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Effect of resistance inducers on biochemical characteristics of apple against *Dematophora necatrix* causing white root rot of apple

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Abstract
White root rot caused by *Dematophora necatrix* Hartig is one of the most devastating soil borne diseases attacking apple plants worldwide. In the present investigation, a pot experiment was conducted during 2016 at Model research Farm of Dr. YS Parmar university of Horticulture and Forestry Solan (H.P.) to evaluate the effect of induced resistance (IR) chemicals viz., salicylic acid, benzothiadiazole-s-methyl ester, potassium oxalate, β-amino butyric acid, calcium carbonate, sodium salicylate and di-potassium hydrogen orthophosphate against white root rot pathogen. These inducers were assayed at 0, 7, 14 and 21 days of pathogen inoculation. The results revealed that the salicylic acid treated apple seedlings developed highest amount of total phenol (493.13µg/g) which was followed by benzothiadiazole-s-methyl ester (451.80µg/g) and β-aminobutyric acid (422.60µg/g) respectively. While the lowest amount of total phenol was recorded in calcium carbonate (296.27µg/g). However, the maximum increase in polyphenol activity in terms of change in absorbance min^{-1} g^{-1} fresh weight of leaf (0.601) was recorded in salicylic acid treated apple seedlings followed by benzothiadiazole-s-methyl ester (0.551) and β-aminobutyric acid (0.505). The study likewise in polyphenol oxidase also revealed the effectiveness of salicylic acid in enhancing the peroxidase activity being highest in salicylic acid (0.703) followed by benzothiadiazole-s-methyl ester (0.643) and β-aminobutyric acid (0.609) respectively. However, the least activity was registered in calcium carbonate (0.366). In similar trends, highest PAL activity in terms of change in absorbance min^{-1} g^{-1} fresh weight of leaf was registered in salicylic acid treated seedlings (0.942) followed by benzothiadiazole-s-methyl ester (0.880) and β-aminobutyric acid (0.820). While least PAL activity was observed in treatment with (0.450). The salicylic acid estimation was also conducted and was found higher in benzothiadiazole-s-methyl ester (57.07µg/g) followed by β-aminobutyric acid (51.87 µg/g) respectively. Interestingly, it was found that irrespective of sampling intervals, maximum content of above tested biochemical parameters was noticed at 7^{th} days followed by 14^{th} days of pathogen inoculation. Our findings suggest that enhancing resistance in apple seedlings before infection could be an innovative, safe and alternative control method against white root rot of apple.

Keywords: White root rot, resistance inducers, pathogen inoculation, disease control

Introduction
Apple is an important temperate fruit crop not only for its economic importance but also for its nutritional value. Plant diseases caused by soil-borne plant pathogens considered the major constraints in agricultural production throughout the world, reducing yield and quality of crops. White root rot caused by *Dematophora necatrix* Hartig is the most devastating soil borne disease of apple (*Malus domestica* L.) in many countries (Sharma and Sharma, 2002) [30]. In Himachal Pradesh, the disease was first observed in 1961 (Agarwala, 1961) [2] and estimated loss due to this single disease was about Rs 1.3 million (Agarwala and Sharma, 1966) [1]. These losses are expected to be much more as the disease has now been reported to occur in almost all apple growing regions of the country (Sharma and Sharma, 2008) [31]. This serious diseases leading to delayed growth and subsequent death of severely infected plants.
The control of white root rot of apple is difficult as pathogen remain deep seated in the soil. The classical strategies, viz., use of fungicides and resistant varieties have largely been ineffective in controlling the disease because of its soil-borne nature. The repeated drenching of these fungicides is not only cost prohibitive but also leads to the development of resistant strains of pathogen.

The fungicide when applied as a soil drench fails to reach the infection site due to their adsorption on soil particles. Presently, carbendazim and aureofungin are being recommended for its management (Anonymous, 2014) [4]. The fungicides being systemic and undoubtedly proved somewhat effective, but can't be drenched repeatedly as the latter is not usually available in the market. Some cultural practices to control this disease (Gupta and Sharma, 1999) [15] though have been recommended but they usually fail to control the disease under high disease pressure situation. Furthermore, there are increasingly more restrictions on application of fungicides due to public concern about residues in food and harmful effects on the environment and human health. Hence there is a need for developing novel management strategy which is practically effective and environmentally safe.

Induction of resistance to pathogen is a promising approach for controlling plant diseases. Induced resistance is the general term by which all types of elicited responses that lead to enhanced protection against disease – including both locally and systemically induced resistance – can be designated (Hammerschmidt *et al.,* 2001) [27]. Keeping in view the adverse consequences of fungicides, reduction in use of fungicides is highly advocated. 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) [12, 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) [32, 10]. 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) [25]. Exogenous application of SA induces plant resistance to different kinds of pathogens that are associated with oxidative burst, cell wall enforcement, up- or down-regulation of gene expression (Oostendorp *et al.,* 2001) [20]. Phenylalanine ammonia lyase (PAL) catalyzes the deamination of L-phenylalanine to t-cinnamic acid, which is the first step in the phenylpropanoid pathway which supplies the precursors for phenolics, lignin and furanocoumarin, phytoalexins and other downstream metabolites (Tsuge *et al.,* 2004) [34]. Peroxidase (POD) oxidizes phenolics to more toxic quinones and generates hydrogen peroxide.The activities of PAL and POD may rapidly be enhanced under the influence of elicitors or pathogen attack.

As a consequence, resistance studies pertaining to *D. necatrix* are rather limited till date. So keeping in view the importance of disease, the present study was therefore, undertaken to find out the role of biochemical mechanisms involved in resistance against white root rot after treatment with induced resistance chemicals.

When microbes invade plant cells, polyphenol oxidases are involved in the oxidation of polyphenols into quinines (Soliva *et al.,* 2001) [33]. Peroxidases participate in wall building 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) [24]. These phenol oxidizing enzymes may participate in plant responses to microbes (Reimers *et al*., 1992; Chen *et al*., 2000) [29, 8].

Materials and Methods

A pot experiment was conducted at Model research Farm of Dr YS Parmar university of Horticulture and Forestry Solan (H.P.), India during 2016 to evaluate the effect of IR chemicals viz., salicylic acid, benzothiadiazole-s-methyl ester, potassium oxalate, β-amino butyric acid, calcium carbonate, sodium salicylate and di-potassium hydrogen orthophosphate against white root rot pathogen. In this study, seven induced resistance (IR) chemicals with 300ppm concentrations were diluted with distilled sterilized water were evaluated by root dip treatment and soil application for various biochemical parameters. Seedlings were dipped for about half an hour in respective solution of above mentioned resistance inducing chemicals before planting in the pots. The 250 ml concentration of above resistance inducers were applied again by drenching the soil around root zone just after 15 days of root dip treatment. Subsequently, after 20 days of soil application, treated plants were inoculated by adding the culture of *D. necatrix* grown on wheat grains @ 0.2 per cent (w/w). Each treatment was replicated three times in completely randomized design (CRD).

Estimation of biochemical changes associated with induced resistance

Activities of salicylic acid, total phenol and enzymes viz., polyphenol oxidase, phenylalanine, peroxidase associated with imparting resistance in host were estimated at 0 (day when pathogen was applied), 7, 14, and 21 days of pathogen inoculation. The leaf extracts of apple seedlings the roots of which surviving treatments with resistance inducer chemicals at 300ppm (concentration found most effective in controlling disease) were evaluated for different biochemical characters. Likewise, untreated seedlings leaves were evaluated for above mentioned biochemicals characters as per the standard procedures described by Mahadevan and Sridhar (1986) [21].

Extraction of phenol contents from leaves

One gram of fresh leaf sample both from IR treated chemicals as well as control (untreated) was cut into small pieces, homogenized in 80 per cent ethanol (4 ml alcohol/g tissue) and thereafter put in boiling water bath for 10 minutes. After 10 minutes of boiling it was cooled and crushed in pestle and mortar thoroughly at room temperature. The extract was filtered through double layer of muslin cloth and again through Whatman No**.** l filter paper. Final volume of the filterate was adjusted to 5ml with 80 per cent ethanol. The entire experiment was performed in dark to prevent light induced degradation of phenols.

Estimation of phenols

Reagents

1. Folin-Ciocalteu reagent (FCR)
2. 80% Ethanol
3. 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. These contents were shaken before heating in a boiling water bath exactly for one minute, and cooled in running 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 measured at 650nm using spectrophotometer in a Spectronic 20. A blank containing all the reagents minus Folin- Ciocalteu reagent was

used to adjust the absorbance to zero. Catechol was used as standard. The calibration curve was plotted using standard catechol.

Extraction of enzymes for polyphenol oxidase and peroxidase activity

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

Estimation of polyphenol oxidase activity

Reagents

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

The polyphenol oxidase activity was determined spectro photometrically. The assay mixture contained 1.95 ml of 0.1 M potassium phosphate buffer (pH 7.5). 1 ml of catechol (0.025M) and 50 µl diluted crude enzyme extract. The enzyme activity was expressed as change in absorbance at 420nm which was recorded at 30s intervals for 3 min. 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 0.1M phosphate buffer (pH 6.0). It was 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 crude 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. The change in absorbance at 420nm was recorded at 30s intervals for 3 min. 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) [17].

Extraction and estimation of phenylalanine ammonia lyase (PAL) activity

One gram sample of leaves 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 15000g 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) [13]. Sample containing 0.4 ml of crude enzyme extract was incubated with 0.5ml of 0.1M borate buffer, pH 8.8 and 0.5 ml of 12mM L-phenylalanine in the same buffer for 30 minutes at 30°C. The amount of trans-cinnamic acid formed from L- phenylalanine was measured spectrophotometrically at 290nm. Enzyme activity was expressed as µg of trans-cinnamic acid (in µ mol quantities) min^{-1} g^{-1} fresh weight.

Estimation of salicylic acid from leaf sample

One gram sample of leaf tissue was collected from the plant and frozen in liquid N_2 their homogenized in 10 ml 80 per cent methanol and stored in the deep-freeze (-20 °C). Later, the homogenate was centrifuged at 15000 rpm for 30 min at 4 °C. The pellet was discarded. After addition of ascorbic acid (0.1 g to 5 ml) the homogenate was evaporated 3 times in a rotary evaporator at 65 °C for 5 min. The residues were dissolved in 5 ml of 80 per cent methanol. Then 500 µl homogenate of the above extract were mixed with 250 µl HCl (10 N) and 1000 µl methanol, incubated in a water bath at 80 °C for 2 h, neutralized with 4-5 drops 1 M $NaHCO_3$, and 1000 µl methanol were added. The O.D. at 254nm was measured at 0, 7, 14 and 21 days of pathogen inoculation as per the procedure described by Dat *et al*. (2000) [11]. The content of salicylic acid was calculated and expressed as:

Amount of total salicylic acid = µg / g leaves

Result and Discussion

Apple seedlings pretreated with IR chemicals viz., salicylic acid, benzothiadiazole-s-methyl ester, potassium oxalate, β-amino butyric acid, calcium carbonate, sodium salicylate and di-potassium hydrogen orthophosphate were assayed at sampling intervals of 0 (day of pathogen inoculation), 7, 14 and 21 days, after pathogen inoculation for various biochemical constitutions like total phenols, polyphenol oxidase activity, peroxidase activity, PAL activity and salicylic acid activity. To the best of our knowledge, this is the first report carried out for the use of resistance inducer chemicals for estimating biochemical parameters of apple against white root rot of apple.

Total phenols

The perusal of data presented on total phenol (Fig.1) suggested that in all treatments total phenol content increased progressively up to 7th day of pathogen inoculation and thereafter it declined. The maximum amount of phenol content (651.67µg/g leaf tissue) was found in apple leaves treated with salicylic acid as root dip treatment followed by benzothiadiazole-s-methyl ester (605.67µg/g leaf tissue) and β-amino butyric acid (567.67µg/g leaf tissue) respectively. However, calcium carbonate (425.00µg/g leaf tissue) treated apple seedlings leaves registered least increase in amount of phenol content at 7th day of pathogen inoculation. Increased total phenol content was also observed in water treated control (350.67µg/g leaf tissue) over a period of time but the level was lower as compared to other treatments. The data also shows that the phenol content for all treatments increase from 0 to 7 days period but again decreases from 7 to 21 days. The result summarized that the phenol content in all treated plants increased up to a certain period and thereafter, it was decreased. These findings are in accordance with those of El-hendawy *et al.*, (2010) [14] who found that faba bean plants treated with salicylic acid either foliar spray or seed soaking showed the maximum acculmulation of total phenol in inducing resistance against chocolate spot disease as compared with the untreated ones. Similarly, Biswas *et al.*, (2012) [6] showed that total phenol contents for all the treatments increases from 5-10 days period but thereafter, again decreases from 10-15 days. The increased phenol content in treated plants might be responsible for defence mechanisms in plants. Jaypal and Mahadevan (1968) [18]

reported that sharp increase in phenol contents in incompatible host pathogen interaction promote resistance through hypersensitive reaction. Phenolic compounds being fungitoxic in nature, hence due to accumulation of phenolic compounds they directly involved in plant resistance either provided mechanical strength to host cell wall or to create a barrier to restrict the entry of pathogens.

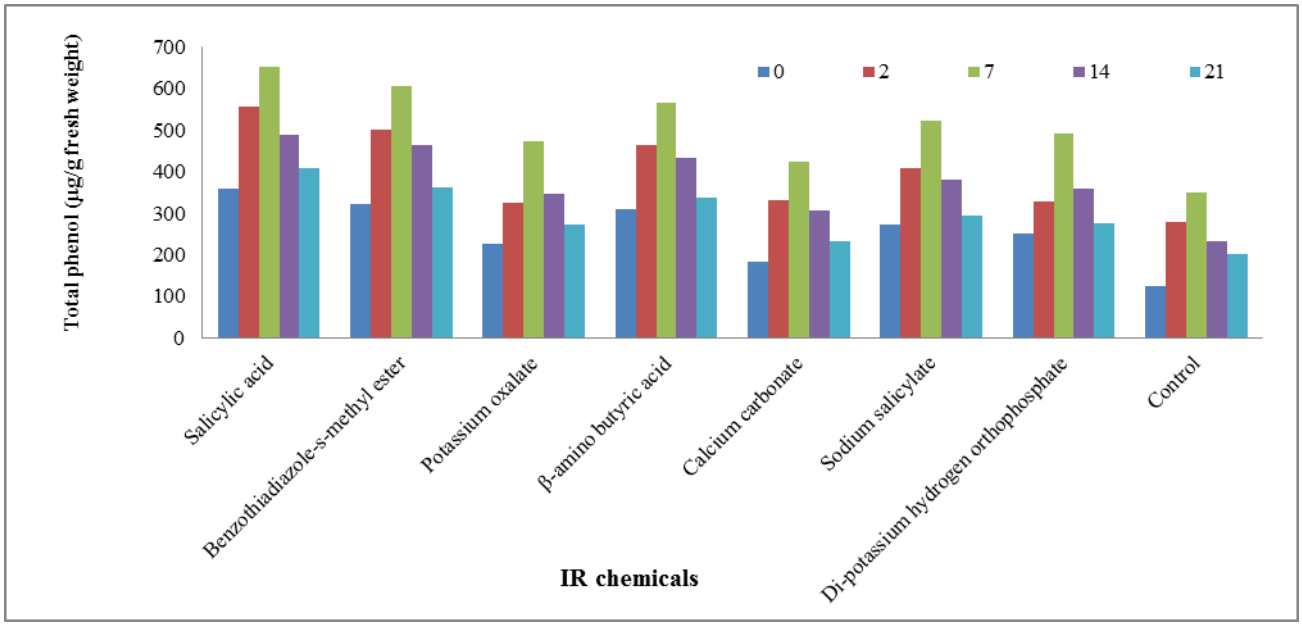

Fig 1: Total phenolic content (µg/g fresh wt. of leaf tissue) in apple seedlings treated with IR chemicals at 0, 7, 14 and 21 days of pathogen inoculation

Polyphenol oxidase (PPO) and Peroxidase (POD) activity

Increased polyphenol oxidase activity of apple seedlings up to 7th days of pathogen inoculation among all the tested IR chemicals shown in figure 2. The highest (0.843) level of PPO activity in terms of change in absorbance/min/mg fresh wt. of leaf tissue was determined in salicylic acid treated apple seedlings followed by benzothiadiazole-s-methyl ester (0.767). However, the lowest record of PPO activity was observed in calcium carbonate (0.403). In general, it was found that PPO activity was higher in chemical treated seedlings as compared to untreated control. Almoneafy *et al.*, (2013) [3] had also reported tomato plants treated with SA showed increased PPO activity on 5th and 9th day of inoculation. Similarly, higher level of PPO was observed in roots and shoots of susceptible cultivars of chickpea on treatment with elicitors SA (Raju *et al.*, 2008) [28]. Furthermore, Chandra *et al.*, (2007) [7] confirmed a decline in infection by *Rhizoctonia solani* with an increase in activity of PPO. The increased PPO activity results in the augmented rate of oxidation of phenolics to more toxic compound quinines which have fungitoxic properties and furthermore, reduced unavailability of nutrients or cellular proteins to the pathogens (Wuyts *et al.*, 2006) [35]. In addition, cross linking of quinines with phenolic compounds form a physical barrier to pathogens in the cell wall and generation of H_2O_2 and other reactive species (Li and Steffens, 2002) [19]. Thus, indicating the substantial role of IR chemicals in imparting resistance through the enhanced activity of PPO enzyme. Perusal of data (Fig.3) revealed that among all the tested IR chemicals, activity of peroxidase (POD) in apple seedlings increased dramatically with similar trends as noticed in PPO. The POD activity was increased significantly up to the 7th days of pathogen inoculation thereafter, it decreased in all the treatments. Maximum (0.943) POD activity in terms of change in absorbance /min/mg fresh wt. of leaf tissue was registered in seedlings treated with salicylic acid followed by benzothiadiazole-s-metyl ester (0.870) while the least increase in POD activity was noticed on calcium carbonate (0.643) treated apple seedlings. The results are similar with the findings of Mandel *et al.*, (2009) [23] who found that the activities of POD was 4 times higher than the control plants at 168 h of salicylic acid feeding through the roots. Similarly, Almoneafy *et al.*, (2013) [3] recorded high induction of POD activity on 9th day after inoculation on SA treated tomato plants. Increase in POD activity was mainly due to enhanced respiratory rate induced by the pathogen activity and its predominant participation in the wall-building processes such as rapid oxidation of phenols, suberization and lignifications of host plant cells thus displays diverse role in defence reaction against pathogenic agents (Asha and kannabiran, 2001) [5].

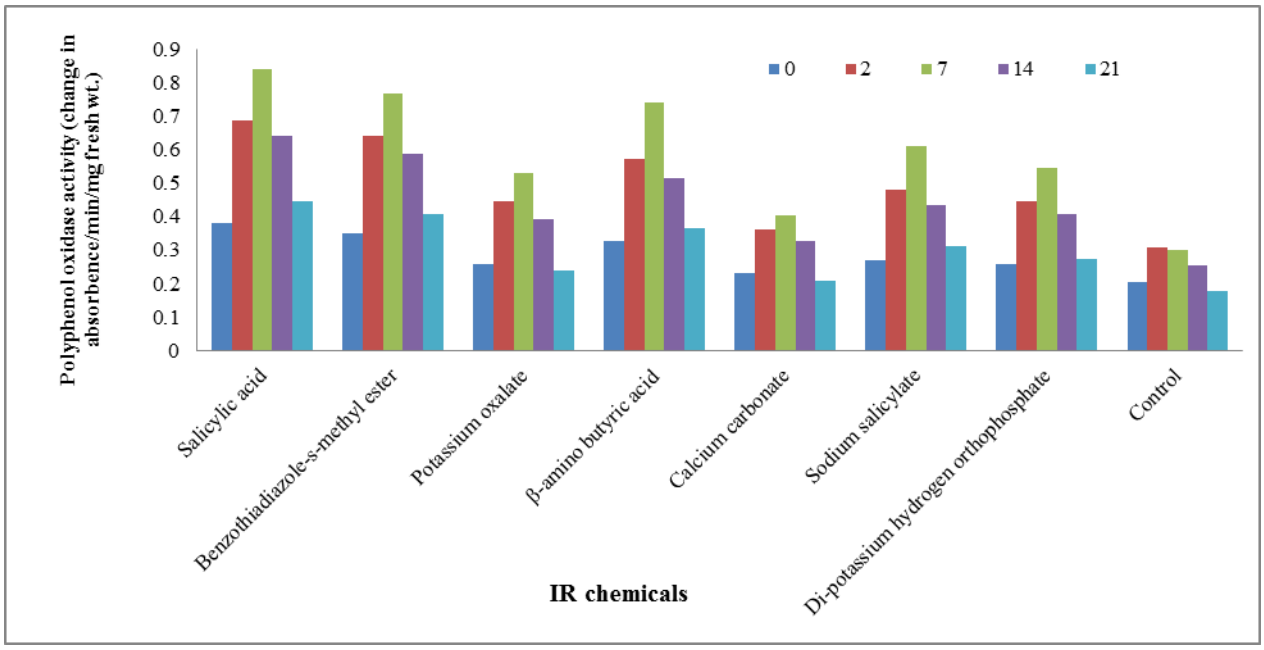

Fig 2: Polyphenol oxidase (change in absorbance /min/mg fresh wt. of leaf tissue) activity in apple seedlings treated with IR chemicals after 0, 7, 14 and 21 days of pathogen inoculation

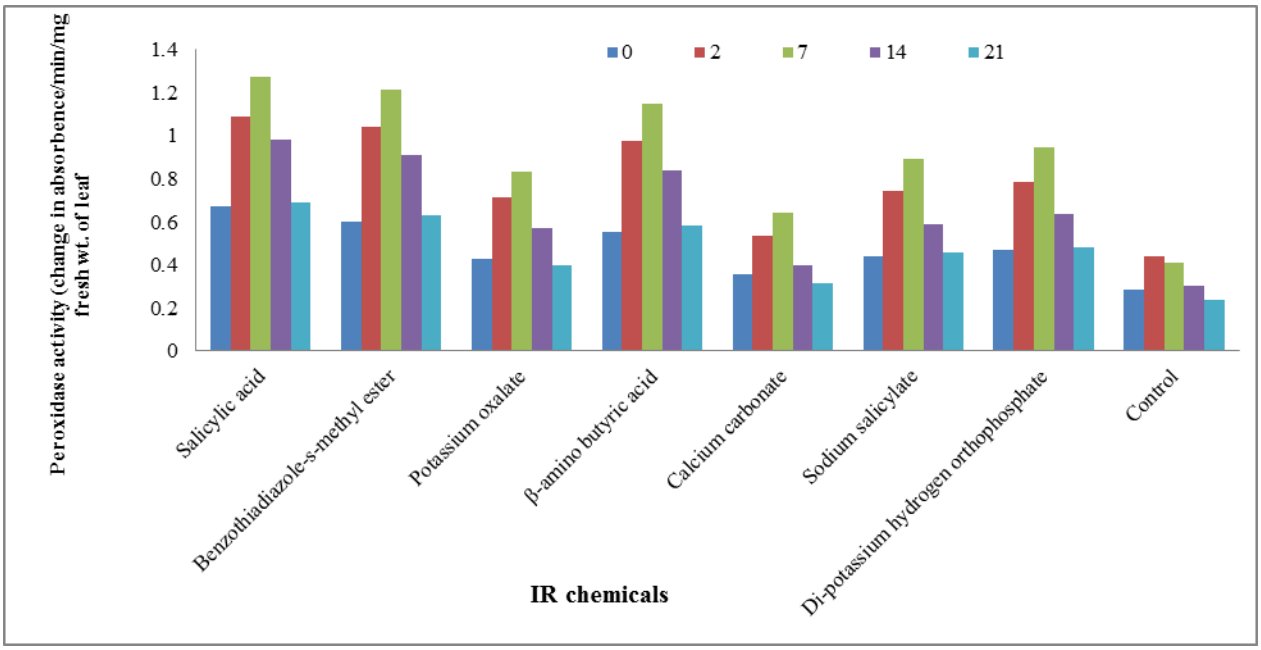

Fig 3: Peroxidase (change in absorbance /min/mg fresh wt. of leaf tissue) activity in apple seedlings treated with IR chemicals after 0, 7, 14 and 21 days of pathogen inoculation

Phenylalanine ammonia-lyase (PAL) activity

The data presented in figure 4 revealed that pretreatment of apple seedlings with all the resistance inducer chemicals, PAL activity increased significantly following pathogen inoculation (0 day) and thereafter continued to increase up to 7 days. Maximum PAL activity (1.273 µmol trans-cinnamic acid $min^{-1}g^{-1}$) was observed in salicylic acid treated apple seedling leaves followed by benzothiadiazole-s-methyl ester (1.217). However, least (0.643 µmol trans-cinnamic acid $min^{-1}g^{-1}$) PAL activity was recorded in calcium carbonate apple seedlings. The results are in agreement with those of Mandel *et al.*, (2009) [23] who detected 3.5 times higher activity of PAL on SA treated tomato plants than control after 72 h of inoculation. They also recorded the activity of enzyme which was 5.9 times higher than control plants on day seven (i.e., 168 h) of the SA feeding of the roots. PAL acts as a key enzyme in the phenyl propanoid metabolism in plants in that way involves in the conversion of amino acid phenyl alanine to trans-cinnamic acid, the precursors for the synthesis of lignin, flavanoid and phytoalexins (Hahlbrock and Scheel, 1989) [16].

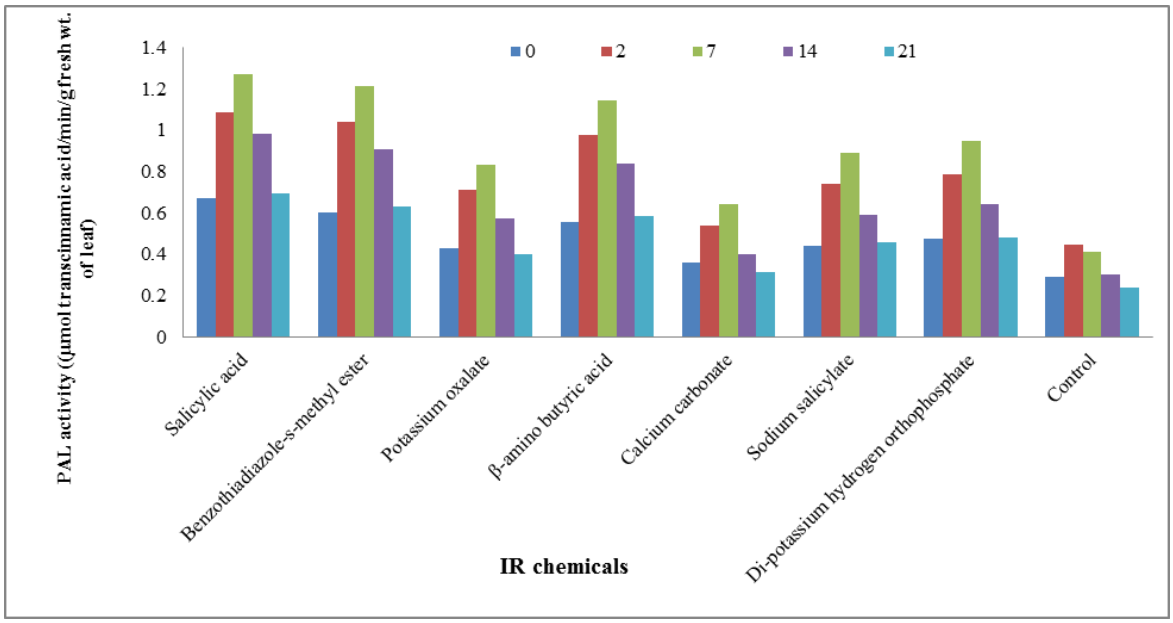

Fig 4: Phenylalanine ammonia-lyase (change in absorbance /min/mg fresh wt.) activity in apple seedlings treated with IR chemicals after 0, 7, 14 and 21 days of pathogen inoculation

Estimation of salicylic acid (SA)

The perusal of data (Fig.5) indicated that salicylic acid content increased sharply after pathogen inoculation (0-day). This increment in extent of SA content was determined mainly up to 14th days of pathogen inoculation and thereafter, it decreased gradually in all the treatments. The highest SA level (67.33µg/g fresh wt. of leaf tissue) was observed in benzothiadiazole-s-methyl ester followed by β-amino butyric acid (59.67µg/g fresh wt. of leaf tissue). However, least SA content (34.67µg/g fresh wt. of leaf tissue) was recorded in apple seedlings treated with calcium carbonate. The results are similar with the findings of Prakongkha *et al.*, (2013) [26] who established that grapevine plants treated with chitosan and BTH, SA level increased significantly seven days after treatment and much more seven days after challenge inoculation. In contrast, SA accumulation in non-treated, but pathogen-inoculated grape vine was considerably lower. Interestingly, in the earlier studies it was noticed that exogenous application of SA does not spread quickly inside plants which does not significantly increase the endogenous levels of SA. The increment of SA concentration in the leaf tissues might have contributed for enhanced resistance to the pathogen. SA was found to be an essential signal molecule involved in triggering defense responses and/or in sensitizing plant cells for a faster and stronger response to further pathogen attack. Furthermore, SA inhibits catalase production which leads to an increase in the concentration of hydrogen peroxide (H_2O_2) or active oxygen species during the hypersensitive response against pathogens thereby, acts as intermediates in the signaling cascade for the expression of genes related to defence (Chen *et al.*, 1993) [9].

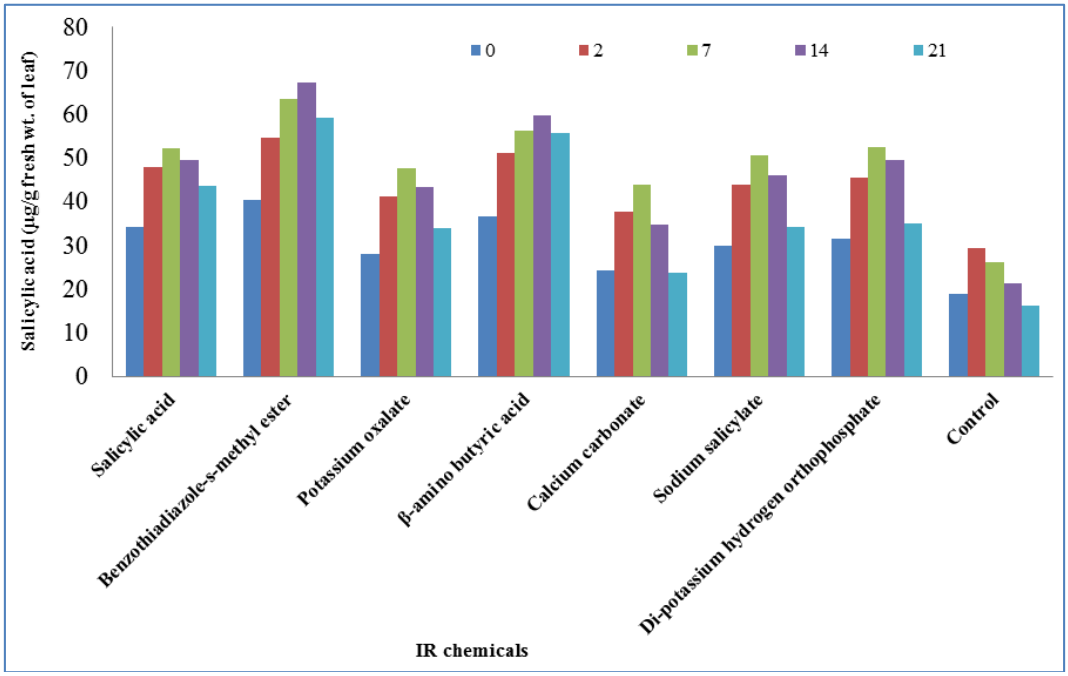

Fig 5: Salicylic acid content (µg/g fresh wt. of leaf tissue) in apple seedlings treated with IR chemicals after 0, 7, 14 and 21 days of pathogen inoculation

Conclusion

This biochemical study indicated that there was pronounced increase in all the biochemical parameters in apple seedlings when pretreated with resistance inducer chemicals. Our findings suggest that enhancing resistance in apple using resistance inducing chemicals especially salicylic acid, benzothiadiazole-s-methyl ester and β-aminobutyric acid could be used as possible alternative strategies for management of white root rot of apple. Although not fully understood, exogenous application of induced resistance chemical opens new horizons and might be commercially used as an eco-friendly, safe, cheap and easily applied method alternative to fungicides to control white root rot pathogen under pot and open field conditions.

References

1. Agarwala RK, Sharma VC. White root rot of apple in Himachal Pradesh. Indian phyto path. 1966; 19:82-86.
2. Agarwala RK. Problems of root rot in Himachal Pradesh and prospects of its control with antibiotics. Himachal Hort. 1961; 2:171-178.
3. Almoneafy AA, Ojaghian MR, Xu S, Ibrahim M, Xie G, Shi Y *et al*. Synergistic effect of acetyl salicylic acid and DL-β-aminobutyric acid on biocontrol efficacy of *Bacillus* strains against tomato bacterial wilt. Trop Plant Pathol. 2013; 38:102-113.
4. Anonymous. Package and practices of fruit crops. Directorate of Extension Education, Dr. YS Parmar University of Horticulture and Forestry, 2014, 240p.
5. Asha AN, Kannabiran B. Effect of Datura metal leaf extract on the enzymatic and nucleic acid changes in the chilli seedlings infected with *Colletotrichum capsici*. Indian phyto path. 2001; 54:373-375.
6. Biswas SK, Pandey NK, Rajik M. Inductions of defense response in tomato against Fusarium wilt through inorganic chemicals as inducers. Plant Pathology and Microbiology. 2012; 3:1-7.
7. Chandra A, Saxena R, Dubey A, Saxena P. Change in phenylalanine ammonia lyase activity and isozyme patterns of polyphenol oxidase and peroxidase by salicylic acid leading to enhanced resistance in cowpea against *Rhizoctonia solani*. Acta Physiol. Plant. 2007; 29:361-367.
8. Chen C, Belanger RR, Benhamou N, Paulitz TC. Defense enzymes induced in cucumber roots by treatment with plant growth-promoting rhizo bacteria (PGPR) and *Pythium aphanidermatum*. Physiol. Mol. Plant Pathol. 2000; 56:13-23.
9. Chen Z, Riciglano JW, Klessig DF. Purification and characterization of a soluble salicylic acid binding protein from tobacco. Proc. Natl. Acad. Sci. USA. 1993; 90:9533-9537.
10. Dao TTH, Linthorst HJM, Verpoorte R. Chalcone synthase and its functions in plant resistance. Phytochemistry Rev1. 2011; 0:397-412.
11. Dat JF, Lopez-Delgade H, Foyer CH, Scott IM. Effects of salicylic acid on oxidative stress and thermotolerance in tobacco. J Plant Physiol. 2000; 156:659-665.
12. Delaney TP, Friedrich L, Ryals JA. Arabidopsis signal transduction mutant defective in chemically and biologically induced disease resistance. Proc. Natl. Acad. Sci. USA. 1995; 92:6602-6606.
13. Dickerson DP, Pascholati SF, Haagerman AE, Butler LG, Nicholson RL. Phenylalanine ammonia lyase and hydroxy 1 cinnamate: CoA ligase in maize mesocotyls inoculated with *Helminthosporium maydis* or *Helminthosporium carbonum*. Physiol. Mol. Plant Pathol. 1984; 25:111-123.
14. El-Hendawy S, Shaban W, Sakagami JI. Does treating faba bean seeds with chemical inducers simultaneously increase chocolate spot disease resistance and yield under field conditions? Turk J Agric For. 2010; 34:475-485.
15. Gupta VK, Sharma SK. Soil borne diseases of apple and their management. In: Diseases of Horticultural Crops: Fruits (Verma LR and Sharma RC Eds.), Indus Publishing Co., New Delhi, 1999, 89-104p.
16. Hahlborck K, Sheel D. Physiology and molecular biology of phenylpropanoid metabolism. Plant Mol. Biol. 1989; 40:347-369.
17. Hammerschmidt R, Nuckles EM, Kuc J. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol. Mol. Plant Pathol. 1982; 20:73-82.
18. Jayapal R, Mahadevan A. Biochemical changes in banana leaves in response to leaf spot pathogens Indian Phyto path. 1968; 21:43-48.
19. Li L, Steffen JC. Overexpression of polyphenol oxidase in transgenic tomato plants results in enhanced bacterial disease resistance. Planta. 2002; 215:239-247.
20. Oostendorp M, Kunz W, Dietrich B, Staub T. Induced disease resistance in plants by chemicals, Eur. J Plant Pathol. 2001; 107:19-28.
21. Mahadevan A, Sridhar R. Methods in Physiological Plant Pathology. Madras: Shivakami Publications, 1986, 316p.
22. Maleck K, Levine A, Eulgem T. The transcriptome of *Arabidopsis thaliana* during systemic acquired resistance. Nature Genetics. 2000; 26:403-410.
23. Mandal S, Mallick N, Mitra A. Salicylic acid-induced resistance to *Fusarium oxysporum* f. sp. *lycopersici* in tomato. Plant Physiol Bio chem. 2009; 47:642-649.
24. Mohammadi M, Kazemi H. Changes in peroxidase and polyphenol oxidase activities in susceptible and resistant wheat heads inoculated with *Fusarium graminearum* and induced resistance. Plant Sci. 2002; 162:491-498.
25. Nicholson RL, Hammerschmidt R. Phenolic compounds and their role in disease resistance. Ann Rev Phytopathol. 1992; 30:369-389.
26. Prakongkha I, Sompong M, Wongkaew S, Athinuwat D, Buensanteai N. Foliar application of systemic acquired resistance (SAR) inducers for controlling grape anthracnose caused by *Sphaceloma ampelinum* de Bary in Thailand. Afr. J. Biotechnol. 2013; 12:5148-5156.
27. Hammerschmidt R, Me´ traux JP, van Loon LC. Inducing resistance: a summary of papers presented at the first international symposium on induced resistance to plant diseases, Corfu, Eur. J Plant Pathol. 2001; 7:1-6.
28. Raju S, Jayalakshmi SK, Sreeramulu K. Comparative study on the induction of defense related enzymes in two different cultivars of chickpea (*Cicer arietinum* L.) genotypes by salicylic acid, spermine and *Fusarium oxysporum* f. sp. *ciceri*. Australian J Crop Sci. 2008; 8:23-27.
29. Reimers PJ, Guo A, Leach JE. Increased activity of a cationic peroxidase associated with an incompatible interaction between *Xanthomonas oryzae* pv. *oryzae* and rice (*Oryza sativa*). Plant Physiol. 1992; 99:1044-1050.
30. Sharma M, Sharma SK. Effect of thermal regimes on the survival of *Dematophora necatrix*, causing white root rot. Indian Phytopath. 2002; 55:310-312.

31. Sharma JN, Sharma RC. Soil borne diseases of temperate fruits and their management. In: Advances in Soil Borne Plant Diseases. MK Naik and GS Devika Rani (eds.). New India Publishing Agency, New Delhi, 2008, 219-231p.
32. Slatnar A, Mikulic Petkovsek M, Halbwirth H, Stampar F, Stich K, Veberic R. Enzyme activity of the phenylpropanoid pathway as a response to apple scab infection. Ann Appl Bio. 2010; 156:449-456.
33. Soliva RC, Elez P, Sebastian M, Martin O. Evaluation of browning effect on avocado puree preserved by combined methods Innov. Food Sci and Emerg technol, 2001; 1:261-268.
34. Tsuge H, Ochiai Y, Inoue T, Ohu K, Tsuno K, Kaku Y *et al*. Involvement of phosphoglucose isomerase in pathogenicity of *Xanthomonas oryzae* pv. *oryzae*, Phytopathol. 2004; 94:478-483.
35. Wuytz N, Waele DD, Swennen R. Extraction and partial characterization of polyphenol oxidase from banana (*Musa acuminata* Grande naine) roots. Plant Physiol Bio chem. 2006; 44:308-314.