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Evaluation of Antioxidants in Different Mustard Cultivars Against Alternaria Blight

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ABSTRACT: The present study reports the variable antioxidant profile resulting from inducers/elicitors on mustard varieties against *Alternaria brassicicola*. An experiment was conducted with four inducers/elicitors *viz*., Benzothiadiazole (BTH), Hydrogen peroxide, Jasmonic Acid and Salicylic acid at three different concentrations to evaluate their effect against Alternaria leaf blight of mustard in four varieties *viz*., TBM-204, Bullet, B-54 and B-9. The pathogen *A. brassicicola* was inoculated at 15 DAS. An attempt was made to study the underline biochemical changes which may have their influence on induced resistance. The component of first line defense mechanism, catalase (CAT) and non-enzymatic antioxidants like ascorbic acid were found to be more prevalent in the plants treated with BTH followed by Jasmonic acid, Salicylic acid and Hydrogen peroxide. Among the varieties tested, these enzymes were more prevalent in TBM-204 and Bullet and less prevalent in B-54 and B-9.

Keywords: Mustard, *Alternaria*, catalase, ascorbic acid.

INTRODUCTION

Brassica juncea, commonly known as Indian mustard, is a major cultivated brassica crop in north-west India, followed by limited cultivation of *B. napus* and *B. rapa* for vegetable oil production. Among oilseed crops, rapeseed mustard (*B. juncea*) occupies an important position globally in terms of production and consumption. India is the fourth largest producer of oilseeds in the world and stands second in Asia. Whereas, West Bengal stands fifth position in which area and production is 0.6 M ha and 0.7M t, respectively, with an average productivity of 1212 kg ha^{-1} (Ministry of Agriculture and Farmers Welfare, 2019). It is generally used as a vegetable, and grown mostly for seeds, which yield essential oil and condiment. Although, India is one of the leading oilseed producing countries of the world, it still not able to meet the requirement for its vast population. To meet the growing demand and make India self-sufficient for edible oils, productivity of the oilseed crops should be increased since the possibility of increasing land under oilseed crops is very limited (Economic survey, 2020-21). The development of high yielding varieties along with the new improved production technology leads to increase in production and productivity of mustard but the gap between potential yield and actual yields are broaden due to the different biotic and abiotic factors. Among them, fungal diseases of oilseed *Brassica* are prevalent in India. The severe attack of these diseases deteriorates the quality and quantity of the seed and oil content. Among the diseases, Alternaria blight caused by *Alternaria brassicicola* (Berk.) Sacc. is the major constraint in production and destructive lethal disease of rapeseed mustard, reported from all the continents of the world causing 47% yield losses (Kolte, 1986) that may range up to 15-71% in productivity and 14.6-36 % in oil content (Meena *et al*., 2010). Apart from indiscriminate use of the pesticides, there is a need to develop strategies providing durable resistance, giving protection for a long time over a broad geographical area. Among such strategies, systemic acquired resistance (SAR) is an example of a defense mechanism offering long lasting disease resistance against a broad spectrum of pathogens and is promising for sustainable crop production in the future (Song and Goodman 2001). Therefore, the following study was conducted against Alternaria blight of mustard by using inducers.

MATERIAL AND METHODS

A. Isolation and purification of A. brassicicola

Different infected plant parts, *viz*., leaves, pods and stems of infected mustard plants were collected in paper bags and brought to the laboratory for isolation of pathogen. The diseased portion of infected plant parts along with healthy portion were cut into bits of 8–10 mm, and surface sterilized with 1% sodium hypochlorite (NaOCl) solution for 30 sec, washed thrice with sterilized distilled water and were blot dried. Thereafter three-four bits were placed in each petriplate containing Potato Dextrose Agar (PDA) medium. The inoculated plates were incubated in BOD incubator at 22 ± 2°C and monitored at regular intervals and initial growth of the pathogen was sub-cultured into agar slants.

Pure culture of Alternaria was obtained by single spore isolation method. The spore suspension was prepared

by scraping the surface of sporulating cultures and was added to lukewarm molten water agar and dispensed into sterilized petri plates. The petri plates were gently swirled for even distribution of the spores and kept for incubation at 25 ± 2°C for 12 h. Individual germinated spore, spaced out clearly was located on inverted water agar plates and marked with a glass marking pencil on the outside of the bottom dish using a compound microscope. Each marked spore was aseptically transferred into separate PDA slants. The culture was maintained and sub-cultured for further studies.

B. Preparation of inoculum spray

Four mustard varieties, *viz.*, TBM-204, Bullet, B-54and B-9 were collected from university instructional farm. The plantsof these varieties were raised in plastic pots (13 cm × 13 cm) containing 3 kg soil (sandyloam soil: FYM 3:1 w/w) in the net house, Department of Plant Pathology. For inoculation, *A. brassicicola* conidial suspension was prepared from nine-day old cultures by flooding the surface of the Petriplates with sterile distilled water and scraping the surface gently with a glass rod. The suspension was filtered through two layers of cheese cloth to eliminate mycelial fragments. Inoculum consisted of a conidial suspension adjusted to 1×10^4 conidia ml^{-1} using a haemocytometer. The plants were sprayed with freshly prepared conidial suspension using an atomizer at 15 DAS (Vishunavat and Kolte 2008).

C. Standardization of inducer concentrations

Benzothiadiazole (BTH) [S-methylbenzo-1, 2, 3-thiadiazole-7-carbothiate], hydrogen peroxide (H_2O_2), Jasmonic Acid (JA) and Salicylic Acid (SA) were used for seed treatment as inducers. Concentrations of these inducers were categorized as three levels *viz*., low, medium and high concentrations and standardized as BTH @ 0.25 mM, 0.75 mM, 1.5 mM, H_2O_2@ 1%, 2%, 3%, JA @ 1 mM, 2.5 mM, 4 mM and SA @ 0.5 mM, 1 mM, 2 mM.

Seed treatment was given by the standardize inducers for 1 h before sowing. For control treatment, seeds were soaked in sterilized distilled water. Spore suspension of the isolated pathogen was artificially inoculated at 15 DAS in three replications. The plants were covered with moist chamber consisting of transparent polythene sheet so that plant could maintain photosynthesis ability. After five days of inoculation, Alternaria blight incidence was visualized on leaves. The leaf samples were collected for three times at 15 DAS and 20 DAS (both uninoculated and inoculated samples) for biochemical analysis and are stored at -20°C.

Biochemical analysis. Leaf samples of different mustard genotypes were analyzed for enzymatic antioxidant (Catalase) and non-enzymatic antioxidant (Ascorbic acid). For assay of catalase activity, fresh leaf sample (0.3 g) was extracted with 0.1 M phosphate buffer (pH 7.5) containing polyvinylpolypyrrolidone (PVP) and Triton X. The homogenate was centrifuged at 10,000 rpm for 30 min and supernatant was collected for enzyme assay. Catalase activity was determined by monitoring the disappearance of H_2O_2 at 240 nm (ε = 40 Mm^{-1} cm^{-1}) (Aebi, 1984). The assay was performed using 2.8 ml of 100 mM phosphate buffer, 0.1 ml of H_2O_2 (1%) and 0.1 ml of plant extract. The molar extinction coefficient of hydrogen peroxide at 240 nm was taken as 0.04 sq. cm/µ mole. Enzyme activity was expressed as µ moles of hydrogen peroxide degraded/min/mg of protein.

Ascorbic acid content was measured by using modified method of Davies and Masten (1991). Leaf samples were extracted using 4% of oxalic acid, using chilled mortar and pestle. Then homogenate was centrifuged at 10,000 rpm at 4°C for 30 min. One ml of supernatant was added with 2 ml of 1.72 mM 2, 6-dichlorophenolindophenol (2, 6-DCPIP) dye indicator in 3 ml cuvette and was measured at 518 nm immediately after mixing. Content of ascorbic acid was expressed as the mg of ascorbic acid per 100 grams of fresh sample.

Statistical analysis of data was performed by Analysis of Variance (ANOVA) using OP STAT software.

RESULTS AND DISCUSSION

In order to assess the effect of inducers on biochemical responses, four different mustard varieties (TBM-204, Bullet, B-54 and B-9) were investigated against Alternaria blight infection.

Catalase: Study on catalase (CAT), indicated that the activity was significantly decreased in all the varieties with regard to inducers and biotic stress in comparison to control. All the treatments in all the four varieties tested were depicting decreasing trend to overcome the infection of the pathogen.

Inducers tested at all concentrations significantly increased the amount of catalase compared to control in all the varieties. Significantly high catalase activity was found in BTH followed by JA, SA and least activity was found in H_2O_2. With the increase in concentration the catalase activity and the percent increase over control was also increased (Tables 1-4).

Interaction between inducers and varieties revealed that Catalase activity was observed to be lesser in infected leaves as compared to the healthy one and the varieties B-54 and B-9 expressed less catalase activity than the varieties TBM-204 and Bullet which can consider having low resistance against Alternaria blight (Table 5, Fig. 1).

Above findings are strongly supported by the report of Subhani *et al*. (2018). The decline in catalase activity is regarded as a general response to many stresses (Jung, 2004; Pan *et al.,* 2006; Liu *et al.,* 2008). The reduction of CAT activity is due to the inhibition of enzyme synthesis or change in the assembly of enzyme subunits under stress conditions. Decrease in catalase activity was also an indication of scavenging the hydrogen peroxide activity after infection.

Result indicate that the high levels of catalase played an important role in reducing damage caused by pathogen by dismutating $O_2^{\bullet -}$ and catalyzing H_2O_2 in TBM-204 and Bullet. However, in B-54 and B-9 varieties an uncontrollable production of reactive oxygen species may couple with lesser activity of catalase created the oxidative stress, and led to the membrane damage and finally appeared as a symptom in plant.

Ascorbic acid: Study on non-enzymatic antioxidant revealed that the level of ascorbate was gradually increased significantly over their respective untreated controls with the increasing stress period in all the varieties tested.

In variety TBM-204, ascorbic acid content at 15 DAS was ranged from 34.95 – 83.25 mg/100 g FW while it was 35.54 - 119.61 and 39.85 - 125.54mg/100 g FW at 20 DAS and 5 DAI respectively (Table 6). In variety Bullet, ascorbic acid content at 15 DAS was ranged from 30.40 - 42.22mg/100 g FW while it was 31.31 - 63.84 and 33.84 - 94.95mg/100 g FW at 20 DAS and 5 DAI respectively (Table 7). In variety B-54, ascorbic acid content was ranged from 27.83 - 39.08, 30.40 - 43.84 and 31.23 - 84.67mg/100 g FW at 15 DAS, 20 DAS and 5 DAI respectively (Table 8). And it was 16.40 - 22.19, 22.70 – 35.54 and 24.67 – 72.10 at 15 DAS, 20 DAS and 5 DAI respectively (Table 9) in variety B-9. In the present study, varieties TBM-204 and Bullet in which maximum ascorbic acid (125.54 and 94.95 mg/100 g FW) under *Alternaria* blight infection were expected to have highest resistance, while varieties B-54 and B-9which possessed comparatively low ascorbic acid (84.67 and 72.10 mg/100 g FW) were considered to have low resistance against *Alternaria* blight (Table 10, Fig. 2).

Among the four inducers tested, BTH at high concentration showed high amount of ascorbic acid followed by medium and low. Later jasmonic acid showed significant high levels of ascorbate followed by salicylic acid and H_2O_2 in all the days of sampling.

Table 1: Effect of inducers at different concentrations on the Catalase activity (µmol of H_2O_2/min/mg of protein) in the mustard (TBM-204) against *A. brassicicola.*

Sr. No.	Treatments	15 DAS	% increase over control	20 DAS	% increase over control	5 DAI	% increase over control
T1	BTH - L	3.014	16.670	3.374	17.311	2.912	16.994
T2	BTH - M	3.166	20.673	3.430	18.663	3.101	22.047
T3	BTH - H	3.403	26.200	4.101	31.967	3.586	32.592
T4	H_2O_2 - L	2.688	6.578	2.816	0.900	2.563	5.672
T5	H_2O_2 - M	2.698	6.936	2.824	1.203	2.580	6.306
T6	H_2O_2 - H	2.718	7.603	2.843	1.848	2.584	6.425
T7	JA - L	2.788	9.923	3.124	10.687	2.686	9.990
T8	JA - M	2.791	10.030	3.139	11.113	2.981	18.902
T9	JA - H	2.832	11.335	3.342	16.521	2.707	10.704
T10	SA - L	2.741	8.383	2.982	6.431	2.634	8.221
T11	SA - M	2.753	8.767	3.071	9.140	2.661	9.140
T12	SA - H	2.765	9.194	3.091	9.742	2.634	8.231
T13	Control	2.511		2.790		2.418	
	SEm±	0.130		0.161		0.147	
	CD (P≤0.05)	0.377		0.469		0.428	
	CV %	7.913		8.875		9.205	

DAS: Days after sowing; DAI: Days after pathogen inoculation; L: Low; M: Medium; H: High

Table 2: Effect of inducers at different concentrations on the Catalase activity (µmol of H_2O_2/min/mg of protein) in the mustard (Bullet) against *A. brassicicola.*

Sr. No.	Treatments	15 DAS	% increase over control	20 DAS	% increase over control	5 DAI	% increase over control
T1	BTH - L	2.607	19.189	2.893	15.120	2.706	11.970
T2	BTH - M	2.898	27.289	2.924	16.011	2.711	12.144
T3	BTH - H	2.943	28.416	3.927	37.472	2.711	12.143
T4	H_2O_2 - L	2.149	1.936	2.720	9.712	2.399	0.708
T5	H_2O_2 - M	2.220	5.082	2.749	10.692	2.462	3.271
T6	H_2O_2 - H	2.186	3.633	2.831	13.257	2.500	4.733
T7	JA - L	2.310	8.775	2.887	14.956	2.576	7.555
T8	JA - M	2.372	11.159	2.907	15.540	2.696	11.644
T9	JA - H	2.565	17.864	2.912	15.690	2.584	7.843
T10	SA - L	2.299	8.350	2.832	13.307	2.524	5.625
T11	SA - M	2.202	4.334	2.845	13.692	2.544	6.394
T12	SA - H	2.304	8.551	2.903	15.414	2.549	6.554
T13	Control	2.107		2.455		2.382	
	SEm±	0.104		0.147		0.070	
	CD (P≤0.05)	0.303		0.427		0.204	
	CV %	7.524		8.761		4.740	

DAS: Days after sowing; DAI: Days after pathogen inoculation; L: Low; M: Medium; H: High

Table 3: Effect of inducers at different concentrations on the Catalase activity (µmol of H_2O_2/min/mg of protein) in the mustard (B-54) against *A. brassicicola.*

Sr. No.	Treatments	15 DAS	% increase over control	20 DAS	% increase over control	5 DAI	% increase over control
T1	BTH - L	2.071	20.859	2.629	19.019	2.612	54.900
T2	BTH - M	2.556	35.876	2.707	21.352	2.656	55.648
T3	BTH - H	2.666	38.522	2.837	24.956	2.666	55.814
T4	H_2O_2 - L	1.774	7.610	2.183	2.474	1.681	29.923
T5	H_2O_2 - M	2.071	20.859	2.152	1.069	1.741	32.338
T6	H_2O_2 - H	2.152	23.838	2.225	4.315	1.803	34.664
T7	JA - L	1.853	11.549	2.383	10.659	1.911	38.357
T8	JA - M	1.955	16.164	2.428	12.315	2.576	54.270
T9	JA - H	2.202	25.568	2.823	24.584	2.005	41.247
T10	SA - L	1.796	8.742	2.188	2.697	1.815	35.096
T11	SA - M	2.078	21.126	2.302	7.515	1.822	35.346
T12	SA - H	2.183	24.920	2.294	7.193	1.825	35.452
T13	Control	1.639		2.129		1.178	
	SEm±	0.094		0.11		0.109	
	CD (P≤0.05)	0.273		0.319		0.316	
	CV %	7.832		7.907		9.307	

DAS: Days after sowing; DAI: Days after pathogen inoculation; L: Low; M: Medium; H: High

Table 4: Effect of inducers at different concentrations on the Catalase activity (µmol of H_2O_2/min/mg of protein) in the mustard (B-9) against *A. brassicicola.*

Sr. No.	Treatments	15 DAS	% increase over control	20 DAS	% increase over control	5 DAI	% increase over control
T1	BTH - L	1.883	27.403	2.071	22.308	1.95	44.410
T2	BTH - M	1.955	30.077	2.294	29.861	2.078	47.834
T3	BTH - H	2.071	33.993	2.310	30.346	2.114	48.723
T4	H_2O_2 - L	1.619	15.565	1.675	3.940	1.572	31.043
T5	H_2O_2 - M	1.686	18.921	1.741	7.582	1.675	35.284
T6	H_2O_2 - H	1.721	20.569	1.815	11.350	1.774	38.895
T7	JA - L	1.700	19.588	1.911	15.803	1.844	41.215
T8	JA - M	1.882	27.365	1.955	17.698	1.883	42.432
T9	JA - H	1.911	28.467	2.078	22.570	1.911	43.276
T10	SA - L	1.601	14.616	1.700	5.353	1.675	35.284
T11	SA - M	1.665	17.898	1.725	6.725	1.748	37.986
T12	SA - H	1.782	23.288	1.853	13.168	1.809	40.077
T13	Control	1.367		1.609		1.084	
	SEm±	0.085		0.099		0.085	
	CD (P≤0.05)	0.246		0.288		0.247	
	CV %	8.351		9.034		8.263	

DAS: Days after sowing; DAI: Days after pathogen inoculation; L: Low; M: Medium; H: High

Table 5: Effect of inducers at different concentrations on the Catalase activity (µmol of H_2O_2/min/mg of protein) in the mustard varieties against *A. brassicicola*

Inducers / varieties	TBM-204			Bullet			B-54			B-9		
	15 DAS	20 DAS	5 DAI	15 DAS	20 DAS	5 DAI	15 DAS	20 DAS	5 DAI	15 DAS	20 DAS	5 DAI
BTH	3.194	3.635	3.200	2.816	3.248	2.709	2.431	2.724	2.645	1.970	2.225	2.047
H_2O_2	2.701	2.828	2.576	2.185	2.767	2.454	1.999	2.187	1.742	1.675	1.744	1.674
JA	2.804	3.202	2.791	2.416	2.902	2.619	2.003	2.545	2.164	1.831	1.982	1.879
SA	2.753	3.048	2.643	2.268	2.860	2.539	2.019	2.261	1.821	1.682	1.759	1.744
CONTROL	2.511	2.790	2.418	2.107	2.455	2.382	1.639	2.129	1.178	1.367	1.609	1.084
Factors	Inducers (I)		Varieties (V)		Days of sampling (D)		I × V		I × D		V × D	I × V × D
SE(m)	0.028		0.025		0.022		0.057		0.049		0.044	0.098
SE(d)	0.04		0.036		0.031		0.08		0.069		0.062	0.139
C.D.	0.079		0.071		0.061		NS		NS		0.123	0.275

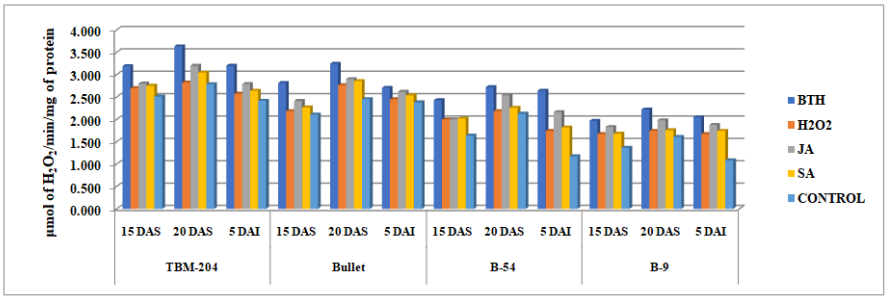

Fig. 1. Effect of inducers at different concentrations on the Catalase activity in the mustard varieties against *A. brassicicola.*

Table 6: Effect of inducers at different concentrations on the ascorbic acid content (mg/100 g FW) in the mustard (TBM-204) against *A. brassicicola*

Sr. No.	Treatments	15 DAS	% increase over control	20 DAS	% increase over control	5 DAI	% increase over control
T1	BTH - L	66.372	47.344	86.411	58.869	96.609	58.751
T2	BTH - M	71.233	50.938	110.008	67.692	114.040	65.056
T3	BTH - H	83.249	58.019	119.613	70.286	125.542	68.258
T4	H_2O_2 - L	50.087	30.224	42.182	15.742	51.233	22.219
T5	H_2O_2 - M	53.842	35.090	48.822	27.202	57.004	30.093
T6	H_2O_2 - H	54.711	36.122	56.727	37.347	68.941	42.197
T7	JA - L	65.186	46.386	72.300	50.842	86.609	53.989
T8	JA - M	68.980	49.335	80.008	55.578	92.063	56.715
T9	JA - H	73.842	52.671	83.328	57.348	97.597	59.169
T10	SA - L	55.542	37.077	61.866	42.550	71.194	44.026
T11	SA - M	59.455	41.218	65.779	45.968	77.202	48.382
T12	SA - H	68.941	49.306	77.202	53.963	87.834	54.631
T13	Control	34.949		35.542		39.850	
	SE m ±	2.280		2.964		3.417	
	CD (P≤0.05)	6.627		8.615		9.932	
	CV %	6.366		7.102		7.220	

DAS: Days after sowing; DAI: Days after pathogen inoculation; L: Low; M: Medium; H: High

Table 7: Effect of inducers at different concentrations on the ascorbic acid content (mg/100 g FW) in the mustard (Bullet) against *A. brassicicola.*

Sr. No.	Treatments	15 DAS	% increase over control	20 DAS	% increase over control	5 DAI	% increase over control
T1	BTH - L	41.391	26.547	52.221	40.039	80.09	57.744
T2	BTH - M	41.945	27.516	56.530	44.609	91.04	62.826
T3	BTH - H	42.221	27.991	63.842	50.953	94.95	64.358
T4	H_2O_2 - L	30.838	1.410	35.163	10.952	46.53	27.268
T5	H_2O_2 - M	32.024	5.060	37.557	16.628	47.83	29.251
T6	H_2O_2 - H	35.186	13.592	39.455	20.637	57.24	40.878
T7	JA - L	40.798	25.480	47.834	34.540	78.11	56.674
T8	JA - M	41.115	26.053	53.842	41.844	79.89	57.639
T9	JA - H	41.352	26.477	58.111	46.116	89.45	62.169
T10	SA - L	34.711	12.412	40.087	21.889	67.56	49.906
T11	SA - M	35.186	13.592	40.285	22.272	68.94	50.912
T12	SA - H	37.834	19.641	40.403	22.500	78.55	56.914
T13	Control	30.403		31.312		33.84	
	SEm±	1.185		1.952		2.21	
	CD (P≤0.05)	3.444		5.674		3.13	
	CV %	5.502		7.368		5.45	

DAS: Days after sowing; DAI: Days after pathogen inoculation; L: Low; M: Medium; H: High

Table 8: Effect of inducers at different concentrations on the ascorbic acid content (mg/100 g FW) in the mustard (B-54) against *A. brassicicola.*

Sr. No.	Treatments	15 DAS	% increase over control	20 DAS	% increase over control	5 DAI	% increase over control
T1	BTH - L	34.672	19.722	38.111	20.224	78.625	60.275
T2	BTH - M	35.282	21.110	38.308	20.636	80.087	61.001
T3	BTH - H	39.076	28.770	43.842	30.653	84.672	63.113
T4	H_2O_2 - L	27.874	0.142	34.175	11.037	42.070	25.759
T5	H_2O_2 - M	29.348	5.158	35.186	13.592	45.107	30.757
T6	H_2O_2 - H	30.324	8.212	37.241	18.361	51.194	38.990
T7	JA - L	32.436	14.188	37.913	19.808	68.545	54.434
T8	JA - M	34.034	18.218	38.071	20.141	72.024	56.635
T9	JA - H	38.150	27.041	41.036	25.910	78.941	60.435
T10	SA - L	30.838	9.741	37.399	18.706	56.648	44.865
T11	SA - M	31.312	11.108	37.439	18.792	57.834	45.995
T12	SA - H	34.063	18.287	37.794	19.557	68.111	54.143
T13	Control	27.834		30.403		31.233	
	SEm±	1.479		1.376		2.652	
	CD (P≤0.05)	4.299		3.998		7.708	
	CV %	7.832		6.362		7.326	

DAS: Days after sowing; DAI: Days after pathogen inoculation; L: Low; M: Medium; H: High

Table 9: Effect of inducers at different concentrations on the ascorbic acid contentmg/100 g FW) in the mustard (B-9) against *A. brassicicola.*

Sr. No.	Treatments	15 DAS	% increase over control	20 DAS	% increase over control	5 DAI	% increase over control
T1	BTH - L	19.356	15.254	33.249	31.740	66.372	62.828
T2	BTH - M	19.474	15.770	34.711	34.616	68.980	64.233
T3	BTH - H	22.194	26.091	35.542	36.143	72.103	65.782
T4	H_2O_2 - L	16.569	1.002	26.648	14.832	38.545	35.993
T5	H_2O_2 - M	17.340	5.402	27.004	15.954	40.087	38.454
T6	H_2O_2 - H	17.399	5.725	30.680	26.024	44.949	45.111
T7	JA - L	19.119	14.203	31.826	28.689	58.111	57.543
T8	JA - M	19.119	14.203	32.696	30.585	61.352	59.786
T9	JA - H	21.141	22.411	33.644	32.542	69.455	64.478
T10	SA - L	18.763	12.576	29.889	24.068	38.071	35.195
T11	SA - M	19.059	13.936	30.324	25.156	41.352	40.336
T12	SA - H	19.059	13.936	31.747	28.511	47.557	48.122
T13	Control	16.403		22.696		24.672	
	SEm±	1.033		1.307		1.945	
	CD (P≤0.05)	3.004		3.800		5.653	
	CV %	9.498		7.347		6.520	

(DAS: Days after sowing; DAI: Days after pathogen inoculation; L: Low; M: Medium; H: High

Table 10: Effect of inducers at different concentrations on the ascorbic acid content (mg/100 g FW) in the mustard varieties against *A. brassicicola.*

Inducers / varieties	TBM-204			Bullet			B-54			B-9		
	15 DAS	20 DAS	5 DAI	15 DAS	20 DAS	5 DAI	15 DAS	20 DAS	5 DAI	15 DAS	20 DAS	5 DAI
BTH	73.618	105.344	112.063	41.852	57.531	88.690	36.343	40.087	81.128	20.341	34.501	69.152
H_2O_2	52.880	49.244	59.059	32.682	37.392	50.535	29.182	35.534	46.123	17.103	28.111	41.194
JA	69.336	78.545	92.090	41.088	53.262	82.485	34.874	39.007	73.170	19.793	32.722	62.972
SA	61.312	68.282	78.743	35.910	40.258	71.681	32.071	37.544	60.864	18.960	30.653	42.327
CONTROL	34.949	35.542	39.850	30.403	31.312	33.842	27.834	30.403	31.233	16.403	22.696	24.672

Factors	Inducers	Varieties	Days of sampling	I × V	I × D	V × D	I × V × D
SE(m)	0.478	0.428	0.37	0.956	0.828	0.741	1.657
SE(d)	0.676	0.605	0.524	1.353	1.171	1.048	2.343
C.D.	1.339	1.198	1.037	2.678	2.319	2.075	4.639

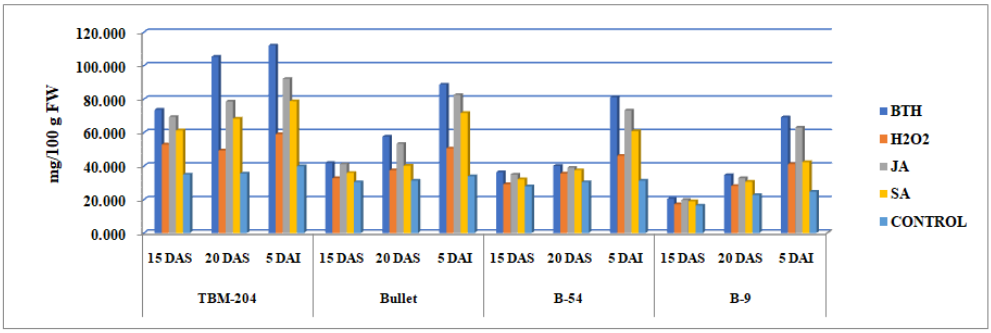

Fig. 2. Effect of inducers at different concentrations on the ascorbic acid content in the mustard varieties against *A. brassicicola.*

Above findings are strongly supported by the report of Mallick *et al*. (2017). Ascorbic acid, acts as powerful antioxidant in tissues and an enhanced level has also been observed in stressed plants as a resistance index against the pathogen (Gupta *et al*., 2012). When the ROS level increases in plants that are exposed to stress, enhanced production of non enzymatic antioxidants in plant cells like ascorbic acid will play a crucial role in minimizing ROS induced oxidative stress (Gill and Tuteja 2010). In this study it is indicated that the less or reduced accumulation of ascorbic acid in B-54 and B-9 varieties favours the oxidative stress and invasion of pathogen deep into the host and finally cause the injury or symptoms in the plant. Necrotrophs appear to stimulate ROS production in the infected tissue to induce cell death that facilitates subsequent infection (Govrin and Levine 2000). This might suggest that the redox state of acorbate could be a defensive response in resistant varieties TBM-204 and Bullet against Alternaria. Moreover, the changes in total concentration and redox state of ascorbic acid can regulate the expression of pathogenesis-related (PR) proteins (Foyer and Noctor 2005), induce the accumulation of phytoalexins (De Gara *et al*., 2003).

CONCLUSIONS

The activation of antioxidant mechanism of the plant, where both, enzymatic antioxidant like catalase and non-enzymatic antioxidant like ascorbic acid were comparatively found to be more in TBM-204 and Bullet through which we can consider them as resistant varieties. The high levels of catalase played an important role in reducing the damage caused by the pathogen by dismutating $O_2^{\bullet-}$ and catalyzing H_2O_2 in resistant in coordination with the non-enzymatic antioxidants. However, it can be assumed that in B-54 and B-9 varieties an uncontrollable production of reactive oxygen species may be coupled with the less potentiality of antioxidant system created the oxidative stress.

Here also noticed that with increase the age of the plant ascorbic acid content was also increased and more focused when the plants were inoculated with pathogen irrespective of inducers and their doses applied in four different cultivars. Here all the inducers increased the ascorbic acid content significantly in comparison to control and maximum in BTH whereas as minimum in H_2O_2.

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