer, 25µl of enzyme extract, 15µl guaiacol, and 25µl H_2O_2. The reaction was initiated by adding H_2O_2 and rate of change in absorbance were recorded at 470 nm for 1 min at an interval of 5 seconds. Peroxidase activity has been defined as the change in absorbance min1mg-1protein.

Catalase activity

Catalase activity was determined by the method of Dhindsa *et al.*, (1981) [6]. The reaction mixture was used in a final volume of 1 ml comprised 50 mM sodium phosphate buffer (pH7.0) with 50 µl of enzyme extract. The enzyme activity was determined by adding 35 µl H_2O_2 at an interval of 5 seconds for 1 min by measuring the decrease in absorbance at 240 nm. The catalase activity was measured using extinction coefficient 39.4 M-1cm-1.

Result and Discussion

Screening of different mustard varieties against *Alternaria blight* in field condition.

Table 2: Summary of cultivated mustard evaluated for *Alternaria blight* resistance using a disease index in field condition.

S.No.	Scoring in field condition		Character
	Varieties	Disease index	
1	Varuna	15.24	Moderately resistance
2	Giriraj	21.66	Highly susceptible
3	Kranti	22.77	Highly susceptible
4	NPJ–112	19.44	Susceptible
5	NPJ–113	17.22	Susceptible
6	NRCDR–02	19.44	Susceptible
7	Pusa mustard–27	15.53	Moderately resistance
8	Pusa mustard–24	16.11	Susceptible
9	RGN–13	17.22	Susceptible
10	RGN–48	20.55	Highly susceptible
	C.D.	0.135	
	SE(m) ±	0.045	
	C.V.	0.423	

Screening of Indian Mustard varieties done at field of NDUA&T at student's instruction form revealed that among ten varieties, none was found immune or highly resistant or resistant against Alternaria blight of Mustard (Table 2). Only two varieties i.e., Varuna (15.2%) and Pusa Mustard-27 (15.5%) were found to be moderately resistant. Rest five entries were categorized as susceptible NPJ-112(19.4%) NPJ-113(17.2%), NRCDR-02(19.4%), Pusa Mustard -24(16.1%) and RGN-13(17.2%). and three entries were found as highly susceptible Giriraj (21.6%), Kranti (22.7%) and RGN-48(20.5%). The conditions were adopted to screen the different hosts namely field condition. The disease index were measured in all the conditions according to 0 to 9 scales of (Wheeler 1969) [14]. 100 % infection was achieved by the immature conidia of *Alternaria brassicae* in all the host and non-host plants. The disease index shown by different hosts varied but the lowest was recorded in mustard varieties Varuna and Pusa mustard-27. The result was supported (Das *et al.*, 2017) [3] who concluded that the Mustard varieties contain the potential source of genes for improving *Alternaria blight* resistance in the mustard gene pool. In another similar result,

Peroxidase isoenzyme activity in mustard leaves.

Peroxidase enzyme activity in leaves is presented in (Table.3) and depicted in respect of Peroxidase activity in mustard leaves witnessed significant variation. The ranged between 29.10 to 216.90 mg/g fresh weight/min. It was observed at highest peroxidase activity in Kranti (216.90 mg/g fresh weight/min) followed by RGN–13 (200.70 mg/g fresh weight/min) and Giriraj (157.20 mg/g fresh weight/min) while minimum peroxidase activity was noticed in Pusa Mustard–27 (29.10 mg/g fresh weight/min). while in case of infected (Table.4) leaves maximum range was obtained in 31.05 to 218.25 mg/g fresh weight/minute it was observed at highest Peroxidase isoenzyme activity in Kranti (218.25 mg/g fresh weight/min) followed by RGN–13 (201.90 mg/g fresh weight/min) and Giriraj (159.00 mg/g fresh weight/min) while minimum peroxidase activity was noticed in Pusa Mustard–27 (31.05 mg/g fresh weight/min). Meena *et al.* (2008) [10] was observed that Peroxidase activity was increased, as disease progressed. Higher levels of Peroxidase activity resulted exclusively from fungal infection. Higher peroxidase activity was observed in diseased leaf as compared to healthy leaf

Table 3: Peroxidase activity in Non infected mustard leaves in (mg/g fresh weight/minute).

S. No.	Name of Varieties	Peroxidase activity (mg/g fresh weight/minute)
1.	Varuna	91.95
2.	Giriraj	157.202
3.	Kranti	216.90
4.	NPJ–112	70.05
5.	NPJ–113	110.25
6.	NRCDR–02	72.00
7.	Pusa mustard–27	29.10
8.	Pusa mustard–24	138.15
9.	RGN–13	200.70
10.	RGN–48	153.00
SEm ±		0.214
CD (%)		0.640

Table 4: Peroxidase activity in infected mustard leaves in (mg/g fresh weight/minute)

S. No.	Name of Varieties	Concentration (mg/g fresh weight/minute).
1	Varuna	94.50
2	Giriraj	159.00
3	Kranti	218.25
4	NPJ-112	72.15
5	NPJ-113	112.50
6	NRCDR -02	73.65
7	Pusa mustard -27	31.05
8	Pusa mustard -24	141.15
9	RGN-13	201.90
10	RGN-48	156.15
	SEm ±	0.055
	CD	0.163

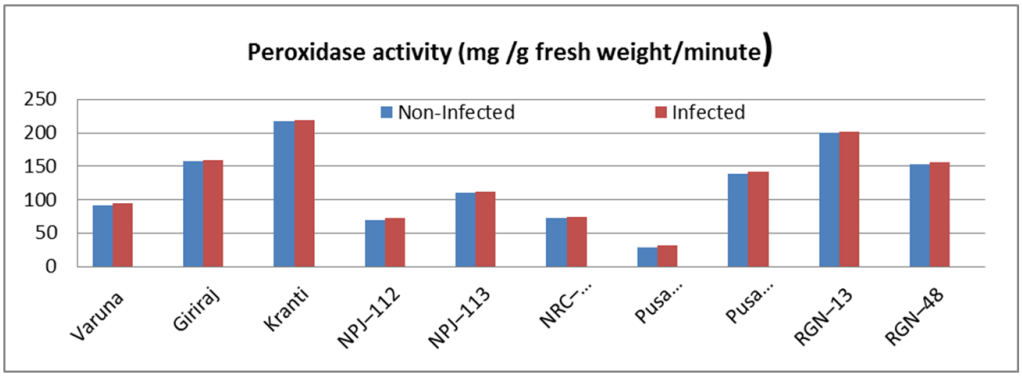

Fig 1: Activity of peroxidase from *Brassica juncea* genotypes infected and non-infected with Alternaria blight.

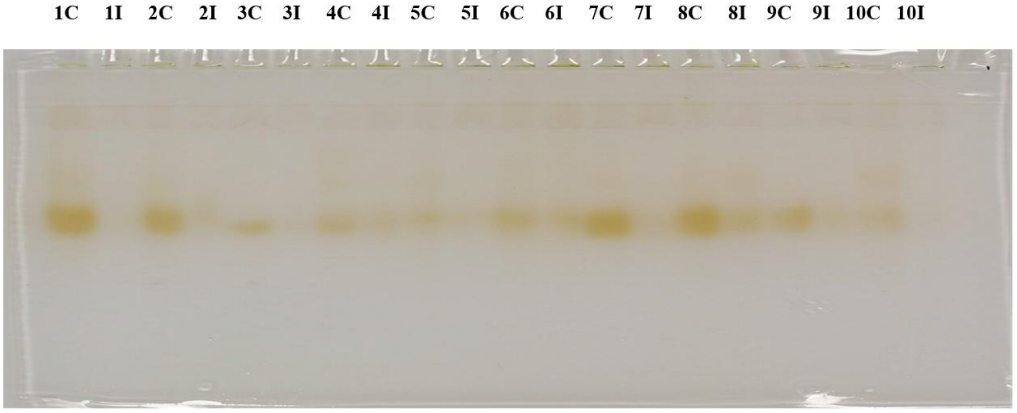

Fig 2: Appearance of Peroxidaes in isoenzymes of mustard non infected and infected leaves In native-PAGE (8%).
(I- Infected and C- Control or Non Infected)

According to isoenzyme of peroxidise in Non-Infected varieties Varuna, Giriraj and Pusa Mustard-27, Pusa Mustard-24 these Four genotypes showed high intensity bands on Native- PAGE while Kranti, NPJ-112, NRCDR-02 and RGN-13 showed minimum intensity bands on Gel. NPJ-113 and RGN-48 had no bands on gel it means they had no peroxidase activity. where in Infected verities NRCDR-02, Pusa Mustard-24 these two genotypes showed high intensity bands on Native- PAGE while Giriraj, NPJ-112, showed minimum intensity bands on Gel. *Varuna*, Kranti, NPJ-113. NPJ-113 and RGN-48 had no bands on gel it means they had no peroxidase activity (Fig.2.) Paranidharan *et al.* (2009) [12] Based on above results it may be concluded that high activity of peroxidase, polyphenol oxidase activity of the leaf sample of *Brassica juncea* appeared to be important biochemical constituents in imparting resistance to Alternaria leaf blight infection. Some genotypes show tolerance towards Alternaria leaf blight infection, having high PAL, PPO and peroxidase activity.

Catalase activity in mustard leaves

Catalase isoenzyme enzyme activity in leaves is presented in (Table. 5) and depicted In respect of catalase activity in mustard leaves significant variation. The catalase activity ranged between 34.20 to 58.00 mg/g fresh weight/min. It was observed at highest catalase isoenzyme activity in Giriraj (58.00 mg/g fresh weight/min) followed by Varuna (50.80 mg/g fresh weight/min), and Pusa Mustard-27 (44.20 mg/g fresh weight/min) while minimum catalase activity was noticed in RGN-48 (34.20 mg/g fresh weight/min) while in case of infected leaves maximum range was obtained in 33.60 to 56.00 mg/g fresh weight/min. It was observed at highest catalase isoenzyme activity in Giriraj (56.00 mg/g fresh weight/min) followed by Varuna (49.00 mg/g fresh weight/min), and Pusa Mustard-27 (42.20 mg/g fresh weight/min) while minimum catalase activity was noticed in

RGN-48 (33.60 mg/g fresh weight/min). Similar result supported that the catalase activity was lower in infected leaves as compared to healthy and very low in later stages of infection which account for maximum disease severity there by suggesting that catalase may not have significant role in disease resistance and also observed that catalase activity was decreased, as disease progressed (Mehdy, 1994) [11].

Table 5: Catalase activity in non-infected mustard leaves in (mg/g fresh weight/min)

S. No.	Name of Varieties	Concentration (mg/g fresh weight/minute)
1	Varuna	50.80
2	Giriraj	58.00
3	Kranti	39.40
4	NPJ-112	43.00
5	NPJ-113	38.40
6	NRCDR -02	37.40
7	Pusa mustard -27	44.20
8	Pusa mustard -24	40.40
9	RGN-13	37.60
10	RGN-48	34.20
	SEm ±	0.158
	CD	0.474

Table 6: Catalase activity in infected mustard leaves in (mg/g fresh weight/minute)

S. No.	Name of Varieties	Concentration (mg/g fresh weight/minute).
1	Varuna	49.00
2	Giriraj	56.00
3	Kranti	38.80
4	NPJ-112	41.00
5	NPJ-113	37.60
6	NRCDR -02	36.00
7	Pusa mustard -27	42.20
8	Pusa mustard -24	39.80
9	RGN-13	37.00
10	RGN-48	33.60
	SEm ±	0.152
	CD	0.454

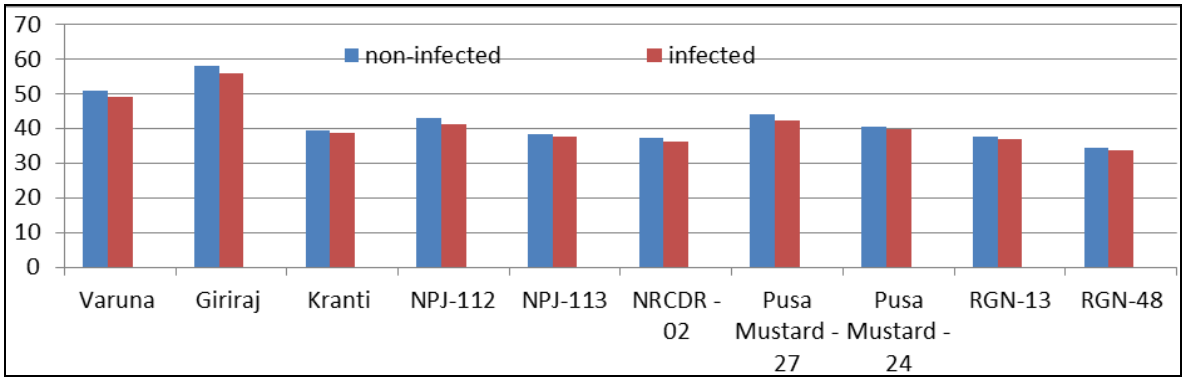

Fig 3: Activity of Catalase from *Brassica juncea* genotypes infected and non-infected with Alternaria blight

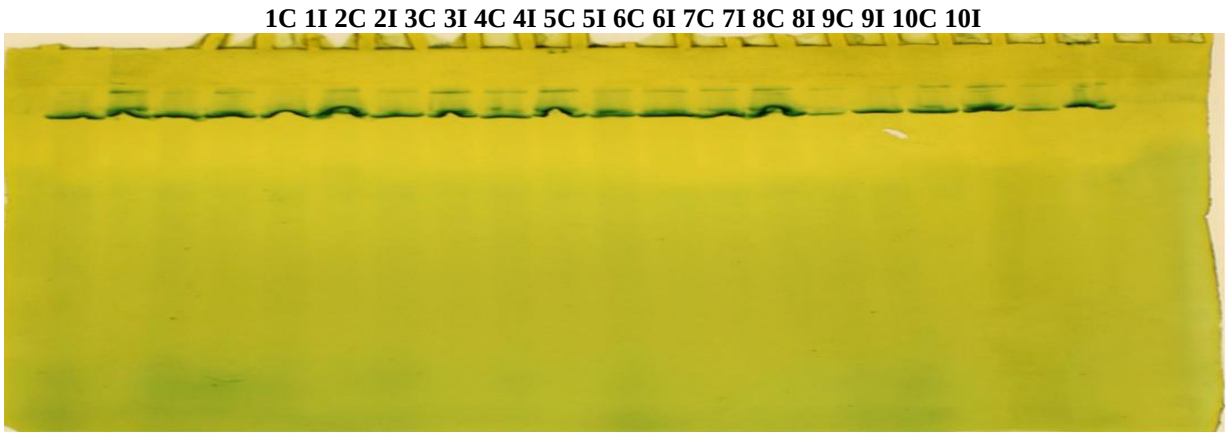

Fig 4: Appearance of catalase in isoenzymes of mustard non infected and infected leaves in native-PAGE (8%).
(I- Infected and C- Control or Non Infected)

According to isoenzymes of catalase in Non-Infected NPJ-113, Pusa Mustard-27 and RGN-13 these three genotypes showed high intensity bands on Native- PAGE while Giriraj, NPJ-112, NRCDR-02, Pusa Mustard-24 and RGN-48 showed minimum intensity bands on native-PAGE. Varuna and Kranti, had no bands on gel it means they had no catalase activity. Where in infected varieties Varuna, NPJ-112, NPJ-113 and Pusa Mustard-27 these four genotypes showed high intensity bands on Native- PAGE. Giriraj, Kranti, NRCDR-02, and RGN-13 showed minimum intensity bands on Native-PAGE. Pusa Mustard-24 and RGN-48, had no bands on gel it mean.

Conclusion

Thus based on the present results, the resistance source was found against *Alternaria Brassiceas* through physiological screening that was reconfirmed by the and biochemical analysis.

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