Evaluation of Phenol Metabolic Enzymes in different mustard (*Brassica juncea* L.) cultivars against Alternaria blight (*Alternaria brassicicola*)

**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, 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. Phenols secondary metabolites with responsible key enzymes of metabolism including phenylalanine ammonia lyase (PAL), Polyphenol oxidase (PPO),peroxidase (POD) were found to be more prevalent in the plants treated with BTH followed by Jasmonic acid, Salicylic acid and Hydrogen peroxide. Among the varieties, these enzymes were more prevalent in TBM-204 and Bullet and less prevalent in B-54 and B-9.

**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 0.7 M 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 oil seed 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, 2013-14). 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 brassicas 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/elicitors.

**Material and Methods**

**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 0C 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 ± 20C 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.

**Preparation of inoculum spray**

Four mustard varieties, *viz*., TBM-204, Bullet, B-54 and B-9 were collected from university instructional farm. The plants of these varieties were raised in plastic pots (13 cm × 13 cm) containing 3 kg soil (sandy loam 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 Petri plates 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 ×104 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).

**Standardization of inducer concentrations**

Benzothiadiazole (BTH) [S-methylbenzo-1, 2, 3-thiadiazole-7-carbothiate], hydrogen peroxide (H2O2), 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, H2O2 @ 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.

**Preparation of phosphate buffer 0.1 M (pH 7.5):** Dissolved 2.82 g of Na2HPO4.2H2O (solution A) and 3.1 g of NaH2PO4.2H2O (solution B) in 100 ml of distilled water separately which makes 0.2 M. Then added 87 ml of solution A and 13 ml of solution B and made the volume to 200 ml which makes 0.1 M.

**Preparation of extraction buffer**: Added 2 g of poly vinyl polypyrrolidone (PVP) and 0.25 ml of Triton X in 100 ml of phosphate buffer. Extraction buffer was prepared freshly before enzyme extraction.

**Preparation of enzyme extract:** For determination of phenol metabolism enzyme activities, extraction procedure was prepared according to Nayyar and Gupta (2006) with some modifications. Each sample (0.3 g) was homogenized with 10 ml of extraction buffer. The homogenate was centrifuged at 10,000 rpm for 30 min and supernatant was collected for enzymes assays.

**Assay of Phenol Metabolism Enzymes**

**Peroxidase (POD) assay**

Peroxidase activity, determined using the guaicol oxidation method by Lin and Kao (2001). The reaction mixture contained 0.15 ml of 4% (v/v) guaiacol, 2.65 ml of 0.1 M potassium phosphate buffer (pH 7.0) and 0.05 ml enzyme extract. The reaction was initiated by the addition of 0.15 ml of 1% H2O2. Increase in absorbance at 470 nm was recorded in 30 sec interval upto three minutes using spectrophotometer. The POD activity was calculated by using an absorption coefficient (26.6 M-1cm-1) at 470 nm for the tetraguaiacol. Enzyme activity was expressed as μ moles of guaicol oxidized/min/mg of protein.

**Polyphenol oxidase (PPO) assay**

For determination of Polyphenol oxidase (PPO) activity modified method of Siriphanich and Kader (1985) was followed. Three ml of reaction mixture contained 1 ml of catechol (0.025 M) and 1.95 ml of 0.1 M potassium phosphate buffer pH 7.0. Reaction was started by adding 0.05 ml of enzyme extract to the reaction mixture and PPO activity was presented as the μ moles of catechol degraded/min/mg of protein.

**Phenylalanine ammonia-lyase (PAL) assay**

Activity was assayed by using a method, modified from the method of Godwin *et al*. (1996). The reaction mixture contained 1.4 ml of 0.1 M Tris-HCl buffer (pH 8.8), 1 ml of 10 mM L-phenylalanine (prepared in 100 mM Tris- HCl buffer pH 8.8) and 0.5 ml enzyme extract. The reaction mixture was then measured at 250 nm for every 15 min till 1.5 h. One unit represents the amount of enzyme that produces 1 μmol of cinnamic acid (extinction coefficient = 9630 M cm) per hour (Unit/gFW). The unit of enzyme was expressed as μ moles of cinnamic acid produced/min/mg protein.

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.

**Peroxidase (POD)**

The peroxidase activity was significantly increased over the control and it was also more prominent with the increase the doses of inducers irrespective of different varieties and days after sampling,

It was observed that, the peroxidase activity increased significantly with the age of the plant and with the concentration of the inducers over the control. POD activity at 15 DAS was comparatively lower than at 20 DAS and 5 DAI in all the four varieties tested. The range of POD in TBM-204 at 15 DAS was from 0.112-0.218 μmol/mg of protein whereas it was 0.126-0.195, 0.129-0.176 and 0.114-0.175 μmol/mg of protein in Bullet, B-54 and B-9 respectively. It was observed that POD activity triggered to higher levels after pathogen inoculation is due to more stress by forming more oxide radicals. The range of POD in TBM-204 at 5 DAI was from 0.542-0.996 μmol/mg of protein whereas it was 0.570-0.887, 0.347-0.478 and 0.342-0.386 μmol/mg of protein in Bullet, B-54 and B-58 respectively, in which the variety with highest POD activity can be considered to have higher resistance against *Alternaria* (Table 1, 2, 3 and 4).

The peroxidase activity was significantly increased with increased the days after sowing and significantly decreased when the plants were inoculated with pathogen *Alternaria* in the same 20 DAS (5 DAI). The maximum peroxidase activity was noticed in 20 DAS irrespective of inducers and their doses and reduced at 5 DAI with same 20 DAS (Days after sowing).

It was observed in all the four varieties and their differences were statistically significant. Amongthe inducers BTH showed maximum Peroxidase activity followed by Jasmonic acid and lowest in H2O2 and their differences were statistically significant (Table 4:15). Among the four varieties maximum Peroxidase activity was noticed in TBM-204 followed by Bullet and lowest in B-9 and their differences were statistically significant. Among the three days after sampling maximum Peroxidase activity was noticed in 20 DAS with 5 DAI followed by 20 DAS without inoculation with Pathogen and lowest in 15 DAS and their differences were also statistically significant. Interaction between Inducers and varieties showed significant differences among themselves.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **Treatments** | **15 DAS** | **% increase over control** | **20 DAS** | **% increase over control** | **5 DAI** | **% increase over control** |
| **T1** | **BTH - L** | 0.184 | 39.130 | 0.238 | 47.479 | 0.789 | 31.305 |
| **T2** | **BTH - M** | 0.187 | 40.107 | 0.267 | 53.184 | 0.881 | 38.479 |
| **T3** | **BTH - H** | 0.218 | 48.624 | 0.355 | 64.789 | 0.966 | 43.892 |
| **T4** | **H2O2 - L** | 0.129 | 13.178 | 0.137 | 8.759 | 0.546 | 0.733 |
| **T5** | **H2O2 - M** | 0.139 | 19.424 | 0.16 | 21.875 | 0.586 | 7.509 |
| **T6** | **H2O2 - H** | 0.142 | 21.127 | 0.164 | 23.780 | 0.586 | 7.509 |
| **T7** | **JA - L** | 0.17 | 34.118 | 0.175 | 28.571 | 0.631 | 14.105 |
| **T8** | **JA - M** | 0.181 | 38.122 | 0.194 | 35.567 | 0.65 | 16.615 |
| **T9** | **JA - H** | 0.197 | 43.147 | 0.234 | 46.581 | 0.693 | 21.789 |
| **T10** | **SA - L** | 0.161 | 30.435 | 0.164 | 23.780 | 0.614 | 11.726 |
| **T11** | **SA - M** | 0.167 | 32.934 | 0.169 | 26.036 | 0.618 | 12.298 |
| **T12** | **SA - H** | 0.17 | 34.118 | 0.178 | 29.775 | 0.628 | 13.694 |
| **T13** | **Control** | 0.112 |  | 0.125 |  | 0.542 |  |
|  | **SEm±** | 0.007 |  | 0.01 |  | 0.028 |  |
|  | **CD (P≤0.05)** | 0.022 |  | 0.029 |  | 0.081 |  |
|  | **CV %** | 7.771 |  | 8.663 |  | 7.154 |  |

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

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 Peroxidase activity (μmol/mg of protein) in the mustard (Bullet) against *A. brassicicola***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **Treatments** | **15 DAS** | **% increase over control** | **20 DAS** | **% increase over control** | **5 DAI** | **% increase over control** |
| **T1** | **BTH - L** | 0.168 | 25.000 | 0.192 | 30.729 | 0.673 | 15.305 |
| **T2** | **BTH - M** | 0.173 | 27.168 | 0.195 | 31.795 | 0.721 | 20.943 |
| **T3** | **BTH - H** | 0.195 | 35.385 | 0.195 | 31.795 | 0.887 | 35.738 |
| **T4** | **H2O2 - L** | 0.127 | 0.787 | 0.135 | 1.481 | 0.572 | 0.350 |
| **T5** | **H2O2 - M** | 0.133 | 5.263 | 0.14 | 5.000 | 0.574 | 0.697 |
| **T6** | **H2O2 - H** | 0.137 | 8.029 | 0.142 | 6.338 | 0.58 | 1.724 |
| **T7** | **JA - L** | 0.162 | 22.222 | 0.173 | 23.121 | 0.618 | 7.767 |
| **T8** | **JA - M** | 0.163 | 22.699 | 0.179 | 25.698 | 0.657 | 13.242 |
| **T9** | **JA - H** | 0.167 | 24.551 | 0.183 | 27.322 | 0.662 | 13.897 |
| **T10** | **SA - L** | 0.143 | 11.888 | 0.153 | 13.072 | 0.579 | 1.554 |
| **T11** | **SA - M** | 0.144 | 12.500 | 0.158 | 15.823 | 0.616 | 7.468 |
| **T12** | **SA - H** | 0.151 | 16.556 | 0.159 | 16.352 | 0.617 | 7.618 |
| **T13** | **Control** | 0.126 |  | 0.133 |  | 0.57 |  |
|  | **SEm±** | 0.008 |  | 0.007 |  | 0.02 |  |
|  | **CD (P≤0.05)** | 0.025 |  | 0.02 |  | 0.057 |  |
|  | **CV %** | 9.60 |  | 7.09 |  | 5.344 |  |

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 Peroxidase activity (μmol/mg of protein) in the mustard (B-54) against *A. brassicicola***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **Treatments** | **15 DAS** | **% increase over control** | **20 DAS** | **% increase over control** | **5 DAI** | **% increase over control** |
| **T1** | **BTH - L** | 0.161 | 27.240 | 0.176 | 36.932 | 0.439 | 20.957 |
| **T2** | **BTH - M** | 0.170 | 31.338 | 0.181 | 38.674 | 0.463 | 25.054 |
| **T3** | **BTH - H** | 0.176 | 33.431 | 0.195 | 43.077 | 0.478 | 27.406 |
| **T4** | **H2O2 - L** | 0.124 | 5.877 | 0.131 | 15.267 | 0.353 | 1.700 |
| **T5** | **H2O2 - M** | 0.129 | 9.405 | 0.132 | 15.909 | 0.356 | 2.528 |
| **T6** | **H2O2 - H** | 0.135 | 13.585 | 0.144 | 22.917 | 0.357 | 2.801 |
| **T7** | **JA - L** | 0.150 | 22.087 | 0.151 | 26.490 | 0.38 | 8.684 |
| **T8** | **JA - M** | 0.151 | 22.371 | 0.157 | 29.299 | 0.387 | 10.336 |
| **T9** | **JA - H** | 0.161 | 27.353 | 0.162 | 31.481 | 0.41 | 15.366 |
| **T10** | **SA - L** | 0.136 | 14.126 | 0.148 | 25.000 | 0.373 | 6.971 |
| **T11** | **SA - M** | 0.144 | 18.633 | 0.151 | 26.490 | 0.373 | 6.971 |
| **T12** | **SA - H** | 0.145 | 19.469 | 0.151 | 26.490 | 0.378 | 8.201 |
| **T13** | **Control** | 0.117 |  | 0.111 |  | 0.347 |  |
|  | **SEm±** | 0.007 |  | 0.006 |  | 0.017 |  |
|  | **CD (P≤0.05)** | 0.021 |  | 0.017 |  | 0.049 |  |
|  | **CV %** | 8.769 |  | 6.651 |  | 7.417 |  |

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 Peroxidase activity (μmol/mg of protein) in the mustard (B-9) against *A. brassicicola***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **Treatments** | **15 DAS** | **% increase over control** | **20 DAS** | **% increase over control** | **5 DAI** | **% increase over control** |
| **T1** | **BTH - L** | 0.145 | 21.379 | 0.162 | 25.309 | 0.377 | 9.284 |
| **T2** | **BTH - M** | 0.163 | 30.061 | 0.173 | 30.058 | 0.386 | 11.399 |
| **T3** | **BTH - H** | 0.175 | 34.857 | 0.183 | 33.880 | 0.383 | 10.705 |
| **T4** | **H2O2 - L** | 0.114 | 0.000 | 0.123 | 1.626 | 0.345 | 0.870 |
| **T5** | **H2O2 - M** | 0.119 | 4.202 | 0.128 | 5.469 | 0.352 | 2.841 |
| **T6** | **H2O2 - H** | 0.121 | 5.785 | 0.134 | 9.701 | 0.36 | 5.000 |
| **T7** | **JA - L** | 0.130 | 12.308 | 0.144 | 15.972 | 0.368 | 7.065 |
| **T8** | **JA - M** | 0.135 | 15.556 | 0.147 | 17.687 | 0.369 | 7.317 |
| **T9** | **JA - H** | 0.143 | 20.280 | 0.159 | 23.899 | 0.37 | 7.568 |
| **T10** | **SA - L** | 0.122 | 6.557 | 0.135 | 10.370 | 0.36 | 5.000 |
| **T11** | **SA - M** | 0.122 | 6.557 | 0.139 | 12.950 | 0.362 | 5.525 |
| **T12** | **SA - H** | 0.129 | 11.628 | 0.143 | 15.385 | 0.368 | 7.065 |
| **T13** | **Control** | 0.114 |  | 0.121 |  | 0.342 |  |
|  | **SEm±** | 0.004 |  | 0.007 |  | 0.013 |  |
|  | **CD (P≤0.05)** | 0.012 |  | 0.021 |  | 0.039 |  |
|  | **CV %** | 5.282 |  | 8.53 |  | 6.294 |  |

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 Peroxidase activity (μmol/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** | 0.196 | 0.286 | 0.879 | 0.179 | 0.194 | 0.760 | 0.169 | 0.184 | 0.460 | 0.161 | 0.173 | 0.382 |
| **H2O2** | 0.137 | 0.153 | 0.573 | 0.132 | 0.139 | 0.575 | 0.125 | 0.136 | 0.355 | 0.118 | 0.128 | 0.353 |
| **JA** | 0.183 | 0.201 | 0.658 | 0.164 | 0.178 | 0.646 | 0.154 | 0.157 | 0.392 | 0.136 | 0.150 | 0.369 |
| **SA** | 0.166 | 0.170 | 0.620 | 0.146 | 0.157 | 0.604 | 0.142 | 0.150 | 0.374 | 0.124 | 0.139 | 0.363 |
| **CONTROL** | 0.112 | 0.125 | 0.542 | 0.126 | 0.133 | 0.570 | 0.129 | 0.111 | 0.347 | 0.114 | 0.121 | 0.342 |
| **Factors** | **Inducers** | | **Varieties** | | **Days of sampling** | | **I X V** | | **I X D** | | **V X D** | **I X V X D** |
| **SE(m)** | 0.002 | | 0.002 | | 0.002 | | 0.005 | | 0.004 | | 0.04 | 0.008 |
| **SE(d)** | 0.003 | | 0.003 | | 0.003 | | 0.007 | | 0.006 | | 0.005 | 0.012 |
| **C.D.** | 0.007 | | 0.006 | | 0.005 | | 0.014 | | 0.012 | | 0.011 | 0.023 |

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

Similarly Interaction between induces and days after sampling also showed significant differences among themselves. It indicated that with increase in days after sampling the inducers and their doses were also significantly decreased, though to some extent Peroxidase activity was increased in pathogen inoculated plant. The interaction between varieties and days after sampling was also statistically significant. Interaction of inducers, varieties and days after sampling were statistically significant with regards to peroxides activity.

Inducers tested at all concentrations significantly increased the POD activity compared to control in all the varieties. Significantly high POD activity was found in BTH followed by JA, SA and least activity was found in H2O2. With the increase in concentration, the POD activity and the percent increase over control was also increased.

Interaction between inducers and varieties revealed that POD activity was observed to be high in infected leaves as compared to the healthy one and the varieties TBM-204 and Bullet expressed high POD activity than the varieties B-54 and B-9 (Table 5, Fig 1).

Peroxidase is a member of the PR-9 family involving in the lignin formation in the host plant as a defense response. POD activity is associated with disease resistance in plants (Lin and Kao, 2001) and increased by pathogen infection in host plants (Zheng *et al*., 2004). POD has been reported to catalyze the last step in the biosynthesis of lignin and hydrogen peroxide (Breusegem *et al*., 2001).

The present investigation is in confirmatory with the investigation of Saharan *et al.* (2000), who reported increased peroxidase activity in response to Alternaria blight infection in both resistant and susceptible cluster bean varieties. Higher levels of peroxidase activity resulted exclusively from fungal infection as reported by Rosta’s *et al.* (2002) in *A. brassicae* infected Chinese cabbage. Increases in peroxidase activity could be correlated with infection in plants, polymerization of cinnamyl alcohols to lignin is catabolised by peroxidase lignification leading to disease resistance.

**Polyphenol oxidase (PPO)**

The polyphenol oxidase activity was increased in different concentration in four different varieties due to treatment with different inducers and days after sampling and their differences were statistically significant. With the increase in concentration of the inducers there was a significant increase in PPO activity and it was different in different varieties irrespective of inducers at different days after sampling.

It was observed that, the polyphenol oxidase activity increased significantly with the age of the plant and with the concentration of the inducers over the control. PPO activity at 15 DAS was comparatively lower than at 20 DAS and 5 DAI in all the four varieties tested. The range of PPO in TBM-204 at 15 DAS was from 2.307-3.699 m mol of catechol oxidised/g/min/mg protein whereas it was 2.2*7*3-3.424, 1.378-3.291 and 1.202-3.118 m mol of catechol oxidised/g/min/mg protein in Bullet, B-54 and B-9 respectively. It was observed that PPO activity triggered to higher levels after pathogen inoculation is due to more stress by forming more oxide radicals. The range of PPO in TBM-204 at 5 DAI was from 3.419-4.830 m mol of catechol oxidised/*g/*min/mg protein whereas it was 3.138-4.724, 3.134-4.189 and 2.808-4.159 m mol of catechol oxidised/g/min/mg protein in Bullet, B-54 and B-58 respectively, in which the variety with highest PPO activity can be considered to have higher resistance against Alternaria (Table 6, 7, 8 and 9).

The effect of inducers irrespective of doses, in four different varieties, the PPO activity was different at different days after sampling and their differences were statistically significant.

The concentration of PPO activity by different inducers was different and their differences were statistically significant. Maximum PPO activity was noticed in inducer BTH treatment followed by jasmonic acid and minimum in H2O2 irrespective of varieties used.

The concentration of PPO activity among the varieties irrespective of inducers used was observed that PPO activity was increased in BTM-204 followed by Bullet and minimum in B-9 and B-54 and their differences were statistically significant.

Days of sampling were statistically significant among themselves and with increase in age or days of sampling the Polyphenol activity was increased and maximum in 20 days after sampling and minimum in 15 days after sampling, whereas different reactions were noticed in different varieties. In TBM-204, with increase in days of sampling there was increase in PPO activity, though with pathogen inoculation proactively was reduced with same days of planting.

**Table 6 Effect of inducers at different concentrations on the PPO activity (m mol of catechol oxidised/g/min/mg protein) in the mustard (TBM-204) against *A. brassicicola***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **Treatments** | **15 DAS** | **% increase over control** | **20 DAS** | **% increase over control** | **5 DAI** | **% increase over control** |
| **T1** | **BTH - L** | 3.357 | 31.269 | 4.854 | 25.955 | 4.753 | 28.063 |
| **T2** | **BTH - M** | 3.562 | 35.232 | 4.910 | 26.807 | 4.823 | 29.107 |
| **T3** | **BTH - H** | 3.699 | 37.638 | 5.238 | 31.393 | 4.830 | 29.212 |
| **T4** | **H2O2 - L** | 2.738 | 15.731 | 3.790 | 5.183 | 3.942 | 13.264 |
| **T5** | **H2O2 - M** | 2.942 | 21.575 | 3.667 | 1.983 | 3.960 | 13.673 |
| **T6** | **H2O2 - H** | 3.077 | 25.025 | 3.806 | 5.562 | 3.849 | 11.177 |
| **T7** | **JA - L** | 3.204 | 27.987 | 4.511 | 20.328 | 4.255 | 19.658 |
| **T8** | **JA - M** | 3.215 | 28.249 | 4.531 | 20.677 | 4.349 | 21.390 |
| **T9** | **JA - H** | 3.282 | 29.714 | 4.798 | 25.098 | 4.416 | 22.588 |
| **T10** | **SA - L** | 3.087 | 25.275 | 4.307 | 16.559 | 4.096 | 16.527 |
| **T11** | **SA - M** | 3.106 | 25.725 | 4.480 | 19.781 | 4.186 | 18.326 |
| **T12** | **SA - H** | 3.132 | 26.331 | 4.508 | 20.275 | 4.233 | 19.235 |
| **T13** | **Control** | 2.307 |  | 3.594 |  | 3.419 |  |
|  | **SEm±** | 0.112 |  | 0.158 |  | 0.203 |  |
|  | **CD (P≤0.05)** | 0.324 |  | 0.458 |  | 0.589 |  |
|  | **CV %** | 6.171 |  | 6.226 |  | 8.274 |  |

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 PPO activity (m mol of catechol oxidised/g/min/mg protein) in the mustard (Bullet) against *A. Brassicicola***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **Treatments** | **15 DAS** | **% increase over control** | **20 DAS** | **% increase over control** | **5 DAI** | **% increase over control** |
| **T1** | **BTH - L** | 3.486 | 34.788 | 4.259 | 37.434 | 4.491 | 30.133 |
| **T2** | **BTH - M** | 3.406 | 33.247 | 4.792 | 44.393 | 4.531 | 30.749 |
| **T3** | **BTH - H** | 3.424 | 33.595 | 5.063 | 47.369 | 4.724 | 33.580 |
| **T4** | **H2O2 - L** | 2.469 | 7.931 | 3.636 | 26.728 | 3.750 | 16.320 |
| **T5** | **H2O2 - M** | 2.810 | 19.088 | 3.145 | 15.266 | 3.821 | 17.887 |
| **T6** | **H2O2 - H** | 2.912 | 21.916 | 3.406 | 21.769 | 3.827 | 18.005 |
| **T7** | **JA - L** | 3.114 | 27.000 | 4.001 | 33.410 | 4.037 | 22.275 |
| **T8** | **JA - M** | 3.153 | 27.898 | 4.041 | 34.071 | 4.162 | 24.616 |
| **T9** | **JA - H** | 3.274 | 30.565 | 4.251 | 37.325 | 4.336 | 27.642 |
| **T10** | **SA - L** | 2.999 | 24.201 | 3.857 | 30.916 | 3.863 | 18.770 |
| **T11** | **SA - M** | 3.057 | 25.631 | 3.904 | 31.754 | 3.971 | 20.985 |
| **T12** | **SA - H** | 3.073 | 26.026 | 3.915 | 31.943 | 3.984 | 21.242 |
| **T13** | **Control** | 2.273 |  | 2.665 |  | 3.138 |  |
|  | **SEm±** | 0.174 |  | 0.193 |  | 0.176 |  |
|  | **CD (P≤0.05)** | 0.506 |  | 0.561 |  | 0.511 |  |
|  | **CV %** | 9.939 |  | 8.526 |  | 7.517 |  |

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 PPO activity (m mol of catechol oxidised/g/min/mg protein) in the mustard (B-54) against *A. brassicicola***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **Treatments** | **15 DAS** | **% increase over control** | **20 DAS** | **% increase over control** | **5 DAI** | **% increase over control** |
| **T1** | **BTH - L** | 2.807 | 50.897 | 3.766 | 31.898 | 4.068 | 22.957 |
| **T2** | **BTH - M** | 2.902 | 52.499 | 3.817 | 32.808 | 4.112 | 23.783 |
| **T3** | **BTH - H** | 3.291 | 58.120 | 3.863 | 33.612 | 4.189 | 25.183 |
| **T4** | **H2O2 - L** | 1.561 | 11.683 | 2.897 | 11.470 | 3.465 | 9.565 |
| **T5** | **H2O2 - M** | 1.783 | 22.710 | 3.025 | 15.210 | 3.552 | 11.771 |
| **T6** | **H2O2 - H** | 2.094 | 34.187 | 3.310 | 22.521 | 3.576 | 12.363 |
| **T7** | **JA - L** | 2.565 | 46.264 | 3.538 | 27.516 | 3.921 | 20.066 |
| **T8** | **JA - M** | 2.604 | 47.069 | 3.694 | 30.568 | 3.969 | 21.029 |
| **T9** | **JA - H** | 2.760 | 50.066 | 3.751 | 31.639 | 4.058 | 22.765 |
| **T10** | **SA - L** | 2.179 | 36.754 | 3.323 | 22.820 | 3.734 | 16.078 |
| **T11** | **SA - M** | 2.321 | 40.623 | 3.449 | 25.645 | 3.821 | 17.975 |
| **T12** | **SA - H** | 2.535 | 45.621 | 3.474 | 26.173 | 3.893 | 19.492 |
| **T13** | **Control** | 1.378 |  | 2.565 |  | 3.134 |  |
|  | **SEm±** | 0.134 |  | 0.174 |  | 0.189 |  |
|  | **CD (P≤0.05)** | 0.390 |  | 0.506 |  | 0.550 |  |
|  | **CV %** | 9.815 |  | 8.820 |  | 8.610 |  |

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

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **Treatments** | **15 DAS** | **% increase over control** | **20 DAS** | **% increase over control** | **5 DAI** | **% increase over control** |
| **T1** | **BTH - L** | 2.503 | 51.971 | 3.210 | 32.338 | 3.888 | 27.777 |
| **T2** | **BTH - M** | 2.553 | 52.905 | 3.270 | 33.585 | 3.971 | 29.293 |
| **T3** | **BTH - H** | 3.118 | 61.439 | 3.419 | 36.478 | 4.159 | 32.481 |
| **T4** | **H2O2 - L** | 1.223 | 1.690 | 2.414 | 10.029 | 3.403 | 17.480 |
| **T5** | **H2O2 - M** | 1.237 | 2.829 | 2.461 | 11.745 | 3.476 | 19.227 |
| **T6** | **H2O2 - H** | 1.313 | 8.429 | 2.626 | 17.273 | 3.509 | 19.990 |
| **T7** | **JA - L** | 2.021 | 40.498 | 3.024 | 28.173 | 3.602 | 22.054 |
| **T8** | **JA - M** | 2.206 | 45.489 | 3.107 | 30.091 | 3.677 | 23.635 |
| **T9** | **JA - H** | 2.483 | 51.571 | 3.208 | 32.295 | 3.702 | 24.152 |
| **T10** | **SA - L** | 1.411 | 14.789 | 2.843 | 23.596 | 3.521 | 20.248 |
| **T11** | **SA - M** | 1.654 | 27.322 | 2.829 | 23.236 | 3.552 | 20.942 |
| **T12** | **SA - H** | 1.881 | 36.080 | 2.999 | 27.570 | 3.559 | 21.095 |
| **T13** | **Control** | 1.202 |  | 2.172 |  | 2.808 |  |
|  | **SEm±** | 0.107 |  | 0.144 |  | 0.193 |  |
|  | **CD (P≤0.05)** | 0.310 |  | 0.419 |  | 0.560 |  |
|  | **CV %** | 9.683 |  | 8.632 |  | 9.264 |  |

**Table 9 Effect of inducers at different concentrations on the PPO activity (m mol of catechol oxidised/g/min/mg protein) in the mustard (B-9) against *A. brassicicola***

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

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **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** | 3.539 | 5.001 | 4.802 | 3.439 | 4.704 | 4.582 | 3.000 | 3.815 | 4.123 | 2.725 | 3.300 | 4.006 |
| **H2O2** | 2.919 | 3.754 | 3.917 | 2.730 | 3.396 | 3.799 | 1.813 | 3.077 | 3.531 | 1.258 | 2.500 | 3.463 |
| **JA** | 3.234 | 4.613 | 4.340 | 3.181 | 4.098 | 4.179 | 2.643 | 3.661 | 3.982 | 2.236 | 3.113 | 3.660 |
| **SA** | 3.108 | 4.432 | 4.172 | 3.043 | 3.892 | 3.939 | 2.345 | 3.415 | 3.816 | 1.649 | 2.890 | 3.544 |
| **CONTROL** | 2.307 | 3.594 | 3.419 | 2.273 | 2.665 | 3.138 | 1.378 | 2.565 | 3.134 | 1.202 | 2.172 | 2.808 |
| **Factors** | **Inducers (I)** | | **Varieties (V)** | | **Days of sampling (D)** | | **I X V** | | **I X D** | | **V X D** | **I X V X D** |
| **SE(m)** | 0.037 | | 0.033 | | 0.029 | | 0.075 | | 0.065 | | 0.058 | 0.129 |
| **SE(d)** | 0.053 | | 0.047 | | 0.041 | | 0.105 | | 0.091 | | 0.082 | 0.183 |
| **C.D.** | 0.104 | | 0.093 | | 0.081 | | NS | | NS | | 0.162 | NS |

**Table 10 Effect of inducers at different concentrations on the PPO activity (m mol of catechol oxidised/g/min/mg protein) in the mustard varieties against *A. brassicicola***

**Fig 2 Effect of inducers at different concentrations on the PPO activity in the mustard varieties against *A. brassicicola***

Whereas in B-54 and B-9 the PPO activity was increased in pathogen inoculated plant at same days of sampling (20 DAS). Interaction between inducers and varieties was statistically significant with regards to PPO activity. It indicated that PPO activity was maximum in BTH treatment in TBM-204 variety and minimum in H2O2 treatment in B-9 variety. Interaction between inducers and days after sampling showed that treatment with BTH and sampling in 20 days after sowing without pathogen inoculation produced maximum PPO activity in comparison to others and their differences were statistically significant. Interaction between varieties and days after sowing showed that in BTH treatment in TBM-204 produced maximum PPO activity (5.00 m mol of catechol oxidized/g/min/mg protein) in 20 Days after sowing without pathogen inoculation and minimum (1.258 m mol of catechol oxidized/g/min/mg protein) in 20 days after sowing with pathogen inoculation in B-9 variety and their difference was statistically significant. Interaction among inducers, varieties and days after sowing were also statistically significant among themselves with regards to PPO activity.

Poly phenol oxidase (PPO) is involved in the oxidation of Poly phenols in the quinines (antimicrobial compounds) and signification of plant cell during microbial invasion. A number of studies indicated that phenol oxidizing enzymes may participate in the responding defense reaction and hypersensitivity by inducing plant resistance against fungi. These include common Potato – *Fusarium sumbucinum* (Ray *et al.,* 1998) and wheat - *F. graminearum* (Mohammadi and Kazami, 2002). The induction pattern of some defense related enzymes and phenolics in roots and shoots of two different genotypes of chickpea cultivar, which were susceptible (L550), and resistant (ICCV10) to wilt disease was observed after treating the plants with salicyclic acid (SA), Spermine (Spm), SA + Spm and *Fusarium oxysporum* f.sp. *ciceri*. Where higher levels of PPO, PAL, P-1,3, glucanase (PR-2) and phenolics were found in roots and shoots of resistant cultivar than that of susceptible cultivar on treatment with elicitors and pathogen (Raju *et al.,* 2008).

Finding of Zheng *et al.* (2005) noticed that the activities of enzymes, responsible for significations in pepper showed differential pattern of expression when inoculated with the pathogen. *Phytophthora capsicii* PPO and PAL activities gradually increased during the 1st three days infection and drastically decreased on 9th day of infection in susceptible variety. However in resistant variety, temporal increase in these enzymatic activities was found.

With the increase in concentration of inducers and plant age, the PPO activity was increased in all the genotypes (Table 10, Fig 2). In the present investigation PPO activity was observed to be higher in infected leaves when compared to the healthy one and the resistant genotype expressed more PPO activity than the susceptible one.

Niranjanraj and Sarosh (2006) and Surendra *et al*. (2012) observed similar results that seedlings of resistant varieties had greater PPO activity than susceptible seedlings and the activity of the enzyme increased with increase in concentration. Similarly the higher PPO activity was recorded in resistant cultivars of pearl millet tissues and pear fruits infected with downy mildew fungus and *Erwinia amylovora* pathogen respectively (Shetty *et al.,* 2001; Honty *et al.,* 2005). The increase levels of PPO induction upon infection could be enhanced the oxidation of phenolic compounds into the more toxic forms, quinones, against pathogen (Tyagi *et al.,* 2000).

**Phenylalanine ammonia lyase (PAL)**

The PAL activity was increased significantly with increasing age of the plant. It indicated that in every variety PAL activity was maximum in 20 DAS and more pronounced in pathogen inoculated plant (5 DAI at same 20 DAS), irrespective of inducers and its doses.

In every inducers highest dose showed maximum PAL activity in pathogen inoculated plant followed by without pathogen inoculation with same age of plants, among the inducers maximum PAL activity was noticed in BTH followed by jasmonic Acid and lowest in H2O2, treated plants. Among the varieties PAL activity was maximum in TBM-204 followed by Bullet and minimum in B-54 followed by B-9 irrespective of inducers and their doses (Table 15).

Interaction between inducers and varieties were also statistically significant in relation to PAL activity. It indicated that PAL activity was maximum in TBM-204 in BTH treatment and minimum in B-54 in H2O2 treatment. Interaction between inducers and days after sampling was statistically significant and is indicated that with increase in age of plant PAL activity was increased in BTH 20 DAS irrespective of varieties and more in pathogen inoculated plants. Interaction between varieties and days after sampling was also statistically significant and it indicated that PAL activity was maximum in TBM-204 at 20 DAS and more in 5 DAI of pathogen at same age of plant. The interaction among inducers X varieties X days after sampling were statistically significant and it indicated that PAL activity was significantly increased at BTH treated of TBM-204 at 20 DAS and more in 5 DAI of pathogen at same age and minimum in H2O2 treated B-54 seeds at 15 DAS (Table 15).

PAL activity was increased significantly with increase in the concentration of inducers and with the age of the plant over un-inoculated control. Increase in the PAL activity was high in the treatment BTH at high concentration and was low in the H2O2 at low concentration in all the varieties tested and in all the days of sampling. Varieties TBM-204 and Bullet showed maximum activity of PAL ranged from (3.58-10.707 and 3.54-9.57 µg of cinnamic acid/min/mg protein respectively) under *Alternaria* blight infection were expected to have high resistance against *Alternaria*, while varieties B-54 and B-9 have shown comparatively low activity of PAL that ranged from (3.17-9.28 and 2.65-8.12 µg of cinnamic acid/min/mg protein respectively) were considered to have low resistance against *Alternaria* blight (Table 11, 12, 13 and 14).

Among the inducers BTH, jasmonic acid followed by salicylic acid and H2O2 showed high amount of PAL content. Is has also been observed that increase in PAL activity was still higher in inoculated when compared to uninoculated treatments. The range of PAL activity in TBM-204 variety at 15 DAS was from 3.58-7.49 µg of cinnamic acid/min/mg protein whereas, it was 4.89-10.57 and 5.860-10.707 µg of cinnamic acid/min/mg protein at 20 DAS and 5 DAI respectively. Similarly, the same trend was followed in Bullet, B-54 and B-9 varieties also. Interaction between inducers and varieties revealed that PAL activity was observed to be high in infected leaves as compared to the healthy one and the varieties TBM-204 and Bullet expressed high PAL activity than the varieties B-54 and B-9 (Table 15, Fig 3).

Our results in treatment with elicitors are in agreement with Raju *et al*. (2008) who reported the more increase in the rate of activity of PAL in the resistant and less increase in the rate of activity in the susceptible cultivar. Increase in the PAL activity in rice seedling after SA spray has also been reported (Cai and Zheng, 1997). An increase in PAL activity results in increase in concentration of phenolic compounds, which are substrates for oxidative enzymes such as polyphenol oxidase and peroxidase. PAL catalyzed first reaction of phenylproponoid pathway, phenylalanine to t-cinannamic acid, which results in accumulation of phenolics and other antimicrobial compounds (Slatnar *et al.,* 2010). Similar observations were recorded in previous research, during the plant development, cell differentiation, stress conditions such as irradiation, wounding, nutrient deficiencies, herbicide treatment and viral, fungal and insect attacks (Longemann *et al*., 2000; Morello *et al.,* 2005; Parihar *et al*., 2012; Surendra *et al*., 2012).

**Table 11 Effect of inducers at different concentrations on the PAL activity (µg of cinnamic acid/min/mg protein) in the mustard (TBM-204) against *A. brassicicola***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **Treatments** | **15 DAS** | **% increase over control** | **20 DAS** | **% increase over control** | **5 DAI** | **% increase over control** |
| **T1** | **BTH - L** | 6.55 | 45.344 | 9.08 | 46.145 | 9.49 | 38.251 |
| **T2** | **BTH - M** | 6.87 | 47.889 | 10.30 | 52.524 | 10.431 | 43.821 |
| **T3** | **BTH - H** | 7.49 | 52.203 | 10.57 | 53.737 | 10.707 | 45.269 |
| **T4** | **H2O2 - L** | 4.47 | 19.911 | 7.09 | 31.030 | 7.271 | 19.406 |
| **T5** | **H2O2 - M** | 4.77 | 24.948 | 7.54 | 35.146 | 7.737 | 24.260 |
| **T6** | **H2O2 - H** | 5.06 | 29.249 | 7.71 | 36.576 | 7.883 | 25.663 |
| **T7** | **JA - L** | 5.90 | 39.322 | 8.68 | 43.664 | 8.771 | 33.189 |
| **T8** | **JA - M** | 6.19 | 42.165 | 8.72 | 43.922 | 8.734 | 32.906 |
| **T9** | **JA - H** | 6.71 | 46.647 | 9.38 | 47.868 | 9.409 | 37.719 |
| **T10** | **SA - L** | 5.05 | 29.109 | 7.21 | 32.178 | 7.763 | 24.514 |
| **T11** | **SA - M** | 5.34 | 32.959 | 8.28 | 40.942 | 8.625 | 32.058 |
| **T12** | **SA - H** | 5.56 | 35.612 | 8.39 | 41.716 | 8.716 | 32.767 |
| **T13** | **Control** | 3.58 |  | 4.89 |  | 5.86 |  |
|  | **SEm±** | 0.25 |  | 0.45 |  | 0.391 |  |
|  | **CD (P≤0.05)** | 0.72 |  | 1.29 |  | 1.136 |  |
|  | **CV %** | 7.61 |  | 9.29 |  | 7.898 |  |

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

**Table 12 Effect of inducers at different concentrations on the PAL activity (µg of cinnamic acid/min/mg protein) in the mustard (Bullet) against *A. brassicicola***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **Treatments** | **15 DAS** | **% increase over control** | **20 DAS** | **% increase over control** | **5 DAI** | **% increase over control** |
| **T1** | **BTH - L** | 6.47 | 45.286 | 6.72 | 34.524 | 8.74 | 35.812 |
| **T2** | **BTH - M** | 6.56 | 46.037 | 7.81 | 43.662 | 9.23 | 39.220 |
| **T3** | **BTH - H** | 6.80 | 47.941 | 7.73 | 43.079 | 9.57 | 41.379 |
| **T4** | **H2O2 - L** | 4.37 | 18.993 | 4.78 | 7.950 | 6.77 | 17.134 |
| **T5** | **H2O2 - M** | 4.56 | 22.368 | 4.96 | 11.290 | 6.84 | 17.982 |
| **T6** | **H2O2 - H** | 4.94 | 28.340 | 5.18 | 15.058 | 7.21 | 22.191 |
| **T7** | **JA - L** | 5.50 | 35.636 | 6.16 | 28.571 | 7.91 | 29.077 |
| **T8** | **JA - M** | 5.74 | 38.328 | 6.71 | 34.426 | 8.11 | 30.826 |
| **T9** | **JA - H** | 6.33 | 44.076 | 7.31 | 39.808 | 8.88 | 36.824 |
| **T10** | **SA - L** | 4.98 | 28.916 | 5.25 | 16.190 | 7.78 | 27.892 |
| **T11** | **SA - M** | 5.20 | 31.923 | 5.72 | 23.077 | 7.89 | 28.897 |
| **T12** | **SA - H** | 5.67 | 37.566 | 6.25 | 29.600 | 8.07 | 30.483 |
| **T13** | **Control** | 3.54 |  | 4.40 |  | 5.61 |  |
|  | **SEm±** | 0.25 |  | 0.29 |  | 0.30 |  |
|  | **CD (P≤0.05)** | 0.72 |  | 0.84 |  | 0.88 |  |
|  | **CV %** | 7.88 |  | 8.23 |  | 6.62 |  |

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

**Table 13 Effect of inducers at different concentrations on the PAL activity (µg of cinnamic acid/min/mg protein) in the mustard (B-54) against *A. brassicicola***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **Treatments** | **15 DAS** | **% increase over control** | **20 DAS** | **% increase over control** | **5 DAI** | **% increase over control** |
| **T1** | **BTH - L** | 5.73 | 44.677 | 6.50 | 42.153 | 8.12 | 41.872 |
| **T2** | **BTH - M** | 5.95 | 46.723 | 7.32 | 48.633 | 9.07 | 47.960 |
| **T3** | **BTH - H** | 6.07 | 47.776 | 7.65 | 50.849 | 9.28 | 49.138 |
| **T4** | **H2O2 - L** | 3.49 | 9.169 | 4.52 | 16.814 | 6.67 | 29.235 |
| **T5** | **H2O2 - M** | 3.66 | 13.388 | 4.98 | 24.498 | 6.80 | 30.588 |
| **T6** | **H2O2 - H** | 4.37 | 27.460 | 5.09 | 26.129 | 7.13 | 33.801 |
| **T7** | **JA - L** | 4.94 | 35.830 | 5.91 | 36.379 | 7.88 | 40.102 |
| **T8** | **JA - M** | 5.61 | 43.494 | 6.47 | 41.885 | 7.96 | 40.704 |
| **T9** | **JA - H** | 5.75 | 44.870 | 6.81 | 44.787 | 8.57 | 44.924 |
| **T10** | **SA - L** | 4.80 | 33.958 | 5.08 | 25.984 | 7.82 | 39.642 |
| **T11** | **SA - M** | 4.72 | 32.839 | 5.33 | 29.455 | 7.33 | 35.607 |
| **T12** | **SA - H** | 5.55 | 42.883 | 5.67 | 33.686 | 7.62 | 38.058 |
| **T13** | **Control** | 3.17 |  | 3.76 |  | 4.72 |  |
|  | **SEm±** | 0.27 |  | 0.30 |  | 0.45 |  |
|  | **CD (P≤0.05)** | 0.78 |  | 0.87 |  | 1.31 |  |
|  | **CV %** | 9.46 |  | 8.94 |  | 10.26 |  |

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

**Table 14 Effect of inducers at different concentrations on the PAL activity (µg of cinnamic acid/min/mg protein) in the mustard (B-9) against *A. brassicicola***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **S.No** | **Treatments** | **15 DAS** | **% increase over control** | **20 DAS** | **% increase over control** | **5 DAI** | **% increase over control** |
| **T1** | **BTH - L** | 5.55 | 52.252 | 6.39 | 48.044 | 7.69 | 40.962 |
| **T2** | **BTH - M** | 5.96 | 55.537 | 6.41 | 48.206 | 8.00 | 43.250 |
| **T3** | **BTH - H** | 5.81 | 54.389 | 6.66 | 50.150 | 8.12 | 44.089 |
| **T4** | **H2O2 - L** | 3.03 | 12.541 | 3.70 | 10.270 | 6.26 | 27.476 |
| **T5** | **H2O2 - M** | 3.20 | 17.188 | 4.08 | 18.628 | 6.40 | 29.063 |
| **T6** | **H2O2 - H** | 3.49 | 24.069 | 4.57 | 27.352 | 6.47 | 29.830 |
| **T7** | **JA - L** | 4.80 | 44.792 | 5.08 | 34.646 | 7.34 | 38.147 |
| **T8** | **JA - M** | 4.96 | 46.573 | 5.70 | 41.754 | 7.31 | 37.893 |
| **T9** | **JA - H** | 5.64 | 53.014 | 6.33 | 47.551 | 7.45 | 39.060 |
| **T10** | **SA - L** | 3.56 | 25.562 | 4.88 | 31.967 | 6.38 | 28.840 |
| **T11** | **SA - M** | 4.63 | 42.765 | 5.11 | 35.029 | 6.48 | 29.938 |
| **T12** | **SA - H** | 4.93 | 46.247 | 5.45 | 39.083 | 6.59 | 31.108 |
| **T13** | **Control** | 2.65 |  | 3.32 |  | 4.54 |  |
|  | **SEm±** | 0.24 |  | 0.24 |  | 0.37 |  |
|  | **CD (P≤0.05)** | 0.70 |  | 0.71 |  | 1.07 |  |
|  | **CV %** | 9.34 |  | 8.08 |  | 9.29 |  |

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

**Table 15 Effect of inducers at different concentrations on the PAL activity (µg of cinnamic acid/min/mg 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** | 6.970 | 9.985 | 10.210 | 6.613 | 7.423 | 9.182 | 5.917 | 7.156 | 8.822 | 5.772 | 6.486 | 7.939 |
| **H2O2** | 4.769 | 7.448 | 7.631 | 4.620 | 4.975 | 6.941 | 3.842 | 4.866 | 6.867 | 3.240 | 4.115 | 6.375 |
| **JA** | 6.265 | 8.925 | 8.971 | 5.855 | 6.728 | 8.301 | 5.431 | 6.394 | 8.136 | 5.132 | 5.702 | 7.364 |
| **SA** | 5.315 | 7.959 | 8.368 | 5.283 | 5.739 | 7.912 | 5.022 | 5.360 | 7.589 | 4.372 | 5.148 | 6.485 |
| **CONTROL** | 3.579 | 4.887 | 5.860 | 3.544 | 4.397 | 5.614 | 3.167 | 3.760 | 4.722 | 2.653 | 3.323 | 4.543 |
| **Factors** | **Inducers (I)** | | **Varieties (V)** | | **Days of sampling (D)** | | **I X V** | | **I X D** | | **V X D** | **I X V X D** |
| **SE(m)** | 0.06 | | 0.053 | | 0.046 | | 0.12 | | 0.104 | | 0.093 | 0.207 |
| **SE(d)** | 0.085 | | 0.076 | | 0.065 | | 0.169 | | 0.146 | | 0.131 | 0.293 |
| **C.D.** | 0.167 | | 0.15 | | 0.13 | | 0.335 | | 0.29 | | 0.259 | NS |

**Fig 3 Effect of inducers at different concentrations on the PAL activity in the mustard varieties against *A. brassicicola***

Peroxidase, polyphenol oxidase and phenylalanine ammonia lyase enzymes are known to be associated with resistance mechanisms in host plants like deposition of lignin and suberin in plant cells (Surendra *et al*., 2012). Increased PAL activity level in response to pathogen or elicitor spray has been reported (Song and Goodman, 2001). Similarly, reduction of phenylpropanoid levels by suppression of PAL increases disease susceptibility (Maher *et al*., 1994). Similarly, in the present study, invasion of pathogen in B-54 and B-9 varieties might have stopped the further induction of PAL during critical stage of disease infection so that, to weaken the plant mechanical strength by reducing the phenol accumulation.

The PAL activity was increased in pathogen inoculation as may be due to increased phonemic and flavonoid content. The PAL has been reported to be associated with the synthesis of phenolic compounds via phenylpropanoid pathway (Hahlbrock and Scheel, 1989). Although PAL activity maintained constitutively at the later stage of infection in moderately resistant and susceptible varieties. This is because of the metabolism of phenonic compounds in addition to the action of oxidative enzymes such as Peroxidase and Polyphenol oxidase, which catalyse the oxidation or phenols to quinines (Thypyapong *et al*, 1995).

The present observation clearly indicates that continuous increase in polyphenol oxidase and peroxidase activity due to application of inducers have depleted the phenol and flavonoid compound, but in case of resistant varieties it was balanced by temporal increase in PAL activity.

The present finding and the previous reports support the hypothesis that inducers may act directly by preventing the infection, inhibiting the fungal growth or inducing the plant defense reactions. More studies are required to determine the nature and role of plant extracts in pathogenesis and sustainable disease management.

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