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Studies on biochemical mechanism of resistance for the management of rose powdery mildew

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Abstract
To study the mechanism of resistance developed in rose cultivar High and Peace against *Podosphaera pannosa* was pretreated with SAR chemicals viz. salicylic acid, β-aminobutyric acid, calcium silicate, potassium bicarbonate, phosphoric acid, dipotassium orthophosphate and potassium silicate were assayed at six sampling intervals of 0 (at time of spray), 2, 4, 6 days, at bud formation and one month after bud formation for various biochemical constitutions like reducing and non-reducing sugars, total phenols, peroxidase and polyphenol oxidase activity and PAL activity. The results revealed that the dipotassium orthophosphate treated rose leaves developed highest amount of the reducing sugar (146.33 µg/g) with passage of time which was followed by salicylic acid (133.72 µg/g), β-aminobutyric acid (128.50 µg/g) and potassium silicate (124.00µg/g). While the lowest amount of reducing sugar was recorded in calcium silicate (117.67 µg/g) including control (115.33 µg/g). However, dipotassium orthophosphate treated rose leaves contain highest amount of the non-reducing sugar (65.00 µg/g) followed by salicylic acid (52.00 µg/g), β-aminobutyric acid (49.33 µg/g), potassium silicate (41.00 µg/g) and potassium bicarbonate (37.00 µg/g) while least amount of non-reducing sugar was found in calcium silicate (31 µg/g) treated plants followed by phosphoric acid (35.00 µg/g) at 6th day of spray. The total phenol (137.67 µg/g) was higher in dipotassium orthophosphate and salicylic acid with 130.72 µg/g irrespective of sampling intervals and different plant stages in comparison to rest of the treatments. maximum polyphenol activity in dipotassium orthophosphate treated rose leaves (0.261) followed by salicylic acid (0.242), β-aminobutyric acid (0.218) and potassium silicate (0.196) while lowest in calcium silicate (0.112) followed by phosphoric acid (0.145) and potassium bicarbonate (0.175). revealed the effectiveness of dipotassium orthophosphate in enhancing the peroxidase activity being highest (0.416) followed by salicylic acid (0.390), β-aminobutyric acid (0.360), potassium silicate (0.343) and potassium bicarbonate (0.313). While the least activity registered in calcium silicate (0.225). The highest PAL activity registered in dipotassium orthophosphate (0.586) followed by salicylic acid (0.523), β-aminobutyric acid (0.480) and potassium silicate (0.438) and potassium bicarbonate (0.403). While least PAL activity was observed in treatment with calcium silicate (0.287).

Keywords: Powdery mildew, bio-control agents, conidial germination, disease control

Introduction
Powdery mildew fungi are obligate and considered as one of the most conspicuous groups of plant pathogens. They are characterized by the appearance of spots or patches of a white to grayish, powdery growth on the outside of plant organs. The attacking fungus is most commonly observed on the upper side of the leaves, but it also affects the underside of leaves and every plant parts; young shoots and stems, buds, flowers, and young fruits in many plant species (Braun, 1995; Horst, 1983 and Gastelum *et al.,* 2014) [5, 17, 15]. The powdery mildew fungi seldom kill their hosts but utilize their nutrients, reduce photosynthesis, increase respiration and transpiration, impair plant growth and reduce the yield, ranging between 20 to 40 per cent depending upon the congenial environment favorable for their growth and multiplication (Agrios, 2005) [1]. The losses particular in roses ranged between 20 to 25 per cent as reported by Kumar (1998) [19].
The disease is managed by the growers by various management practices. Among these, use of fungicides has become an important component in disease management. Scanning of literature revealed that the pathogen-host interaction (powdery mildew/rose) alone causes huge amount of pesticides volume up to 40 per cent applied to rose crop to combat this disease (Tjosvold and Koike, 2001) [31]. Rose powdery mildew is usually controlled by synthetic chemical products in commercial greenhouses (Scarito *et al.,* 2007) [34]. In India, at present there are more than 260 technical grade pesticides and 585 pesticide formulations which have been registered according to the Pesticide Registration Committee (PRC) and their consumption in year 2014-2015 is about 57353 MT (http://www.cibrc.nic.in/mup.htm).
But in recent years application of fungicides has plagued with very serious problems such as development of fungicide resistance in the pathogenic fungi, evolution of new strains of the

pathogen, environmental hazards and soil health deterioration. There are many examples of fungicide resistance in the fungal pathogens from developed, developing and underdeveloped countries like USA, UK, France, Germany, Japan, and Australia and also in South East Asian countries. In India it appears that studies on fungicides resistance have been neglected. Only a few reports on fungicide resistance in fungal pathogen are available in India (Gangawane and Saler, 1981; Gangawane and Reddy, 1988, Rao and Reddy, 1988; Annamalai and Lalithakumari, 1990; Waghmare, 1991; Arora *et al.*, 1992; Gangawane and Kamble, 2001; Apte and Kamble, 2008) [14, 13, 2, 41, 4, 12, 3].

Keeping in view the adverse consequences of fungicides, reduction in use of fungicides is highly advocated. Also because of increasing international demand to reduce the use of toxic pesticides, because of human health and environmental hazards, the recent shift has been towards the potential biological and the ecofriendly methods as an alternative strategy. Therefore, exploitation of host resistance by application of induced resistance chemicals would be an ideal approach in the context of subsistence farming of resource-limited regions of the world. Resistance to pathogens is associated with the accumulation of enzymes, antibiotics and inhibitors. Salicylic acid is a natural phenolic compound present in many plants and is an important component in the signal transduction pathway and is involved in local and systemic resistance to pathogens (Delaney *et al.,* 1995 and Maleck *et al.,* 2000) [10, 22]. Phenolic compounds are a chemically diverse and biologically important group of secondary metabolites. In apple trees, these compounds are involved in natural defence reactions against various diseases (Slatnar *et al.,* 2010; Dao *et al.,* 2011) [35, 9]. Their rapid accumulation at the infection site limits the development of the pathogen, potentially isolating it at the original site of ingress (Nicholson and Hammerschmidt, 1992) [29]. When microbes invade plant cells, polyphenol oxidases are involved in the oxidation of polyphenols into quinines (Soliva *et al.,* 2001) [36]. Peroxidases participate in wallbuilding processes, e.g., oxidation of phenols, and the suberization and lignification of host cells during the defence reaction against pathogenic agents (Mohammadi and Kazami, 2002) [26]. These phenol oxidizing enzymes may participate in plant responses to microbes (Reimers *et al*., 1992; Chen *et al*., 2000) [33, 8]. However, resistance studies pertaining to Podosphaera pannosa are rather limited therefore the present study was therefore, undertaken to find out the role of biochemical mechanisms involved in resistance against powdery mildew after treatment with SAR chemicals.

Materials and methods

Leave samples from different treatments treated SAR chemicals were taken at 0 (before spray), 2, 4, 6 days, at bud formation and one month after bud formation for estimating the changes in the biochemical constituents viz., reducing sugars, non-reducing sugars, total phenol, peroxidase activity, polyphenol oxidase and phenyl ammonia lyase. Each treatment was replicated thrice and the leaves were randomly picked up from treated plants. Leave samples from untreated plants were taken as control for comparison. The biochemical constituents were estimated by various methods as mentioned below.

Extraction of sugars

One g of leaf sample was extracted with 25 ml of boiling ethyl alcohol for 10 minutes, decanted and the residue was re-extracted with 10 ml of ethyl alcohol. The extract was again filtered and both the filtrates were pooled and final volume was used for estimating reducing and non reducing sugars.

Estimation of reducing and non-reducing sugars

Reducing and non-reducing sugars were determined by the colorimetric method using Nelson's chromogenic reagent (Nelson, 1944). Twenty ml of alcohol extract was taken and its alcoholic part was evaporated by keeping it in a 100 ml beaker placed in a water bath at 68°C. It was ensured that the contents did not dry completely and the volume of the residue was raised to 8ml with distilled water so as to represent 1gm of the extract in 2 ml of water. This was used for the estimation of reducing and non- reducing sugars.

Estimation of reducing sugars

Reagents: 1. Copper reagent A: Sodium carbonate (anhydrous) -25 g, sodium potassium tartrate - 25 g**,** sodium bicarbonate - 20 g, sodium sulphate (anhydrous) -200g and distilled water - 800 ml. The volume was made upto 800 ml, filtered and stored in a brown glass stoppered bottle. **2. Copper reagent B:** Copper sulphate-15 g, distilled water - 100 ml, concentrated sulphuric acid- 1-2 drops. **3. Arsenomoblybdate reagent:** Ammonium molybdate -25 g, concentrated sulphuric acid - 21 ml, distilled water - 450 ml, sodium arsenate -3 g and distilled water - 25 ml. The arsenomolybdate reagent was prepared by mixing the above (a) and (b) solutions together and incubated at 37°C for about 48 hours. The reagent was stored in glass stoppered brown bottle at room temperature (25°C).

Procedure

To one ml of tissue extract in a 25 ml test tube (prepared as already mentioned) 1ml of fresh copper reagent (prepared by mixing 25 parts of copper reagent A and 1 part of copper reagent B) was added. The solutions were mixed and then heated exactly for 20 minutes in a boiling water bath. It was taken out and cooled in a pan of cold water. Thereafter one ml of arsenomolybdate reagent was added. The contents were mixed thoroughly till the effervescence ceased. The volume was raised to 20 ml with double glass distilled water and intensity of blue colour was measured at 620 nm in a Spectrophotometer. The quantity of reducing sugars was calculated from a standard curve prepared with known concentrations of glucose.

Estimation of non- reducing sugars

To determine the non- reducing sugars, hydrolysis of the extract was done as follows: One ml of the extract was put in 25 ml test tube and to it was added 2 ml of 1 N sulphuric acid. It was heated at 50 °C for 30 minutes and then cooled in a pan of cold water. Thereafter, 1-2 drops of methyl red indicator solution was added. The reddish solution was then neutralized with 1N NaOH, adding it drop by drop. This solution was then treated as in case of reducing sugars for finding out the total sugars present in the sample. By subtracting the reducing sugar from total sugars the non-reducing sugar content was calculated.

Total Phenols

Phenols were extracted from the fresh leaves following the method of (Mahadevan and Sridhar, 1982) [21]. One g of fresh leaf was cut into small pieces put in boiling alcohol in a water bath for 5-10 minutes (4 ml alcohol /gm tissue). After 15 minutes of boiling it was cooled and crushed in mortar and

pestle thoroughly at room temperature. The extract was passed through two layers of cheese cloth and then filtered through Whatman No. 1 filter paper. Final volume was adjusted with 80 per cent ethanol. The whole experiment was performed in dark to prevent light induced degradation of phenols.

Estimation of phenols

Total phenols were estimated by the method described by Bray and Thorpe (1954) [6].

Reagents: 1. Folin-Ciocalteu Reagent (FCR), 80% ethanol and 20% sodium carbonate.

Procedure: To one ml of alcohol extract, one ml of Folin-Ciocalteu reagent was added followed by the addition of 2 ml of 20 per cent sodium carbonate solution. The contents were shaken before heating in a boiling water bath for exactly one minute and then cooled in running tap water. The blue solution so obtained was diluted to 25 ml with double distilled water. After half an hour optical density of the solution was read at 650 nm. A blank containing all the reagents minus Folin- Ciocalteu reagent was used to adjust the absorbance to zero. Total phenols were calculated from the standard curve prepared from caffeic acid.

Extractions of enzymes for polyphenol oxidase and peroxidase activity

0.5 g sample was homogenized in 5 ml of 0.1 M potassium phosphate buffer (pH 7.5) containing 2% (w/v) polyvinylpyrrolidone (PVP) and 0.25% (v/v) Triton X. The homogenate was centrifuged at 10,000 rpm for 30 minutes at 4°C. The supernatants were used as crude enzyme extracts to assay the enzymatic activities.

Estimation of polyphenol oxidase activity

Reagents

1. 0.025 M catechol ($C_6H_6O_2$) dissolved in 0.1 M phosphate buffer (Ph 6.0).
2. 0.1 M potassium phosphate buffer (pH 6.5)

The Polyphenol oxidase activity was determined spectrophotometrically. The assay mixture contained 1.95 ml of 0.1 M potassium phosphate buffer (pH 7.5), 1 ml of catechol (0.025 M) and 50 µl diluted enzyme extract. The enzyme activity was expressed as change in absorbance at 420 nm was recorded at 30 second intervals for 3 minutes. The enzymatic activity was expressed as the change in the absorbance of the reaction mixture min^{-1} g^{-1} on a fresh weight basis.

Estimation of peroxidase activity

Reagents

1. 0.05 M solution of pyrogallol was prepared in phosphate buffer (pH 6.0). It was then filtered through filter paper (Whatman No. 42) and kept in dark and used within two hours.
2. One per cent hydrogen peroxide was prepared in double distilled water and was tightly corked and stored.

Procedure

Peroxidase activity was assayed spectrophotometrically. The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of enzyme extract, and 0.5 ml of 1 per cent hydrogen peroxide. The reaction mixture was incubated at room temperature (28±1°C) for 30 minutes. Change in absorbance at 420 nm was recorded at 30 second intervals for 3 minutes. The enzymatic activity was expressed as the change in the absorbance of the reaction mixture min^{-1} g^{-1} on a fresh weight basis (Hammerschmidt *et al*., 1982).

Extraction and estimation of phenylalanine ammonia lyase (PAL) activity

One gram of the leaf sample was homogenized in 3 ml of ice-cold 0.1M sodium borate buffer, (pH 7.0) containing 1.4 mM of 2-mercaptoethanol and 0.1g of insoluble polyvinylpyrrolidone. The extract was filtered through cheese cloth and the filtrate was centrifuged at 15000 g for 15 minutes. The supernatant was used as enzyme source. Phenylalanine ammonia lyase activity was determined as the rate of conversion of L-phenylalanine to trans-cinnamic acid (Dickerson *et al*., 1984). Sample containing 0.4 ml of enzyme extract was incubated with 0.5 ml of 0.1 M borate buffer, pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 minutes at 30 ºC. The amount of trans-cinnamic acid formed from L- phenylalanine was measured spectrophotometrically at 290 nm. Enzyme activity was expressed as µg of trans-cinnamic acid (in µ mol quantities) min^{-1} g^{-1} fresh weight.

Results and discussion

Rose plants pretreated with SAR chemicals viz. salicylic acid, β-aminobutyric acid, calcium silicate, potassium bicarbonate, phosphoric acid, dipotassium orthophosphate and potassium silicate were assayed at six sampling intervals of 0 (at time of spray), 2, 4, 6 days, at bud formation and one month after bud formation for various biochemical constitutions like reducing and non-reducing sugars, total phenols, peroxidase and polyphenol oxidase activity and PAL activity.

Reducing and Non-reducing sugar

Data presented in Table 1 revealed that in all treatments the amount of reducing sugar increased with span of time; it was higher side on 6th day after the spray and decreases at time of bud formation and one month after bud formation. From the perusal of results it is indicated that the dipotassium orthophosphate treated rose leaves contain highest amount of the reducing sugar (189 µg/g) followed by salicylic acid (163 µg/g), β -aminobutyric acid (146 µg/g), potassium silicate (140 µg/g), potassium bicarbonate (135 µg/g), phosphoric acid (130.00 µg/g) in descending order of their content and least level however was observed in calcium silicate (127 µg/g) at 6th day of spray. Increased reducing sugar content was also observed in water treated control over a period of time of assessment but the level was lower compared to other treatments. At time of the bud formation there was gradual decrease in the level of the reducing sugar in all the treatments except control, which remained almost stable. But one month after the bud formation there was drastically decrease in the reducing sugar level including control.

Overall mean revealed that the dipotassium orthophosphate treated rose leaves developed highest amount of the reducing sugar (146.33µg/g) with passage of time which was followed by salicylic acid (133.72µg/g), β-aminobutyric acid (128.50 µg/g) and potassium silicate (124.00µg/g). While the lowest amount of reducing sugar was recorded in calcium silicate (117.67 µg/g) including control (115.33 µg/g).

Similar trend with respect to non-reducing sugar content was followed as evident from Table 2. In all SAR chemicals, amount of non-reducing sugar was increased up to the 6th day

and decreased at the time of bud formation and one month after bud formation stages without any adverse effect on treated plants. However, dipotassium orthophosphate treated rose leaves contain highest amount of the non-reducing sugar (65.00 μg/g) followed by salicylic acid (52.00 μg/g), β-aminobutyric acid (49.33 μg/g), potassium silicate (41.00 μg/g) and potassium bicarbonate (37.00 μg/g) while least amount of non-reducing sugar was found in calcium silicate (31 μg/g) treated plants followed by phosphoric acid (35.00 μg/g) at 6th day of spray. Increased non-reducing sugar content was also observed in water treated control over a period of time but the level was lower compared to other treatments. However, in all SAR chemicals the gradual and drastic decrease in the level of non reducing sugar was determined from the leaves of treated plants when reaches to bud formation stage and also in one month assessment after this stage.

In order to observe the changes occurring in reducing and non-reducing sugar in rose leaves pretreated with SAR chemicals, the estimated content at 6th day after spray, at time of bud formation and one month after bud formation indicated that highest content of both sugars was obtained in leaves treated with dipotassium orthophosphate. Literature on reducing and non-reducing sugars content after elicitor treatment affecting the resistant and susceptible behaviors of the cultivars against pathogens is inadequate. However, decreased sugar levels in diseased plants were observed by Prasad *et al.* (1976) [30] and Nema (1989) [28]. The depletion of sugars during host-parasite interaction might be due to increased respiration or utilization of sugars by the fungi which depends on the capability of fungi to secrete carbohydrate degrading enzyme. Nema (1989) [28] suggested that reduction in sugars during disease development might be due to utilization of sugars probably for energy and synthetic reactions involved in multiplication of the pathogen.

Total phenols

The data presented on total phenol suggested that in all treatments the amount of total phenol was increased upto the 6th day after spray and decreased at time of bud formation and one month after bud formation (Table 3). The perusal of the results indicated that the dipotassium orthophosphate treated rose leaves accumulates maximum amount of the total phenol (182.00 μg/g) upto 6th day of sampling followed by salicylic acid (168.00 μg/g), β-aminobutyric acid (151.00 μg/g), potassium silicate (142.00 μg/g) and potassium bicarbonate (139.00 μg/g). However, calcium silicate (115.89 μg/g) registered least increase in amount at 6th day of spray. Increased total phenol content was also observed in water treated control over a period of time but the level was lower compared to other treatments. At time of the bud formation there was decrease in the level of the total phenol but higher as compared to the control in all the treatments and one month after the bud formation there was drastic decrease in the total phenol content.

The total phenol (137.67 μg/g) was higher in dipotassium orthophosphate and salicylic acid with 130.72 μg/g irrespective of sampling intervals and different plant stages in comparison to rest of the treatments.

Phenolic compounds are a chemically diverse and biologically important group of secondary metabolites. Their rapid accumulation at the infection site limits the development of the pathogen, potentially isolating it at the original site of ingress (Nicholson and Hammerschmidt, 1992) [29]. In the present study, in all the SAR chemicals treatments there was considerable increase in phenolic compounds and dipotassium orthophosphate sprayed plants showed maximum phenolic content followed by salicylic acid and β-aminobutyric acid during all the sampling intervals. Increase in phenolic content after elicitor treatments may be due to increase of PAL activity, as PAL has been reported to be associated with the synthesis of phenolic compounds via phenylpropanoid pathway (Hahlbrock and Scheel, 1989) [16].

Many phenolic compounds and their oxidation products are considered to be potentially toxic substances associated with the reduction in development and multiplication of plant pathogens (Mahadevan, 1970) [20]. The results of present study are in agreement with the findings of Meena *et al*. (2001) [25] that salicylic acid applied as pre-inoculation spray in groundnut plants challenged with *Cercosporidium personatum* resulted in three fold increase in the phenol content on fourth day. Similarly, Vimala and Suriachandraselvan (2009) [39] reported that pre-inoculation spray of salicylic acid showed the maximum phenolic content followed by post-inoculation spray. Matern and Kneusal (1988) [23] also expressed the view that the first stage of defence in plants is the accumulation of phenols at the infection site which restricts the growth of the pathogen. Accumulation of phenolics at the site of infection is a general response of plants in many host- pathogen interactions (Farkas and Kiraly, 1962) [11] and this accumulation is fostered by biotic and abiotic elicitors.

Polyphenol oxidase and Peroxidase activity

All the SAR chemicals increased the polyphenol oxidase activity upto the 6th day after the spray and decreases the activity at time of bud formation and one month after bud formation (Table 4). The highest polyphenol oxidase activity (0.531 μg/g) was found in dipotassium orthophosphate followed by salicylic acid (0.498), β-aminobutyric acid (0.475) and potassium silicate (0.420) while minimum polyphenol activity was registered in calcium silicate (0.165) followed by phosphoric acid (0.267) and potassium bicarbonate (0.385) on 6th day. Similar trend was observed with passage of time in all the treatments the activity increased over a period of time but the level was lower compared to other treatments. At time of the bud formation there was gradual decrease but remained higher compared to control and one month after the bud formation a drastic decrease in the polyphenol activity was observed.

Overall mean revealed maximum polyphenol activity in dipotassium orthophosphate treated rose leaves (0.261) followed by salicylic acid (0.242), β-aminobutyric acid (0.218) and potassium silicate (0.196) while lowest in calcium silicate (0.112) followed by phosphoric acid (0.145) and potassium bicarbonate (0.175).

Peroxidase activity resulted into similar trend as noticed in polyphenol oxidase activity. In all treatments peroxidase activity was increased up to the 6th day; however it decreases at time of bud formation and one month after bud formation (Table 5). Dipotassium orthophosphate treated rose leaves registered maximum amount of peroxidase activity (0.875) followed by salicylic acid (0.812), β-aminobutyric acid (0.720), potassium silicate (0.673), potassium bicarbonate (0.612) and phosphoric acid (0.575) while least increase in peroxidase activity noticed on calcium silicate (0.321) at 6th day after spray. Similar decrease in the total level of the peroxidase activity at time of the bud formation and one month after the bud formation observed with time span.

Overall mean also revealed the effectiveness of dipotassium orthophosphate in enhancing the peroxidase activity being highest (0.416) followed by salicylic acid (0.390), β-aminobutyric acid (0.360), potassium silicate (0.343) and potassium bicarbonate (0.313). While the least activity registered in calcium silicate (0.225).

Peroxidase and polyphenol oxidase mediate the oxidation of phenols and oxidized phenols which are highly toxic to the pathogen (Sequeira, 1983). Activity of both polyphenol oxidase and peroxidase was higher in dipotassium orthophosphate treated plants followed by salicylic acid and β-aminobutyric acid treated plants compared to untreated control plants which increased considerably with progress of infection.

Peroxidase has antifungal effects and has been implicated in the defence responses to pathogens in various crops. Increase in peroxidase activity has been shown to be associated with lignification, phenol oxidation and plant defence. Peroxidase is a key enzyme in the biosynthesis of lignin and other oxidized phenols (Bruce and West, 1989) [7]. Similarly, higher levels of polyphenol oxidase (PPO) were observed in roots and shoots of resistant cultivars than those of susceptible cultivars of chickpea on treatment with elicitors salicylic acid, spermine (Spm), SA+Spm and pathogen *Fusarium oxysporum* f. sp. *ciceri* (Raju *et al.,* 2008) [31].

PAL activity

Data on PAL activity estimation was presented in Table 6 which revealed that all the SAR chemicals increased the PAL activity up to the 6th day; however, it decreases at time of bud formation and one month after bud formation. The maximum PAL activity (1.23) was found in treatment with dipotassium orthophosphate. Salicylic acid (1.020), β-aminobutyric acid (0.932) and potassium silicate (0.911) were next best in production of PAL activity. Its activity was least in calcium silicate (0.376) followed by phosphoric acid (0.722) and potassium bicarbonate (0.765) after 6th day of spray. Increase in PAL activity was also observed in water treated plants (control) over a period of time but the level was lower compared to other treatments. In all SAR chemicals the gradual decrease in the level was found on the plants approaching to bud formation stage.

Similar trend with overall mean was recorded i.e. the highest PAL activity registered in dipotassium orthophosphate (0.586) followed by salicylic acid (0.523), β-aminobutyric acid (0.480) and potassium silicate (0.438) and potassium bicarbonate (0.403). While least PAL activity was observed in treatment with calcium silicate (0.287).

Phenylalanine ammonia lyase (PAL) is a key enzyme in the biosynthesis of phenyl propane unit, which is a component of phenolic acids, flavonoids and lignins. PAL activity can be induced by plant pathogen interactions and by fungal elicitor treatment (Ramanathan *et al*., 2000) [32]. Analyzed data from the present study revealed that, in the SAR chemical treated plants, PAL activity was higher in dipotassium orthophosphate followed by Salicylic acid and β-aminobutyric acid treated plants compared to control plants.

Present study also indicated a rapid increase of PAL activity in SAR chemical treated plants. These results are in accordance with those reported by Song *et al.* (1993) [37] in apple leaves that increased PAL activity level in response to pathogen or elicitor spray existed with SAR chemicals as well as bioagents. Vimala and Suriachandraselvan (2009) [39] reported that salicylic acid pretreated bhendi plants challenged or inoculated with *Erysiphe cichoracearum* resulted in earlier and increased activity of phenylalanine ammonia lyase, and higher accumulation of phenolics which enhanced the resistance against invasion by *Erisiphe cichoracearum* in bhendi.

Table 1: Reducing sugar content in rose leaves after treatment with SAR chemicals at different intervals

Treatment (s)	Reducing sugar content (µg/g fresh weight) in rose leaves						Mean
	Sampling Interval (days)						
	0 (At the time of spray)	2	4	6	At time of bud formation	1 month after bud formation	
Salicylic acid	109.00	138.33	147.00	163.00	130.00	115.00	133.72
β-aminobutyric acid	111.00	136.00	136.00	146.00	128.00	114.00	128.50
Calcium silicate	111.00	120.00	116.00	127.00	119.00	113.00	117.67
Potassium bicarbonate	110.00	125.00	127.00	135.00	124.00	113.00	122.33
Phosphoric acid	112.00	123.00	123.00	130.00	122.33	114.00	120.72
Dipotassium orthophosphate	109.00	159.00	163.00	189.00	142.00	116.00	146.33
Potassium silicate	110.00	129.00	128.00	140.00	124.00	113.00	124.00
Control	111.00	112.00	118.00	119.00	120.00	112.00	115.33
Mean	110.38	130.29	132.25	143.63	126.17	113.75	
$CD_{0.05}$	Treatments (T)=0.54 Day (D)=0.62 Treatment (T) x Day (D)= 1.51						

Table 2: Non-reducing sugar content in rose leaves after treatment with SAR chemicals at different intervals

Treatment (s)	Non-reducing sugar content (µg/g fresh weight) in rose leaves						Mean
	Sampling Interval (days)						
	0 (At the time of spray)	2	4	6	At time of bud formation	1 month after bud formation	
Salicylic acid	20.00	37.67	40.00	52.00	33.00	20.00	33.78
β-aminobutyric acid	23.00	33.67	36.33	49.33	31.00	21.00	32.39
Calcium silicate	20.00	25.67	26.00	31.00	28.00	19.00	24.94
Potassium bicarbonate	22.00	32.00	31.00	37.00	30.00	18.00	28.33
Phosphoric acid	21.00	29.00	28.00	35.00	29.00	20.00	27.00
Dipotassium orthophosphate	22.00	47.00	43.00	65.00	38.00	20.67	39.28

Potassium silicate	21.00	35.00	34.00	41.00	29.00	18.00	29.67
Control	22.00	26.00	23.00	25.00	27.00	19.00	23.67
Mean	21.38	33.25	32.67	41.92	30.63	19.46	
CD0.05	Treatments (T)=0.56 Day (D)=0.62 Treatment (T) x Day (D)= 1.56						

Table 3: Total phenol content in rose leaves after treatment with SAR chemicals at different intervals

Treatment (s)	Total phenol content (µg/g fresh weight) in rose leaves						Mean
	Sampling Interval (days)						
	0 (At the time of spray)	2	4	6	At time of bud formation	1 month after bud formation	
Salicylic acid	111.33	140.00	142.00	168.00	123.00	100.00	130.72
β-aminobutyric acid	111.00	137.00	135.00	151.00	121.00	98.00	125.50
Calcium silicate	110.67	121.00	120.00	128.00	115.00	100.67	115.89
Potassium bicarbonate	110.00	130.00	129.00	139.00	118.00	99.00	120.83
Phosphoric acid	111.00	127.00	126.00	134.00	118.00	101.00	119.50
Dipotassium orthophosphate	109.00	146.00	153.00	182.00	132.00	104.00	137.67
Potassium silicate	110.67	133.00	134.00	142.00	120.00	96.00	122.61
Control	110.00	113.00	114.00	115.00	114.00	100.00	111.00
Mean	110.46	130.88	131.63	144.88	120.13	99.83	
$CD_{0.05}$	Treatments (T)=0.35 Day (D)=0.40 Treatment (T) x Day (D)= 0.98						

Table 4: Polyphenol oxidase activity in rose leaves after treatment with SAR chemicals at different intervals

Treatment (s)	Polyphenol oxidase activity (Change in absorbance /min/mg fresh wt.)						Mean
	Sampling Interval (days)						
	0 (At the time of spray)	2	4	6	At time of bud formation	1 month after bud formation	
Salicylic acid	0.022	0.290	0.380	0.498	0.220	0.039	0.242
β-aminobutyric acid	0.019	0.267	0.320	0.475	0.193	0.035	0.218
Calcium silicate	0.020	0.146	0.167	0.165	0.139	0.034	0.112
Potassium bicarbonate	0.019	0.215	0.250	0.385	0.148	0.035	0.175
Phosphoric acid	0.021	0.196	0.210	0.267	0.142	0.031	0.145
Dipotassium orthophosphate	0.020	0.290	0.430	0.531	0.260	0.034	0.261
Potassium silicate	0.021	0.239	0.290	0.420	0.172	0.035	0.196
Control	0.023	0.126	0.135	0.131	0.133	0.036	0.097
Mean	0.021	0.221	0.273	0.359	0.176	0.035	
$CD_{0.05}$	Treatments (T)=0.004 Day (D)=0.005 Treatment (T) x Day (D)= 0.009						

Table 5: Peroxidase activity in rose leaves after treatment with SAR chemicals at different intervals

Treatment (s)	Peroxidase activity (Change in absorbance /min/mg fresh wt.)						Mean
	Sampling Interval (days)						
	0 (At the time of spray)	2	4	6	At time of bud formation	1 month after bud formation	
Salicylic acid	0.163	0.390	0.527	0.812	0.296	0.152	0.390
β-aminobutyric acid	0.164	0.354	0.477	0.720	0.290	0.152	0.360
Calcium silicate	0.164	0.197	0.324	0.321	0.191	0.153	0.225
Potassium bicarbonate	0.163	0.298	0.408	0.612	0.232	0.167	0.313
Phosphoric acid	0.162	0.274	0.382	0.575	0.202	0.152	0.291
Dipotassium orthophosphate	0.160	0.430	0.565	0.875	0.312	0.153	0.416
Potassium silicate	0.171	0.371	0.432	0.673	0.264	0.150	0.343
Control	0.160	0.168	0.176	0.176	0.170	0.152	0.167
Mean	0.163	0.310	0.411	0.596	0.245	0.154	
$CD_{0.05}$	Treatments (T)=0.008 Day (D)=0.009 Treatment (T) x Day (D)= 0.022						

Table 6: PAL activity in rose leaves after treatment with SAR chemicals at different intervals

Treatment (s)	PAL activity (µmol trans-cinnamic acid $min^{-1}g^{-1}$)						Mean
	Sampling Interval (days)						
	0 (At the time of spray)	2	4	6	At time of bud formation	1 month after bud formation	
Salicylic acid	0.232	0.455	0.845	1.020	0.376	0.212	0.523
β-aminobutyric acid	0.231	0.397	0.765	0.932	0.342	0.211	0.480
Calcium silicate	0.234	0.288	0.364	0.376	0.245	0.213	0.287
Potassium bicarbonate	0.234	0.321	0.621	0.765	0.263	0.215	0.403
Phosphoric acid	0.235	0.342	0.445	0.722	0.252	0.211	0.368
Dipotassium orthophosphate	0.230	0.467	0.911	1.230	0.462	0.213	0.586
Potassium silicate	0.231	0.356	0.623	0.911	0.293	0.214	0.438
Control	0.247	0.243	0.259	0.247	0.241	0.212	0.242
Mean	0.234	0.359	0.604	0.775	0.309	0.213	0.242
$CD_{0.05}$	Treatments (T)=0.010 Day (D)=0.011 Treatment (T) x Day (D)= 0.028						

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