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Impact of Elicitor Seed Treatment of Mustard Genotypes on Various Defense Related Compounds against Alternaria Blight of Indian Mustard (*Brassica juncea*)

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ABSTRACT: Alternaria leaf blight being a major concern on mustard whose control largely depends on the application of chemical pesticides however it is not the long term solution due to environment concern and risk due to fungicide residues. Under these circumstances, Induced resistance is one of the most dominant mechanism in managing the disease by increasing the activity of various defense related enzymes and non-enzymatic antioxidants. Role of inducers *viz.* Benzothiadiazole (BTH), Salicyclic acid (SA), Jasmonic acid (JA) and Hydrogen peroxide (H_2O_2) at three different concentrations *viz.* low, medium and high concentrations, *viz*. BTH (0.25 mM, 0.75 mM, 1.5 Mm); H_2O_2 (1%,2%, 3%); JA (1mM, 2.5 mM, 4 mM) & SA (0.5 mM, 1 mM, 2 mM) were evaluated on induction of resistance to manage Alternaria blight of mustard in three different varieties *viz.* resistant, moderately resistant and susceptible against the disease was studied in net house. Elicitor treatments exhibited maximum content of chlorophyll, total soluble protein, proline, total phenol, starch, total sugar, reducing sugars, non- reducing sugars content as compared to water sprayed control on un- inoculated plants. Among all the treatments, high concentration of BTH(1.5 mM) was most effective on increasing the observed parameters as chlorophyll, total soluble protein, proline, total phenol, starch, total sugar, reducing sugars, non- reducing sugars content followed by SA on disease resistance. Significantly high concentration of chlorophyll content (31.11 - 43.38 SPAD) was recorded in healthy plants in comparison to diseased plants (25.89-40.28 SPAD). In healthy plants total soluble proteins (13.30-26.44 mg/g), total sugars (41.36-85.11 mg/g), starch (136.46-222.28 mg/g) were maximum in comparison to inoculated plants recorded as (9.69-22.59 mg/g), (36.72-76.38 mg/g), (124.32-211.20 mg/g) respectively. Lower amount of phenols and low accumulation of proline were recorded in healthy plants 5.4-15.47 mg/g and 9.64-21.84 µmol/g respectively when compared with infected plants 10.61-18.14 mg/g and 16.09-26.58 µmol/g respectively at 18 DAS or 3DAI.These changes can be attributed to the role played by inducers and it is well known fact that BTH played an important role in enhancing the defense mechanism in plants.

Keywords: Elicitors, biochemical changes, Indian mustard, pot experiment

INTRODUCTION

Oilseed crops play an important role in agriculture economy of India which constitutes the second largest agriculture product next to food grains in country. Among oilseed crops, *Brassica* group of species are the largest oilseed crop in the world and occupies the second position next to groundnut and sunflower (Kumar *et al.,* 2010). During 2017-18, the production and productivity is 72.42 mT and 1974 kg/ha in the world and globally India accounts for 19.8% and 9.8% of the acreage and production (USDA, 2016-17). During 2016-17, the production and productivity were 7.98 mT and 1324 kg/ha in India (India stat 16-17). Rajasthan is the highest producing state for mustard-rapeseed (48.12%) in India followed by Haryana and Madhya Pradesh. In West Bengal the area, production and productivity are 4.578 lakh hectare (l ha), 4.999 lakh hectare (l ha) and 1090 kg/ha respectively. Despite a large area under cultivation and increased production of mustard, the productivity remains low when the potential yield of the latest varieties are taken into consideration and there is a big gap between the expected yield and yield realised by the farmers. Therefore, it is essential to know the possible reasons for existing gap in mustard cultivation with regards to biotic and abiotic stresses. The major constraints that put down the productivity are various abiotic and biotic stresses which causes massive yield loss to the crop. Among Biotic stress factors, disease is a vital factor in which mustard encounters several foliar diseases among which Alternaria blight of mustard caused by *Alternaria brassicae* (Berk.) Sacc. Is the major constraint in production of this crop (Kolte, 1985) and is the most devasting and destructive disease. Depending upon disease severity, about 47% yield loss has been estimated in India and has been reported from all the continents of the world (Meena *et al*., 2010). In West Bengal the disease is reported in various agro climatic zones of the state including undulating red and lateritic zone (Mamgain *et al*., 2013). *A. brassicae* perpetuates through seeds, plant debris, soil and weed hosts plant and due to its cosmopolitan habitat, it is

very difficult to control the disease. Although there are various options available for the management of the disease such as developing resistant cultivars, biological control, cultural practices like crop protection and use of chemicals has become the most important component of disease management strategy in mustard with the steady supply of highly effective and newer broad spectrum fungicides over the past decades, indiscriminate constant application of various chemicals has become major alternative which is being employed by majority of the farmers in field but its constant use of fungitoxic chemicals adds to the environmental pollution due to their broad spectrum toxicity and also led to the development of resistant strains of the pathogens. These circumstances led to the recognition and attention towards the use of eco-friendly elicitors in plant disease management. Elicitors are the compounds which activate chemical defence in plants at low concentration, they act as signal compounds providing information for the plant to trigger defence as induced by the pathogen infection (Ebel and Cosio, 1994; Boller, 1993). The present study reports the variable non- enzymatic antioxidant profile and oxidative damages resulting from *Alternaria brassicae* infection on mustard. The experiment reports the resistance, moderately resistant and susceptibility reactions in plant were might be attributed by the differential metabolomics responses of the plant. Where, in particular, the resistance reaction was mainly because of elevated defense metabolites in plant.

MATERIALS AND METHODS

The three genotypes of Indian mustard (*Brassica juncea*) *viz.*, resistant, moderately resistantand susceptible*viz.*, TBM-204, Bullet, B-9 respectively were sown post seed treatment with various inducers with different concentrations *viz.* BTH (0.25 mM, 0.75 mM, 1.5 Mm); H2O2 (1%, 2%, 3%); JA (1mM, 2.5 mM, 4 mM); SA (0.5 mM, 1 mM, 2 mM) as done by Biswas *et al.*, and sprayed inoculated using an atomiser at 15 days after sowing (DAS) by single spore method spore suspension with the concentration of 10^4 conidia/ ml of *A. brassicae* and were kept covered for 48 hr in humid plastic bags under net house conditions. Control plants were raised and sprayed with distilled water.

A. Treatment details

T1: Seed treatment with inducers, with pathogen inoculation

T2: Seed treatment with inducers, without pathogen inoculation

T3: Without seed treatment (water), with pathogen inoculation

T4: Without seed treatment (water), without pathogen inoculation

B. Biochemical studies

Mustard leaves were collected from different treatments and the changes and the content of various biochemical parameters were estimated at 15 DAS, 18 DAS and 3 DAI.

Determination of total phenols. Total phenols was estimated by Folin-Ciocalteau Reagent method (Sadasivam and Manickam, 1992). Sample of 0.5 g from each replicate sample was ground in 10-times volume of 80 per cent ethanol in mortar and pestle and centrifuged at 10,000 rpm for 20 min. A sample of 20 µl was taken for total phenols, estimated colorimetrically with Folin-Ciocalteu reagent. The absorbance was taken at 650 nm against a reagent blank and gallic acid was used as standard.

Determination of total soluble proteins. The total soluble proteins from the mustard leaves were analysed based on the method described by Lowry's method (Lowry, 1951). The sample of 0.2 g of fresh weight from each replicate was ground in pre-chilled mortar and pestle and then centrifuged at 10,000 rpm at 4ºC for 30 minutes. The absorbance was recorded at 660nm, bovine serum albumin (Fraction V) was used as standard.

Determination of chlorophyll content. The Chlorophyll content was measured by using a chlorophyll meter SPAD-502 meter(Konica-Minolta, Japan).

Estimation of Proline content. Free proline was determined using the method given by Bates *et al.* (1973). One gram of fresh leaf was homogenized and centrifuged at 10,000 rpm for 30 min. The absorbance of chromophore containing toluene layer was measured at 520 nm.

Estimation of total sugar. Total sugar was extracted from healthy and diseased leaves with 80% ethanol and estimated using anthrone (Sadasivam and Manickam, 1992). Total sugars were measured as glucose equivalent after comparing with the standard curve prepared from standard glucose and expressed as mg g-1 fresh weight of tissue at 630 nm.

Estimation of Starch. Starch was extracted from healthy and diseased leaves with 80% ethanol to remove sugars and centrifuged and the residue was retained and estimated using anthrone (Sadasivam and Manickam, 1992). Starch were measured as glucose equivalent after comparing with the standard curve prepared from standard glucose and expressed as mg g-1 fresh weight of tissue at 630 nm.

Estimation of reducing sugar. The reducing sugars were estimated based on the protocol described by nelson-somogyi method. The absorbance of blue colour was read at 620 nm and standard curve was prepared by using glucose.

Estimation of non-reducing sugar. The amount of non-reducing sugar was calculated by deducting the reducing sugar content from that of the total soluble sugars.

RESULTS AND DISCUSSION

The effect of elicitors were investigated in terms of various defense related parameters through biochemical analysis in three genotypes of mustard *i.e.*, TBM- 204 (Resistant genotype), Bullet (moderately resistant genotype) and B9 (susceptible genotype) against Alternaria blight caused by *Alternaria brassicae*.

Elicitors used at different concentrations reduced the disease incidence and significantly showed the difference among various defense related compounds (Table 1-8 and Fig. 1-8). It is observed that with increase in concentration of the different elicitors showed a significant increase in defense related compounds both in pathogen and without pathogen inoculated plants and their differences were significant in all the three genotypes.

It is evident from the Table 1, Fig. 1, Among the treatments, BTH @ 0.75mM treated mustard leaves showed the higher content of proteins at 15 DAS, 18 DAS and 3 DAI as 21.25 mg/g fresh leaves, 26.44 mg/g fresh leaves and 19.74 mg/g fresh leaves respectively followed by SA treated plants in TBM- 204 mustard genotype. Likewise, BTH @ 0.75mMtreated mustard leaves showed significant increase of total soluble protein content in Bullet genotype when compared to the control plants but possessed relatively low content of proteins than TBM 204. However BTH @ 1.5mM significantly increased the amount of proteins in B9 genotype at 15 DAS, 18 DAS and 3 DAI as 13.30 mg/g, 17.95 mg/g and 10.82 mg/g respectively when compared to control plants but relatively lower content of proteins when compared to other two genotypes of mustard. The results are in accordance with Saud *et al*., (2000) who reported decrease in protein content after infected with *Aspergillus niger* in guava, while, Mogle and Mayee (1981) who observed the reduction of free amino acids in leaf protein from virus infected resistant and susceptible genotypes. Ghosh *et al*., (2003) found maximum amount of proteins in healthy than diseased plants and also supported by the findings of Yadav *et al.,* (2015), Parihar *et al.* (2012), Meena *et al.,* (2014) and Mishra *et al.,* (2006) reported that proteins was higher in healthy leaves as compared to infected leaves with the increase in infection and plant age, the protein content was increased in all genotypes. During host pathogen interaction, proliferation of microorganism synthesize several enzyme proteins and sometimes causes rearrangement of nutritional composition of substrate due to formation of several degradation products thereby increasing its protein content (Onifade and Agboola 2003).

Table 1: Effect of various treatments on total soluble proteins in mustard genotypes (mg/g) fresh weight.

Treatments	TBM-204			BULLET			B9		
	15DAS	18DAS	3DAI	15DAS	18DAS	3DAI	15DAS	18DAS	3DAI
BTH @ 0.25mM	19.57	24.24	16.99	17.58	19.33	17.78	10.39	13.89	8.45
BTH @ 0.75mM	21.25	26.44	19.74	22.49	24.36	18.55	11.79	14.74	9.08
BTH @ 1.5 mM	19.67	26.12	22.5	20.42	20.9	18.45	13.3	17.95	10.82
H_2O_2 @1%	15.46	17.59	17.39	13.58	19.08	13.3	6.24	11.49	5.93
H_20_2 @ 2%	17.05	17.62	17.59	15.77	20.02	13.33	6.51	9.65	5.03
H_2O_2@ 3%	18.14	20.05	16.42	17.18	17.91	16.06	5.31	8.77	5.23
JA @ 1 mM	18.33	20.04	20.47	16.37	18.75	15.3	9.05	9.99	9.69
JA @2.5 mM	21.42	24.11	19.63	18.55	19.03	18.2	8.11	8.78	5.74
JA @ 4 mM	22.62	21.13	24.7	18.35	20.86	19.3	9.59	10.99	7.47
SA @ 0.5 mM	21.62	22.98	20.08	17.61	18.41	18.8	7.28	8.3	6.7
SA @ 1 mM	22.89	23.17	19.67	19	20.02	17.21	12.19	14.23	9.75
SA @ 2 mM	25.37	22.45	21.2	20.62	21.97	19	9.75	10.68	9.21
Control	17.78	20.78	16.7	17.78	20.78	16.7	10.68	11.91	9.15
SEM	0.78	0.40	1.15	0.82	0.90	0.51	0.31	0.28	0.45
CD (P=0.05)	2.27	1.15	3.34	2.38	2.61	1.47	0.91	0.83	1.30
CV	6.74	3.12	10.22	7.88	7.83	5.20	5.89	4.23	9.84

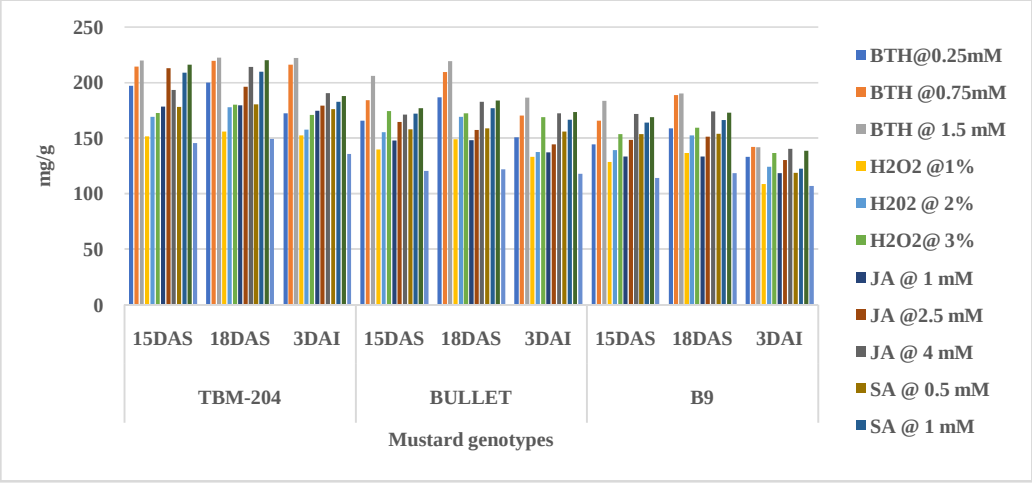

Fig. 1. Graphical representation of Total soluble proteins of mustard genotypes to various elicitor treatment.

The Proline content was measured maximum in BTH @1.5mM treated mustard leaves in all the three mustard genotypes at 15 DAS, 18 DAS and 3 DAI compared to control plants (Table 2 and Fig. 2) however, the accumulation of Proline was maximum in TBM 204 when compared to the other two genotypes *i.e*., 24.69 µ mol/g, 24.85 µ mol/g and 26.58 µ mol/gin TBM-204 and 19.14 µ mol/g, 23.84 µ mol/g and 24.31 µ mol/g in Bullet genotype and 10.02 µ mol/g, 15.09 µ mol/g and 16.09 µ mol/g in B-9 mustard genotype followed by SA @ 2 mM treated leaves in all the mustard genotypes.

Likewise, the total phenol content was found to be maximum in BTH @1.5mM treated mustard leaves in all the three mustard genotypes at 15 DAS, 18 DAS and 3 DAI compared to control plants (Table 3 and Fig. 3) however, the accumulation of Phenols was maximum in B9 mustard genotype when compared to the other two genotypes *i.e*., 15.36 mg/g, 15.67 mg/g and 18.14 mg/g when compared to 11.44 mg/g, 14.67 mg/g, 18.33 mg/g and 6.47 mg/g, 8.47 mg/g, 10.61 mg/g of Bullet and TBM-204 genotypes at 15 DAS, 18 DAS and 3 DAI respectively followed by BTH @ 0.75mM treated mustard plants in TBM-204 genotype and Bullet genotype and JA @4 mM treated plants in B9 and also found that and also found that the accumulation of phenols is maximum in pathogen inoculated plants when compared to un-inoculated plants. Phenols plays a major role in conferring resistance to plants against infection by microbes by inactivation of fungal enzymes or viral nucleoproteins by accumulating in the infected tissue to inhibit the growth of the pathogens of the host and may be related to their release from glycosidic esters by enzymatic activity of host or pathogen (Meena *et al.*, 2014). These compounds have been correlated with the resistance of plants to infectious agents (Singh, 2000). It is evident from the Table 3 and Fig. 3, that accumulation of phenol compounds are maximum in diseased plants of susceptible mustard genotype as compared to healthy resistant genotypes which were in accordance with the findings of Singh (2000).

Table 2: Effect of various treatments on proline content in mustard genotypes (µmol/g) fresh weight.

Treatments	TBM-204			BULLET			B9		
	15DAS	18DAS	3DAI	15DAS	18DAS	3DAI	15DAS	18DAS	3DAI
BTH @ 0.25mM	18.23	20.03	21.24	15.33	15.46	15.59	8.3	11.85	13.04
BTH @ 0.75mM	21.87	22.97	24.95	18.95	19.39	19.69	9.12	14.69	15.64
BTH @ 1.5 mM	24.69	24.85	26.58	19.14	23.84	24.31	10.02	15.09	16.09
H_2O_2 @1%	13.92	16.05	16.91	9.97	12.18	14.72	5.91	9.64	10.07
H_20_2 @ 2%	15.43	18.71	20.96	14.95	14.47	15.57	6.49	10.24	10.72
H_2O_2@ 3%	17.58	20.74	21.7	15.48	16.84	18.13	7.91	11.06	11.67
JA @ 1 mM	16.01	17.13	21.38	11.37	15.81	15.37	6.46	9.89	10.03
JA @2.5 mM	18.39	20.82	23.24	14.75	15.11	16.55	9.01	11.53	12
JA @ 4 mM	20.11	21.99	24.31	15.92	18.2	18.69	11.58	11.84	12.33
SA @ 0.5 mM	19.59	20.67	20.13	13.93	16.46	16.45	6.22	12.85	10.7
SA @ 1 mM	20.11	22.67	24.45	18.79	18.93	19.7	8.75	13.14	12.83
SA @ 2 mM	22.34	23.04	26.45	19.31	21.51	22.28	11.08	14.93	13.84
Control	12.6	13.67	15.35	7.34	10.21	11.57	5.29	9.72	10.45
SEM	0.39	0.80	0.97	1.01	0.78	1.02	0.49	0.45	0.42
CD (P=0.05)	1.12	2.33	2.82	2.92	2.28	2.96	1.41	1.30	1.22
CV	3.60	6.84	7.59	11.61	8.08	10.02	10.32	6.41	5.94

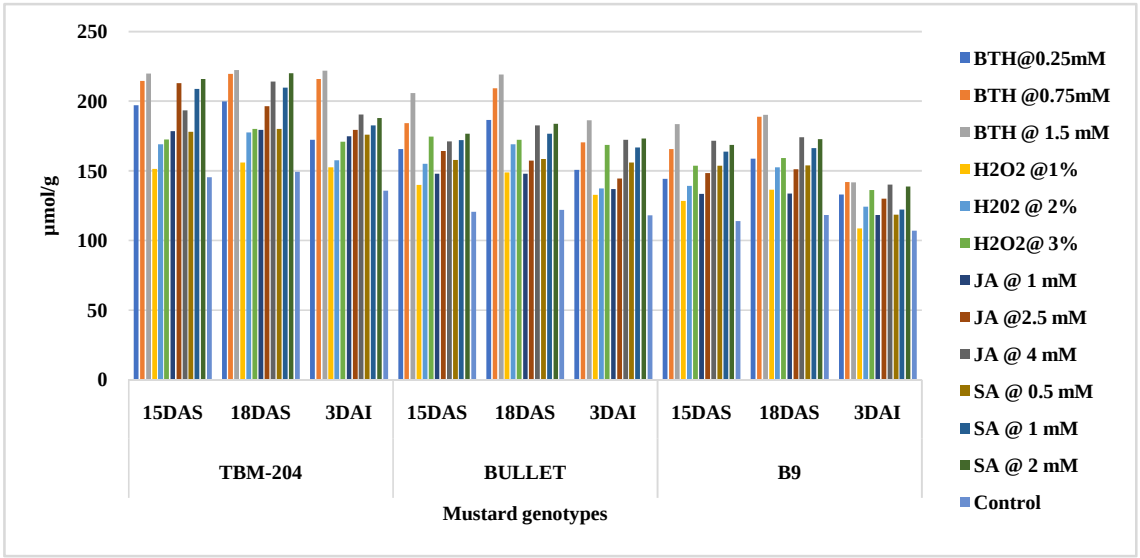

Fig. 2. Graphical representation of Proline of mustard genotypes to various elicitor treatment.

Table 3: Effect of various treatments on total phenols in mustard genotypes (mg/g) dry weight.

Treatments	TBM-204			BULLET			B9		
	15DAS	18DAS	3DAI	15DAS	18DAS	3DAI	15DAS	18DAS	3DAI
BTH @ 0.25mM	5.71	9.47	8.33	7.71	10.5	10.29	11.49	14.6	16.77
BTH @ 0.75mM	6.33	8.15	9.61	10.23	13.46	17.12	14.46	15.22	17.28
BTH @ 1.5 mM	6.47	8.47	10.61	11.44	14.67	18.33	15.36	15.67	18.14
H_2O_2 @1%	2.34	3.41	7.42	5.53	5.79	12.42	9.76	10.3	15.1
H_20_2 @ 2%	2.58	4.58	8.06	5.55	6.76	12.45	9.78	11.47	15.73
H_2O_2@ 3%	4.4	5.4	8.17	6.27	8.84	13.17	10.5	11.96	15.85
JA @ 1 mM	4.22	6.3	8.99	7.42	8.42	14.54	12.11	13.19	17.8
JA @2.5 mM	4.3	6.73	9.14	7.52	8.62	16.01	13.57	13.62	16.18
JA @ 4 mM	4.73	8.43	7.26	9.22	10.66	17.28	14.84	15.11	18.09
SA @ 0.5 mM	3.36	5.36	8.51	7.34	10.57	14.62	12.19	12.59	16.66
SA @ 1 mM	5.07	7.07	10.13	8.8	12.03	14.72	12.28	13.96	17.15
SA @ 2 mM	5.65	7.65	10.41	10.08	13.2	15.64	13.31	14.54	18.28
Control	3.25	2.45	2.6	5.77	8.05	8.29	8.18	10.12	10.27
SEM	0.41	0.43	0.54	0.57	0.56	0.51	0.55	0.69	0.67
CD (P=0.05)	1.19	1.26	1.57	1.65	1.62	1.49	1.59	2.01	1.95
CV	15.85	11.63	11.13	12.46	9.52	6.26	7.78	9.04	7.09

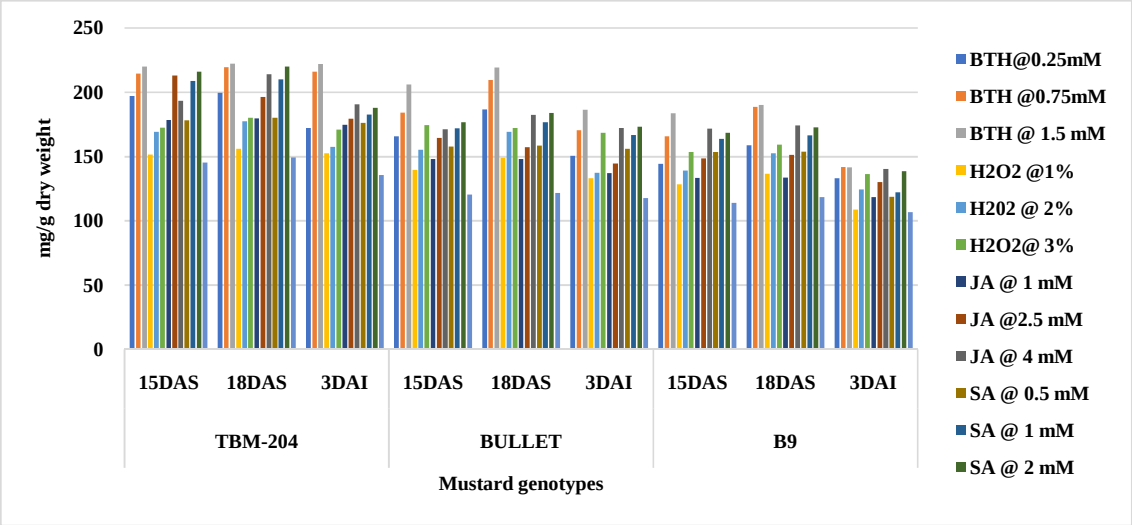

Fig. 3. Graphical representation of Total Phenols of mustard genotypes to various elicitor treatment.

The present findings are in accordance with Moussa and Aziz (2008) who reported that the high levels of proline enable the plant to maintain low water potential causing the accumulation of compatible osmolytes necessary to tolerate the stress. Kumar *et al*. (2010) who reported enhanced proline accumulation during stress which indicates that proline play a cardinal role as an osmoregulatory solute in plants. The increased accumulation of proline in treated plants in the present investigation showed the capacity of elicitors to induce resistance against the pathogen. Therefore, Proline is not only an important molecule in redox signaling, but also an effective quencher of reactive oxygen species formed under stress conditions in all plants (Alia and Saradhi, 1991). The ability of proline to scavenge reactive oxygen species and ability to inhibit reactive oxygen species-mediated apoptosis can be an important function in response to cellular stress and increased accumulation of Proline has been correlated with improved tolerance to various stresses (Hare and Cress, 1997).

The results are evident from the Table 4, Fig. 4 showed that the chlorophyll content (SPAD units) was significantly increased in all the treatments as compared to control among all the treatments at 15 DAS, 18 DAS and 3 DAI in TBM 204, Bullet and B9 genotypes but BTH @1.5mM was found increase the chlorophyll content at all the stages and also in the three genotypes (40.92, 43.38 and 40.28 SPAD units) (40.13,40.45 and 38.80 SPAD units) and (38.80, 38.83 and 38.15 SPAD units) in TBM-204, Bullet and B-9 mustard genotypes at 15 DAS, 18 DAS and 3DAI respectively followed by BTH @ 0.75mM treated plants when compared to control plants in all mustard genotypes. Similar type of observations were also noticed by Rosyara *et al.*, (2010) who reported that Chlorophyll content (SPAD) of wheat spot blotch was positively correlated with disease and also supported by Mathpal *et al.*, 2011, who reported that there was higher chlorophyll content in resistant genotypes and it was decreased following infection.

Table 4: Effect of various treatments on total chlorophyll in mustard genotypes (SPAD units).

Treatments	TBM-204			BULLET			B9		
	15DAS	18DAS	3DAI	15DAS	18DAS	3DAI	15DAS	18DAS	3DAI
BTH @ 0.25mM	32.62	35.08	33.07	31.83	32.15	31.78	30.5	30.43	29.52
BTH @ 0.75mM	38.29	40.75	37.65	37.5	37.82	37.41	36.17	36.2	35.52
BTH @ 1.5 mM	40.92	43.38	40.28	40.13	40.45	40.04	38.8	38.83	38.15
H_2O_2 @1%	30.32	32.78	29.68	29.53	29.85	29.44	28.2	28.24	27.55
H_20_2 @ 2%	28.66	31.11	28.01	27.87	28.18	27.78	26.53	28.33	25.89
H_2O_2@ 3%	31.42	33.88	30.78	30.63	30.95	30.54	29.3	29.3	28.65
JA @ 1 mM	29.92	32.38	29.28	29.13	29.45	29.04	27.8	29.48	27.15
JA @2.5 mM	31.32	33.78	30.68	30.53	30.85	30.44	29.2	29.23	28.55
JA @ 4 mM	34.42	36.88	33.11	32.33	33.63	32.88	31.95	32.33	31.45
SA @ 0.5 mM	29.86	32.31	29.21	29.07	29.38	28.18	27.73	27.77	27.09
SA @ 1 mM	30.79	33.25	30.15	30	30.32	29.91	28.67	28.7	28.02
SA @ 2 mM	35.66	38.11	35.01	34.87	35.18	34.78	33.53	33.57	32.89
Control	25.41	26.08	24.61	24.11	24.3	23.95	22.18	23.48	22.62
SEM	2.10	2.13	1.58	2.11	2.13	2.05	0.53	0.79	0.90
CD (P=0.05)	6.10	6.18	4.60	6.14	6.19	5.95	1.54	2.29	2.63
CV	11.25	10.64	8.64	11.67	11.63	11.34	3.05	4.49	5.30

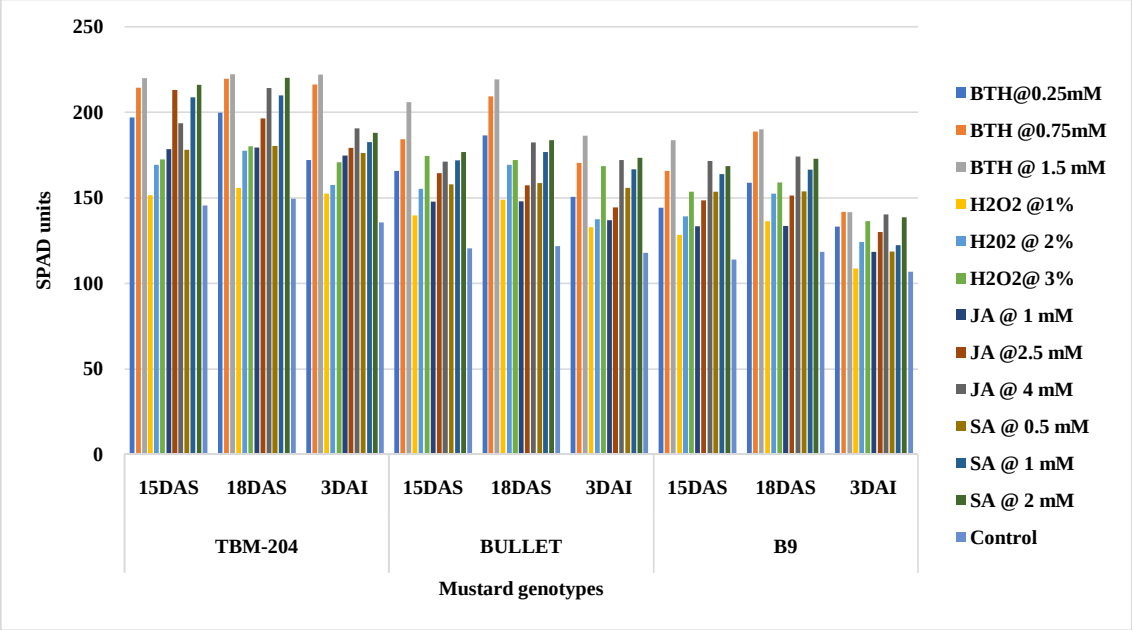

Fig. 4. Graphical representation of Total Chlorophyll of mustard genotypes to various elicitor treatment.

Total sugars and starch were found to increase with all the treated mustard leaves and increased with increase in the concentration of the elicitors in both healthy and *A. brassicae* infected plants. BTH @1.5Mm treated plants showed significant increase of total sugars, reducing sugars and also non- reducing sugars among the genotypes however there is slightly reduction in the sugar content in pathogen inoculated plants (Table 5,6,7 and 8 and Fig. 5, 6, 7 and 8). It is evident from the results represented that higher amount of total sugars and starch were present in resistant genotypes at pre-inoculation stage (15 DAS and 18 DAS) and lowest in inoculated mustard leaves (3 DAI) in susceptible genotypes. Our results are supported by the observations drawn showing higher sugar content in resistant genotypes in leaf blight of Barley (Singh *et al*., 2009), white rust and downy mildew of *B. napus* (Singh *et al.*, 2000), Charcol rot of bean (Baraka *et al*., 2004) and also in Alternaria leaf blight of chickpea (Bhargava and Khare 1988). The reduction in sugar content after inoculation with pathogen was probably due to rapid hydrolysis of sugars during pathogenesis and utilisation of sugars by pathogen development and also can be expected due to decrease in photosynthetic pigments which is directly proportional to the rate of photosynthesis which supports the decline of chlorophyll content in infected plants when compared to healthy plants.

Table 5: Effect of various treatments on total sugars in mustard genotypes (mg/g) dry weight.

Treatments	TBM-204			BULLET			B9		
	15DAS	18DAS	3DAI	15DAS	18DAS	3DAI	15DAS	18DAS	3DAI
BTH @ 0.25mM	72.51	73.75	68.17	69.11	71.77	65.81	65.34	69.89	58.57
BTH @ 0.75mM	74.15	75.86	70.93	71.22	71.57	65.37	67.78	68.93	61.17
BTH @ 1.5 mM	76.55	85.11	76.32	76.22	79.44	74.33	71.77	76.43	71.68
H_2O_2 @1%	49.09	61.38	52.04	44.45	48.77	42.39	41.36	43.74	36.72
H_20_2 @ 2%	56.06	64.66	54.32	51.81	52.42	45.85	45.1	46.8	39.52
H_2O_2@ 3%	62.73	66.41	59.74	53.18	58.09	51.66	49.24	51.42	47.4
JA @ 1 mM	67.81	69.34	63.69	59.84	62.14	54.06	55.84	56.13	47.74
JA @2.5 mM	71.81	75.13	66.75	62.84	65.51	56.9	57.69	58.59	50.23
JA @ 4 mM	77.18	80.23	71.58	72.22	76.59	68.45	61.02	67.04	63.58
SA @ 0.5 mM	70.94	72.78	68.58	62.99	67.1	54.35	55.45	61.3	48.02
SA @ 1 mM	76.48	81.12	72.37	73.48	75.72	68.9	64.38	72.57	64.26
SA @ 2 mM	77.5	81.7	74.95	75.72	77.2	70.25	67.66	74.24	65.61
Control	48.31	50.81	44.77	48.2	43.71	38.23	38.23	41.56	35.29
SEM	2.16	2.11	1.95	3.21	2.93	2.15	2.08	2.22	2.75
CD (P=0.05)	6.27	6.14	5.67	9.33	8.52	6.24	6.05	6.46	7.98
CV	5.92	5.60	5.81	8.21	7.04	5.72	6.33	6.34	8.96

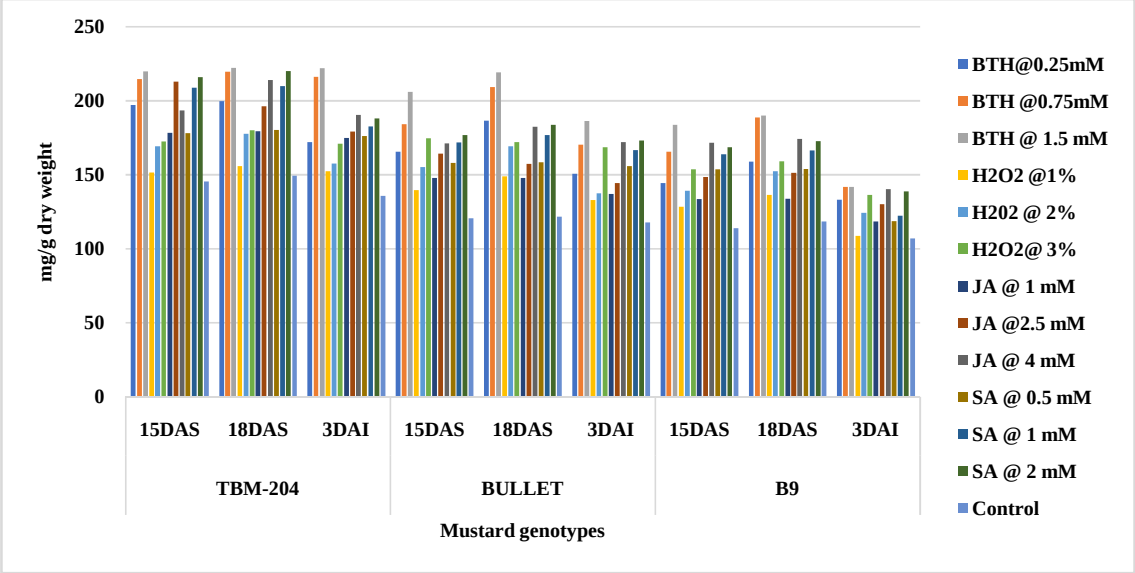

Fig. 5. Graphical representation of Total Sugars of mustard genotypes to various elicitor treatment.

Table 6: Effect of various treatments on total reducing sugars in mustard genotypes (mg/g) dry weight.

Treatments	TBM-204			BULLET			B9		
	15DAS	18DAS	3DAI	15DAS	18DAS	3DAI	15DAS	18DAS	3DAI
BTH @ 0.25mM	13.70	13.82	13.27	16.17	18.52	15.17	12.94	13.35	11.23
BTH @ 0.75mM	13.91	14.54	14.77	18.08	20.50	15.26	13.41	13.49	12.08
BTH @ 1.5 mM	14.40	15.12	14.26	16.78	22.47	16.19	13.37	13.75	13.41
H_2O_2 @1%	11.90	13.73	11.92	14.43	16.75	14.97	10.44	11.83	10.27
H_20_2 @ 2%	11.65	12.24	12.27	15.73	18.83	15.04	10.54	10.86	11.08
H_2O_2@ 3%	12.37	12.39	12.56	17.27	17.47	14.34	10.93	11.20	11.33
JA @ 1 mM	12.70	13.09	13.10	15.40	19.62	14.83	11.50	12.37	10.30
JA @2.5 mM	12.98	13.03	13.72	16.48	18.15	15.26	11.68	12.41	10.88
JA @ 4 mM	13.00	14.52	13.68	17.37	20.12	15.49	12.21	12.85	12.46
SA @ 0.5 mM	13.65	13.72	13.93	16.23	18.82	14.92	12.54	12.58	10.35
SA @ 1 mM	14.31	14.12	14.22	17.08	19.22	14.96	12.86	13.25	12.03
SA @ 2 mM	13.64	16.70	14.87	18.41	21.80	16.21	13.32	13.52	11.63
Control	11.92	11.83	11.59	13.63	14.83	13.93	10.39	10.47	10.13
SEM	0.42	0.35	0.32	0.52	0.29	0.47	0.36	0.20	0.46
CD (P=0.05)	1.21	1.02	0.94	1.51	0.84	1.3	1.04	0.57	1.34
CV	5.50	4.42	4.17	5.51	2.64	5.40	5.17	2.73	7.06

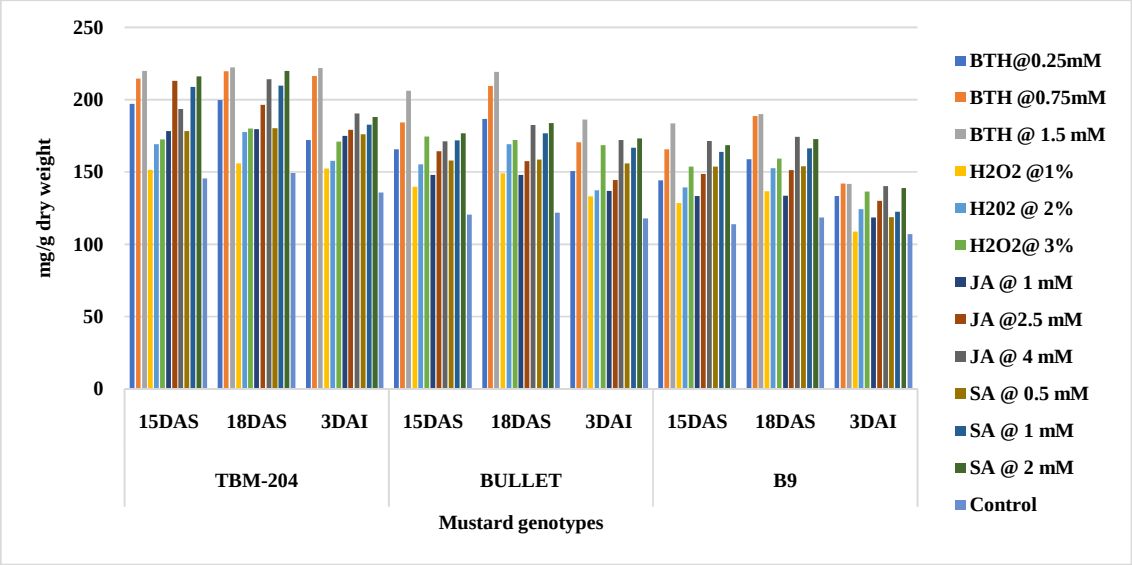

Fig. 6. Graphical representation of Total Reducing Sugars of mustard genotypes to various elicitor treatment.

Table 7: Effect of various treatments on non-reducing sugars in mustard genotypes (mg/g) dry weight.

Treatments	TBM-204			BULLET			B9		
	15DAS	18DAS	3DAI	15DAS	18DAS	3DAI	15DAS	18DAS	3DAI
BTH @ 0.25mM	52.76	57.95	52.54	56.67	55.24	53.00	50.42	56.54	49.94
BTH @ 0.75mM	51.42	57.03	50.61	57.99	55.36	55.67	47.36	55.44	46.49
BTH @ 1.5 mM	61.79	64.31	60.07	58.13	62.64	60.13	57.81	62.68	58.27
H_2O_2 @1%	32.06	35.03	30.46	34.66	44.63	37.70	29.57	31.91	26.45
H_20_2 @ 2%	40.16	40.18	33.59	40.33	45.82	39.28	34.55	35.94	28.44
H_2O_2@ 3%	40.81	45.70	39.10	45.46	48.93	44.77	38.32	40.22	36.06
JA @ 1 mM	47.14	49.05	40.96	51.65	49.72	48.86	44.34	43.76	37.44
JA @2.5 mM	49.86	52.48	43.18	53.73	56.98	51.49	46.01	46.18	39.35
JA @ 4 mM	59.22	62.07	54.77	60.40	60.11	56.09	48.81	54.19	51.12
SA @ 0.5 mM	49.33	53.38	40.42	54.48	53.96	53.66	42.92	48.73	37.67
SA @ 1 mM	59.17	61.60	54.68	59.61	61.90	57.41	51.52	59.33	52.23
SA @ 2 mM	62.08	60.49	55.38	58.04	59.89	58.73	54.34	60.72	53.98
Control	36.28	31.88	26.63	34.68	35.98	30.84	27.84	31.09	25.16
SEM	2.16	2.42	2.65	3.03	3.68	2.47	3.01	2.65	3.30
CD(P=0.05)	6.29	7.04	7.71	8.81	10.69	7.17	8.76	7.71	9.59
CV	7.59	8.12	10.25	10.24	11.98	8.58	11.82	9.53	13.69

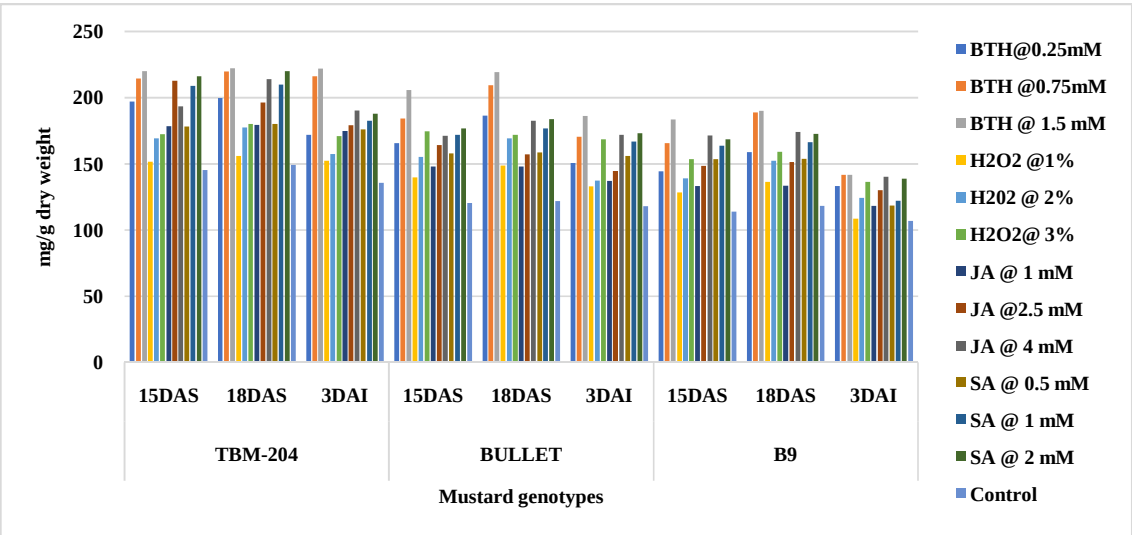

Fig. 7. Graphical representation of non- Reducing Sugars of mustard genotypes to various elicitor treatment.

Table 8: Effect of various treatments on Starch in mustard genotypes (mg/g) dry weight.

Treatments	TBM-204			BULLET			B9		
	15DAS	18DAS	3DAI	15DAS	18DAS	3DAI	15DAS	18DAS	3DAI
BTH@0.25mM	197.14	199.82	172.14	165.74	186.62	150.66	144.32	158.85	133.18
BTH @0.75mM	214.56	219.66	216.18	184.24	209.44	170.4	165.73	188.8	141.9
BTH @ 1.5 mM	219.97	222.28	221.97	206.02	219.27	186.3	183.64	190.1	141.77
H_2O_2 @1%	151.55	155.97	152.45	139.73	148.96	132.97	128.39	136.46	108.71
H_20_2 @ 2%	169.27	177.55	157.56	155.21	169.27	137.45	139.17	152.5	124.32
H_2O_2@ 3%	172.54	180.16	170.85	174.52	172.14	168.65	153.65	159.19	136.41
JA @ 1 mM	178.39	179.56	174.77	147.92	148.06	136.98	133.46	133.66	118.46
JA @2.5 mM	213.02	196.41	179.3	164.32	157.36	144.5	148.54	151.35	130.18
JA @ 4 mM	193.49	214.1	190.49	171.16	182.52	172.15	171.61	174.14	140.34
SA @ 0.5 mM	178.13	180.3	176.15	157.89	158.59	155.99	153.59	153.91	118.72
SA @ 1 mM	208.85	209.9	182.68	171.96	176.82	166.71	163.8	166.41	122.37
SA @ 2 mM	216.15	220.09	187.97	176.82	183.8	173.27	168.65	172.81	138.78
Control	145.52	149.35	135.68	120.47	121.76	117.92	113.9	118.47	106.9
SEM	5.81	6.26	10.87	4.92	5.83	4.88	3.92	6.71	4.23
CD (P=0.05)	16.89	18.20	31.61	14.30	16.96	14.19	11.41	19.51	12.28
CV	5.32	5.63	10.56	5.19	5.88	5.46	4.49	7.35	5.72

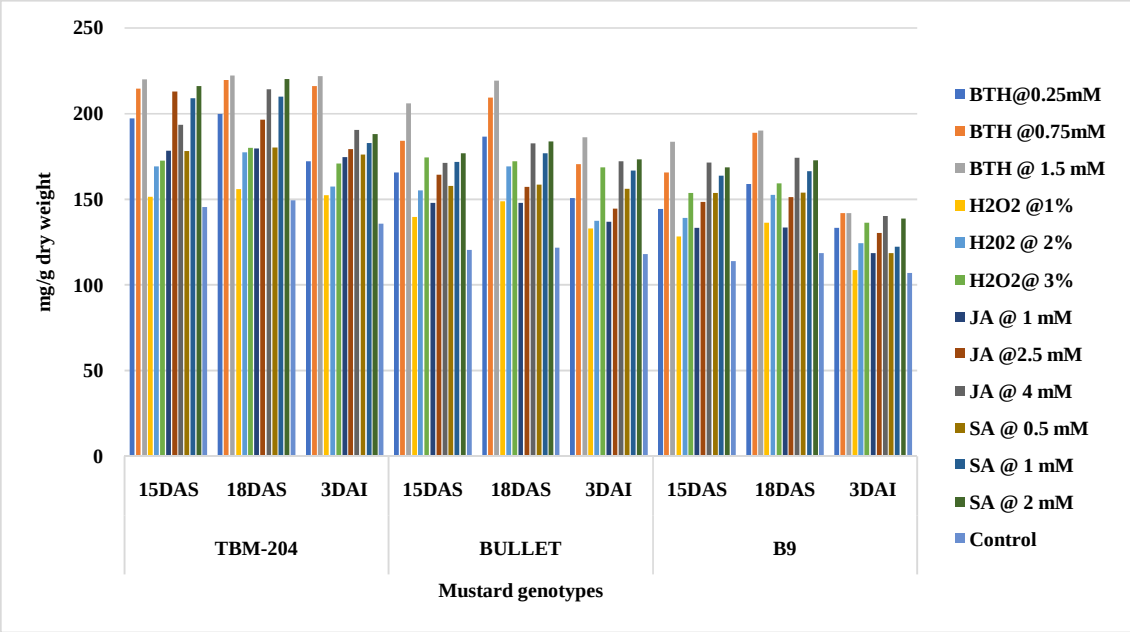

Fig. 8. Graphical representation of Starch of mustard genotypes to various elicitor treatment.

FUTURE SCOPE OF RESEARCH

1) The studies in this area may provide information concerning host-pathogen interaction which can be utilized for resistance breeding and thus desirable trait can be developed by incorporating resistance in promising but susceptible genotype of mustard.
2) Study of factors responsible for triggering of defense genes through signal transduction which are stimulated by elicitors should be studied at gene level.
3) There is need to search for various inducers responsible for inducting resistance should be studied through enzyme markers.
4) The efficiency of various elicitors in field level should be assessed on various genotypes of the crop and there performance can be evaluated by using resistance markers.
5) Effort should be made such that, elicitors should be commercialized by recommending as seed treatment in farmer's field which may become an alternative to chemical fungicides in management of few disease where fungicide resistance was established by pathogens is a major problem.

Conflict of interest. There are no conflict of interests to declare to publish this article.

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