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IMPACT OF RESISTANCE INDUCERS AND CHEMICALS ON BIOCHEMICAL PARAMETERS IN POMEGRANATE SEEDLINGS AGAINST BACTERIAL BLIGHT (*XANTHOMONAS CITRI* PV. *PUNICAE*)

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ABSTRACT

The research explores the potential of resistance inducers and chemicals, including salicylic acid, â-amino butyric acid (BABA), and others, in mitigating bacterial blight in pomegranate cultivation. Bacterial blight poses a significant threat to pomegranate production, causing economic losses. Current disease management relies heavily on chemical compounds, raising concerns about toxicity and ecological impact. The study investigates the impact of various resistance inducers on biochemical parameters in pomegranate plants under both pot conditions. The experiment conducted at Dr YS Parmar University of Horticulture and Forestry evaluates the effects of different resistance inducers on plant defense enzymes, total phenol, polyphenol and salicylic acid content. The treatments include individual and combined applications of salicylic acid, BABA, streptocycline, and bronopal. The results show variations in biochemical parameters, with certain treatments demonstrating higher levels of salicylic acid, polyphenol oxidase activity, peroxidase activity, total phenol content and phenylalanine ammonia-lyase (PAL) activity. The findings suggest that certain combinations, such as salicylic acid+ BABA+ Streptocycline + Bronopal, exhibit promising results in enhancing plant resilience against bacterial blight. The study contributes to the shift towards more sustainable and environmentally friendly disease control methods in pomegranate cultivation. Overall, the research emphasizes the importance of exploring alternative approaches to reduce reliance on chemical interventions in agriculture.

Key words : Pomegranate Seedlings, Bacterial blight, Total phenol, biochemical parameters.

Introduction

The pomegranate, scientifically known as *Punica granatum* L., was historically widely consumed in tropical and subtropical regions, with its peel, seeds, and pulp recognized for medicinal properties (Kiran Kumar *et al*., 2018). Indigenous to Iran, it is cultivated in various countries worldwide, facing challenges such as dry spells, lack of suitable varieties, environmental factors, nutritional deficiencies, post-harvest issues, inappropriate storage and susceptibility to diseases like “bacterial blight” caused by *Xanthomonas citri* pv. *punicae*.

Bacterial blight poses a significant threat to pomegranate production, affecting all above-ground plant parts, especially fruits. Since its initial report in Delhi in 1952, the disease has spread to states like Maharashtra, Karnataka, Andhra Pradesh and Himachal Pradesh, causing economic losses and impacting the export of high-quality pomegranates from India since 2002. The disease manifests as light-passing spots on leaves, evolving into light to dark brown hues with moist margins, leading to premature leaf fall. Stems and branches are also damaged, resulting in encircling damage and cracking. Fruits exhibit dark brown irregular spots with an oily appearance, progressing to ‘L’ or ‘Y’ shaped cracks that eventually split the fruit open. In severe cases, bacterial blight can devastate entire orchards, causing economic losses of 70-80 percent. Despite allocating a significant portion (25-30 percent) of production expenses to plant cultivation, effectively managing this disease remains challenging. The recent application of plant resistance inducers shows promise in enhancing plant resilience against pathogens, triggering plant defense mechanisms and reducing reliance on chemical interventions.The concept of inducing resistance involves using natural products from fungi, bacteria, or plants to elicit non-specific deterrent responses against pathogens. This approach, supported by studies (Bernonville *et al*., 2014), offers a sustainable method of disease control, protecting the environment, farmers, and consumers. Recent research has explored resistance inducers like salicylic acid, β-aminobutyric acid (BABA) and acibenzolar-s-methyl to induce systemic acquired resistance in plants. These inducers enhance disease tolerance, leading to improved yield and quality of pomegranate fruits. Understanding long-distance signalling mechanisms and key enzymes like salicylic acid, polyphenol peroxidase and peroxidase (POD) contributes to deciphering plant defense responses.

Abiotic resistance inducers (ARI) encompass chemical compounds like salicylic acid, L-Glutamate, jasmonic acid, acibenzolar-S-methyl, baminobutyric acid and others. These AIs, either used individually or in combination, help manage plant diseases of various origins. In a specific study, salicylic acid, â-amino butyric acid, and other combinations were evaluated for their impact on pomegranate plants' biochemical parameters after application.

In conclusion, the exploration of resistance inducers provides a promising avenue for sustainable and environmentally friendly disease control in pomegranate cultivation. This research contributes to evolving agricultural practices, moving away from heavy reliance on chemical interventions towards more holistic and eco-friendly approaches.

Materials and Methods

An experiment was conducted at Model Farm of Dr YS Parmar university of Horticulture and Forestry, Solan (H.P.), India during 2021 to evaluate the effect of foliar application of salicylic acid, β-amino butyric acid, salicylic acid + Streptocycline, Salicylic acid+ Bronopal, BABA+ Streptocycline, BABA+ Bronopal, Salicylic acid+ Streptocycline + Bronopal, BABA+ Streptocycline+ Bronopal, Salicylic acid+ BABA+ Streptocycline + Bronopal against bacterial blight pathogen under pot and field conditions. The pots (size 45cm×30cm dia.) were filled with 10 kg of sterilized potting mixtures (soil mixed with farm yard manure and sand in the ration of 4:1:1). Subsequently, pomegranate seedlings raised through cuttings from cv. Kandhari were planted in each pot, each pot was planted with three seedlings and for each treatment 3 pots were used utilizing CRD experimental design. In this study, nine different treatments were diluted with distilled sterilized water and evaluated for various biochemical parameters. Plants were sprayed twice before onset of monsoon in respective solution of above-mentioned treatments. Subsequently, after 20 days of application, treated plants were inoculated by spraying the culture suspension of *Xanthomonas citri* pv. *punicae* grown on nutrient glucose broth (1×10^8 cfu/ml). The assay for plant defence enzyme was performed at 0, 2, 5, 10 and 14 days of pathogen inoculation.

Extraction of phenol contents from leaves

Phenols were extracted from the fresh leaves following the method of Mahadevan and Sridhar (1982). One gram sample of fresh leaves from treated plants was cut into small pieces, put in boiling alcohol, in a water bath for 5-10 minutes (4ml alcohol/g tissue). After 15 minutes of boiling, it was cooled and crushed in pestle and mortar thoroughly at room temperature. The extract was passed through two layers of cheese cloth and filtered through Whatman No. l filter paper. Final volume was adjusted with 80 per cent ethanol. The whole process was performed in dark to prevent light induced degradation of phenols.

Estimation of phenols

Total phenols were estimated by the method described by Bray *et al*. (1955). 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 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 650 nm using spectrophotometer. 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.

Estimation of Salicylic acid from leaf sample

One gram sample of leaf tissue was collected from the treated plant and frozen in liquid nitrogen then homogenized in 10 ml 80 per cent methanol and stored in the deep-freeze (-20^{0}C). Later, the homogenate was centrifuged at 15,000 rpm for 30 minutes at 4^{0}C. The pellet was discarded. After addition of ascorbic acid (0.1g) the homogenate was evaporated 3 times in a rotary evaporator at 65^0C for 5 minutes. 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 (10N) and 1000 µl methanol, incubated in a water bath at 80^0C for 2 h, neutralized with 4-5 drops 1 M $NaHCO_{3,}$ and 1000 µl methanol were added. The O.D. at 254 nm was measured at 0, 2, 5, 10 and 14 days of pathogen inoculation as per the procedure described by Dat *et al*. (1998). The content of Salicylic acid was calculated and expressed as:

Amount of total Salicylic acid = µg/g leaves

Extraction of enzymes for polyphenol oxidase and peroxidase activity

A leaf sample (0.5 g) from treated plants was homogenized in 5 ml of 0.1M potassium phosphate buffer (pH 7.5) containing 2 per cent (w/v) polyvinyl-polypyrrolidone (PVP) and 0.25 per cent (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 : 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 (0.025M) and 50 µl diluted crude enzyme extract. The enzyme activity was expressed as change in absorbance at 420 nm, which was recorded at 30 seconds 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 : 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 ± 1^0C) for 30 minutes. The change in absorbance at 420 nm was recorded at 30 seconds 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 sample of leaves from treated plants was homogenized in 3 ml of ice-cold 0.1 M sodium borate buffer (pH 7.0), containing 1.4 mM of 2-mercaptoethanol and 0.1 g of insoluble polyvinylpyrrolidone. The extract was filtered through cheese cloth, and the filtrate was centrifuged at 15,000 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 crude 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 30^0C. The amount of trans-cinnamic acid formed from L-phenylalanine was measured spectrophotometrically at 290 nm. Enzyme activity was expressed as ìg of trans-cinnamic acid (in µ mol quantities) min^{-1} g^{-1} fresh weight.

Statistical analysis

The data was recorded in triplicate and analyzed, using the IBM SPSS software version 16.0. Analysis of variance was determined and the mean values were compared by Duncan's multiple range tests at $P< 0.05$.

Results and Discussion

Effect of individual and conjoint application of Resistance inducers and chemicals on total phenol content in pomegranate seedlings cv. Kandhari under pot conditions

The data presented in Table 1 indicated, that the overall phenol content in all treatment's gradual increase up to the 5th day of inoculation, followed by a subsequent decline. The treatment with the highest phenol content was Salicylic acid+ BABA+ Streptocycline+ Bronopal, reaching a peak at (677.58 µg/g fresh weight of leaf tissue). This was closely followed by Salicylic acid+ Streptocycline + Bronopal (661.99 µg/g fresh weight of leaf tissue) and BABA+Streptocycline+ Bronopal (653.92 µg/g fresh weight of leaf tissue). On the other hand, the lowest phenol content (622.12 µg/g fresh weight of leaf tissue) was observed in pomegranate plants treated with Salicylic acid+ Streptocycline, followed by salicylic acid alone (636.17 µg/g fresh wt. of leaf tissue). Both the Inoculated control (289.75 µg/g fresh wt. of leaf tissue) and Un-inoculated control (281.26 µg/g fresh wt. of leaf tissue) demonstrated an increase in phenol content over time, although the levels remained comparatively lower than in other treatments. The combination of nitrogen and exogenously applied salicylic acid has been found to positively affect the phenol concentration in leaves but has a negative impact on proline concentration, as demonstrated by Maity *et al*. (2017). These findings align with the results of El-Hendawy *et al*. (2010), who observed that faba bean plants treated with salicylic acid through foliar spray or seed soaking exhibited the highest accumulation of total phenols, contributing to the induction of resistance against chocolate spot disease compared

Table 1 : Effect of individual and conjoint application of Resistance inducers and chemicals on total phenol content in pomegranate seedlings cv. Kandhari under pot conditions.

T. no.	Treatments	Conc. (ppm)	Total phenol content(μg/g fresh weight) in pomegranate leaves					Mean
			Sampling interval after pathogen inoculation (days)					
			0	2	5	10	14	
1.	**Salicylic acid**	**300**	421.83	587.26	636.17	480.35	380.32	**501.19**
2.	**β-amino butyric acid (BABA)**	**300**	411.93	563.66	643.57	468.97	370.89	**491.80**
3.	**Salicylic acid+ Streptocycline**	**300+ 500**	430.59	580.74	622.12	501.43	394.63	**505.90**
4.	**Salicylic acid + Bronopal**	**300+ 500**	448.15	603.09	657.72	498.80	393.66	**520.28**
5.	**BABA+ Streptocycline**	**300+ 500**	445.32	595.45	649.35	495.32	375.21	**512.13**
6.	**BABA+ Bronopal**	**300+ 500**	441.02	596.60	644.92	480.62	384.17	**509.47**
7.	**Salicylic acid+ Streptocycline + Bronopal**	**300+ 500 + 500**	455.12	614.63	661.99	517.61	402.37	**530.34**
8.	**BABA+Streptocycline+ Bronopal**	**300 + 500 + 500**	451.23	604.65	653.92	520.23	409.23	**527.85**
9.	**Salicylic acid+ BABA+ Streptocycline + Bronopal**	**300 + 300 + 500 + 500**	469.23	611.88	677.58	512.23	402.23	**534.63**
10.	**Inoculated control**	**-**	205.26	288.35	289.75	253.62	200.09	**247.41**
11.	**Un-inoculated control**	**-**	199.00	246.29	281.26	212.61	150.00	**217.83**
	Mean		**398.06**	**535.69**	**583.49**	**449.25**	**351.16**	
	$CD_{0.05}$		**Treatment= 1.67, Interval= 1.12 Treatment × Interval= 3.72**					

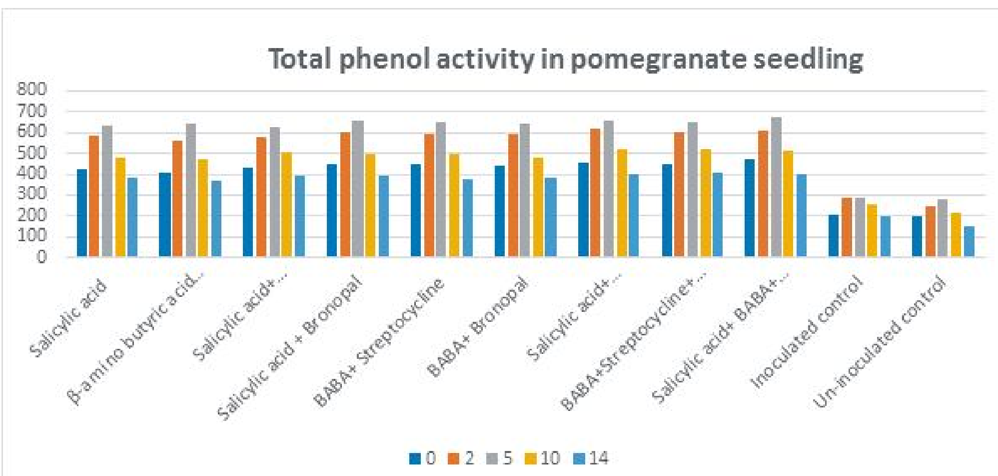

Fig. 1 : Effect of individual and conjoint application of Resistance inducers and chemicals on total phenol content in pomegranate seedlings cv. Kandhari under pot conditions.

to untreated plants. Kalaivani *et al*. (2020) investigated the effectiveness of various chemical inducers, including salicylic acid, potassium silicate, potassium sulfate, fosetyl aluminum, humic acid at 1000 ppm and COC 3g/lit + streptocycline 300ppm (control), against *Pectobacterium carotovorum* subsp. *carotovorum*. They observed that these inducers led to the activation of defense enzymes such as peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), superoxide dismutase (SOD) and phenols by the fifth day post-inoculation. Similarly, Biswas *et al.* (2012) noted an increase in total phenol contents for all treatments during the 5-10 day period, followed by a subsequent decrease from 10-15 days. The heightened phenol levels in treated plants are likely associated with plant defense mechanisms. According to Jaypal and Mahadevan (1968),

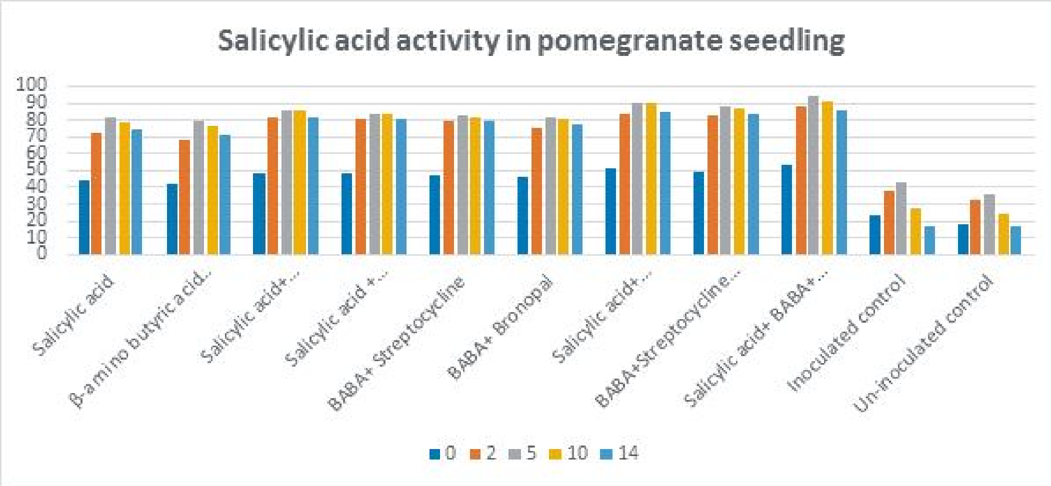

Fig. 2 : Effect of individual and conjoint application of Resistance inducers and chemicals on salicylic acid content pomegranate seedlings cv. Kandhari under pot conditions.

Table 2 : Effect of individual and conjoint application of Resistance inducers and chemicals on salicylic acid content pomegranate seedlings cv. Kandhari under pot conditions.

T. no.	Treatments	Conc. (ppm)	Salicylic acid content(µg/g fresh weight) in pomegranate leaves					Mean
			Sampling interval after pathogen inoculation (days)					
			0	2	5	10	14	
1.	Salicylic acid	300	44.58	72.45	81.57	79.00	74.68	70.46
2.	β-amino butyric acid (BABA)	300	42.47	67.90	79.90	76.19	71.56	67.60
3.	Salicylic acid+ Streptocycline	300+ 500	48.25	81.58	85.42	85.60	81.58	76.49
4.	Salicylic acid + Bronopal	300+ 500	47.94	80.82	83.65	83.60	80.43	75.29
5.	BABA+ Streptocycline	300+ 500	46.90	79.23	82.64	81.67	79.50	73.99
6.	BABA+ Bronopal	300+ 500	45.99	75.38	81.92	80.61	77.14	72.21
7.	Salicylic acid+ Streptocycline + Bronopal	300+ 500 + 500	51.68	84.02	90.01	89.76	84.82	80.06
8.	BABA+Streptocycline+ Bronopal	300 + 500 + 500	49.67	82.70	87.72	86.99	83.58	78.13
9.	Salicylic acid+ BABA+ Streptocycline + Bronopal	300 + 300 + 500 + 500	53.30	88.42	94.27	91.58	86.38	82.79
10.	Inoculated control	-	22.90	38.03	42.90	27.22	16.86	29.58
11.	Un-inoculated control	-	17.61	32.72	36.11	24.48	17.21	25.63
	Mean		42.85	71.20	76.92	73.34	68.52	
	$CD_{0.05}$		Treatment= 1.151, Interval= 0.776 Treatment × Interval= 2.574					

a significant rise in phenol contents in incompatible host-pathogen interactions promotes resistance through a hypersensitive reaction. Phenolic compounds, known for their fungitoxic properties, may strengthen the host cell wall mechanically or create a barrier to impede pathogen entry.

Effect of individual and conjoint application of Resistance inducers and chemicals on salicylic acid content pomegranate seedlings cv. Kandhari under pot conditions

The perusal of data (Table 2) indicated that salicylic acid content increased immediately after pathogen inoculation (0 day). This increment in SA content was

Table 3 : Effect of individual and conjoint application of Resistance inducers and chemicals on polyphenol oxidase activity content pomegranate seedlings cv. Kandhari under pot conditions.

T. no.	Treatments	Conc. (ppm)	Polyphenol oxidase activity (Change in absorbance /min/mg fresh wt.) Sampling interval after pathogen inoculation (days)					Mean
			0	2	5	10	14	
1.	**Salicylic acid**	**300**	0.381	0.640	0.852	0.608	0.405	0.577
2.	**β-amino butyric acid (BABA)**	**300**	0.300	0.557	0.846	0.601	0.397	0.540
3.	**Salicylic acid+ Streptocycline**	**300+ 500**	0.441	0.685	0.900	0.666	0.476	0.634
4.	**Salicylic acid + Bronopal**	**300+ 500**	0.434	0.674	0.886	0.654	0.533	0.636
5.	**BABA+ Streptocycline**	**300+ 500**	0.428	0.668	0.875	0.648	0.459	0.616
6.	**BABA+ Bronopal**	**300+ 500**	0.422	0.662	0.865	0.640	0.450	0.608
7.	**Salicylic acid+ Streptocycline + Bronopal**	**300+ 500 + 500**	0.491	0.702	0.916	0.688	0.495	0.658
8.	**BABA+Streptocycline+ Bronopal**	**300 + 500 + 500**	0.450	0.694	0.909	0.676	0.517	0.649
9.	**Salicylic acid+ BABA+ Streptocycline + Bronopal**	**300 + 300 + 500 + 500**	0.464	0.736	0.938	0.704	0.505	0.669
10.	**Inoculated control**	-	0.231	0.346	0.396	0.305	0.255	0.307
11.	**Un-inoculated control**	-	0.185	0.313	0.333	0.246	0.178	0.251
	Mean		0.384	0.607	0.792	0.585	0.425	
	$CD_{0.05}$		**Treatment= 0.052, Interval= 0.035 Treatment × Interval= 0.116**					

observed mainly up to 5th days of pathogen inoculation and thereafter, it decreased gradually in all the treatments. The highest SA level (94.27 µg/g fresh wt. of leaf tissue) was observed in treatment Salicylic acid+ BABA+ Streptocycline + Bronopal followed by treatment Salicylic acid+ Streptocycline + Bronopal (90.01 µg/g fresh wt. of leaf tissue) and BABA+Streptocycline+ Bronopal (87.72 µg/g fresh wt. of leaf tissue). However, least SA content (79.90 µg/g fresh wt. of leaf tissue) was recorded in pomegranate plants treated with treatment â-amino butyric acid (BABA) followed by salicylic acid (81.57 µg/g fresh wt. of leaf tissue). Increased SA content was also observed in inoculated control (42.90µg/g fresh wt. of leaf tissue) and Un-inoculated control (36.11 µg/g fresh wt. of leaf tissue) over a period of time but the level was lower as compared to other treatments. Prakongkha *et al*. (2013) conducted a study on grapevine plants treated with chitosan and BTH. They observed a significant increase in salicylic acid (SA) levels after 7 days of treatment, with even higher levels after subsequent pathogen inoculation. In contrast, pathogen-inoculated grapevines showed considerably low SA accumulation. Interestingly, previous research indicated that externally applied SA does not rapidly spread within plants, resulting in a minimal increase in endogenous SA levels. The rise in SA concentration in leaf tissues likely contributed to enhanced resistance against pathogens. SA was identified as a crucial signaling molecule, playing a role in triggering defense responses and sensitizing plant cells for a quicker and more robust reaction to subsequent pathogen attacks. Additionally, SA inhibits catalase production, leading to an elevated concentration of hydrogen peroxide (H_2O_2) or active oxygen species during the hypersensitive response against pathogens. Consequently, SA acts as an intermediate in the signaling cascade, influencing the expression of genes related to defense (Chen *et al.*, 1993).

Effect of individual and conjoint application of Resistance inducers and chemicals on polyphenol oxidase activity content pomegranate seedlings cv. Kandhari under pot conditions

The data recorded in Table 3 revealed how resistance inducers and chemicals, applied singly or in combination, affect PPO activity in pomegranate cv. Kandhari seedlings. This activity is shown to increase up to the fifth day following pathogen inoculation in all tested resistance inducers and chemicals. Salicylic acid+

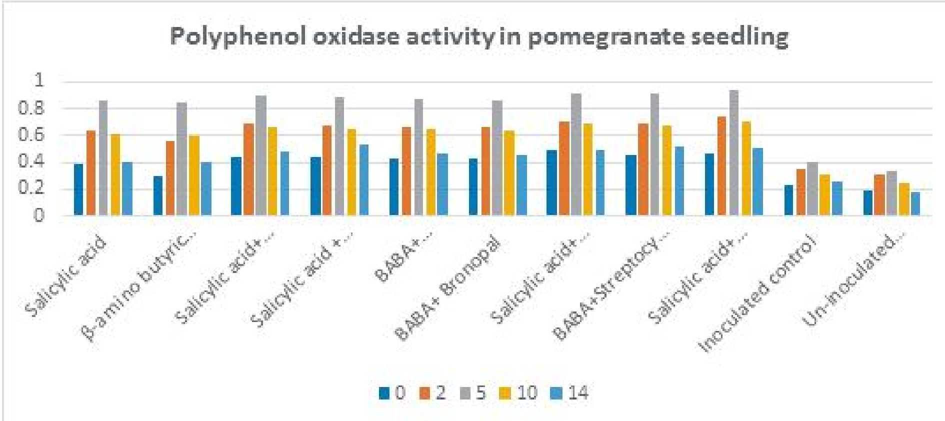

Fig. 3 : Effect of individual and conjoint application of Resistance inducers and chemicals on polyphenol oxidase activity content pomegranate seedlings cv. Kandhari under pot conditions.

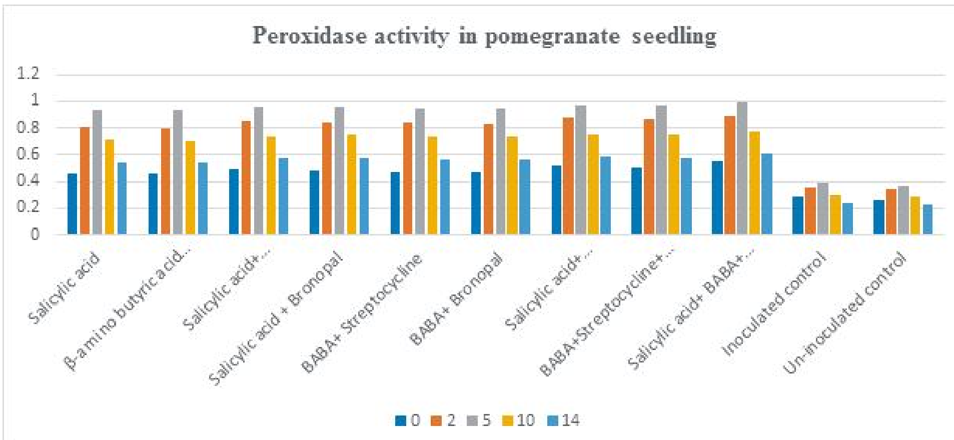

Fig. 4 : Effect of individual and conjoint application of Resistance inducers and chemicals on peroxidase activity in pomegranate seedlings cv. Kandhari under pot conditions.

BABA+ Streptocycline + Bronopal treated pomegranate seedlings exhibited the highest level of PPO activity (0.938) in terms of change in absorbance/min/mg fresh weight of leaf tissue, followed by Salicylic acid+ Streptocycline + Bronopal (0.916) and BABA+Streptocycline+ Bronopal (0.909). On the other hand, β-amino butyric acid (BABA) had the lowest PPO activity record (0.846). PPO activity was often shown to be increased in treated seedlings when compared to water spray control and pathogen-inoculated seedlings. Li *et al*. (2020) also noted a continuous rise in PPO activities in both BABA-treated and control plants following *Botrytis cinerea* inoculation. Over the course of the sample period, PPO activity in BABA-treated plants was continuously higher than in control plants. Similarly, after salicylic acid treatment, sensitive cultivars of chickpea showed increased levels of PPO in their roots and shoots (Raju *et al*., 2008). In addition, Chandra *et al*. (2007) reported a correlation between an increase in PPO activity and a decrease in *Rhizoctonia solani* infection. The elevated PPO activity decreased the pathogens' inability to access nutrients or cellular proteins and accelerated the oxidation of phenolics to the more toxic chemical quinines, which has fungitoxic qualities (Wuytz *et al*., 2006). Furthermore, quinine cross-linking with phenolic compounds creates a physical barrier in the cell wall that prevents pathogens from entering and produces H_2O_2 and other reactive species (Li and Steffens, 2002). Consequently, this suggests that IR compounds play a significant role in imparting resistance via increasing PPO enzyme activity. Kalaivanan *et al*. (2020) investigated the effectiveness of various chemical inducers, including salicylic acid, potassium silicate, potassium sulfate, fosetyl aluminum, humic acid at 1000 ppm and COC 3g/lit + streptocycline 300ppm (control), against *Pectobacterium carotovorum* subsp. *carotovorum*. They observed that these inducers led to the activation of defense enzymes such as peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), superoxide dismutase (SOD) and phenols by the fifth day post-inoculation.

Table 4 : Effect of individual and conjoint application of Resistance inducers and chemicals on peroxidase activity in pomegranate seedlings cv. Kandhari under pot conditions.

T. no.	Treatments	Conc. (ppm)	Peroxidase activity (Change in absorbance/min/mg fresh wt.)					Mean
			Sampling interval after pathogen inoculation (days)					
			0	2	5	10	14	
1.	Salicylic acid	300	0.461	0.809	0.939	0.709	0.545	0.693
2.	β-amino butyric acid (BABA)	300	0.458	0.800	0.929	0.701	0.539	0.685
3.	Salicylic acid+ Streptocycline	300+ 500	0.491	0.857	0.959	0.738	0.576	0.724
4.	Salicylic acid + Bronopal	300+ 500	0.482	0.843	0.952	0.748	0.579	0.721
5.	BABA+ Streptocycline	300+ 500	0.476	0.837	0.948	0.741	0.565	0.713
6.	BABA+ Bronopal	300+ 500	0.469	0.825	0.941	0.735	0.559	0.706
7.	Salicylic acid+ Streptocycline + Bronopal	300+ 500 + 500	0.512	0.871	0.971	0.754	0.585	0.739
8.	BABA+Streptocycline+ Bronopal	300 + 500 + 500	0.501	0.863	0.965	0.743	0.579	0.730
9.	Salicylic acid+ BABA+ Streptocycline + Bronopal	300 + 300 + 500 + 500	0.548	0.889	0.991	0.774	0.612	0.763
10.	Inoculated control	-	0.285	0.351	0.391	0.297	0.241	0.313
11.	Un-inoculated control	-	0.260	0.339	0.371	0.281	0.224	0.295
	Mean		0.449	0.753	0.851	0.656	0.509	
	$CD_{0.05}$		Treatment= 0.012, Interval= 0.008 Treatment × Interval= 0.028					

Effect of individual and conjoint application of Resistance inducers and chemicals on peroxidase activity in pomegranate seedlings cv. Kandhari under pot conditions

Table 4 showed that pre-treating pomegranate cv. Kandhari plants with chemicals that induce resistance increases peroxidase activity significantly after pathogen inoculation (0 day) and continues to increase for up to 5 days. The plants treated with salicylic acid+ BABA+ Streptocycline + Bronopal showed the highest peroxidase activity (0.991) in terms of change in absorbance/min/ mg fresh weight of leaf tissue, followed by BABA+Streptocycline + Bronopal (0.965) and salicylic acid + BABA+ Streptocycline + Bronopal (0.971). Nevertheless, pomegranate seedlings treated with BABA (0.929) showed the lowest level of peroxidase activity. Over time, increased peroxidase activity was also seen in the water-treated control (0.371) and pathogen-inoculated control (0.391), but at a lower level than in the other treatments. On the ninth day following inoculation, Almoneafy *et al.* (2013) observed a significant increase in POD activity in tomato plants treated with salicylic acid. POD activity increased primarily as a result of the pathogen's increased respiratory rate. It also played a significant role in wall-building processes, including the fast oxidation of phenols, suberization, and lignification of host plant cells, demonstrating a variety of defense responses against pathogenic agents (Asha and Kannabiran, 2001). Kalaivanan *et al.* (2020) investigated the effectiveness of various chemical inducers, including salicylic acid, potassium silicate, potassium sulfate, fosetyl aluminum, humic acid at 1000 ppm, and COC 3g/lit + streptocycline 300 ppm (control), against *Pectobacterium carotovorum* subsp. *carotovorum*. They observed that these inducers led to the activation of defense enzymes such as peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), superoxide dismutase (SOD), and phenols by the fifth day post-inoculation.

Effect of individual and conjoint application of Resistance inducers and chemicals on phenylalanine ammonia lyase (PAL) activity in pomegranate seedlings cv. Kandhari under pot conditions

The data in Table 5 indicated a significant increase in phenylalanine ammonia lyase (PAL) activity following pathogen inoculation (day 0), persisting for up to 5 days after pre-treating pomegranate seedlings (cv. Kandhari)

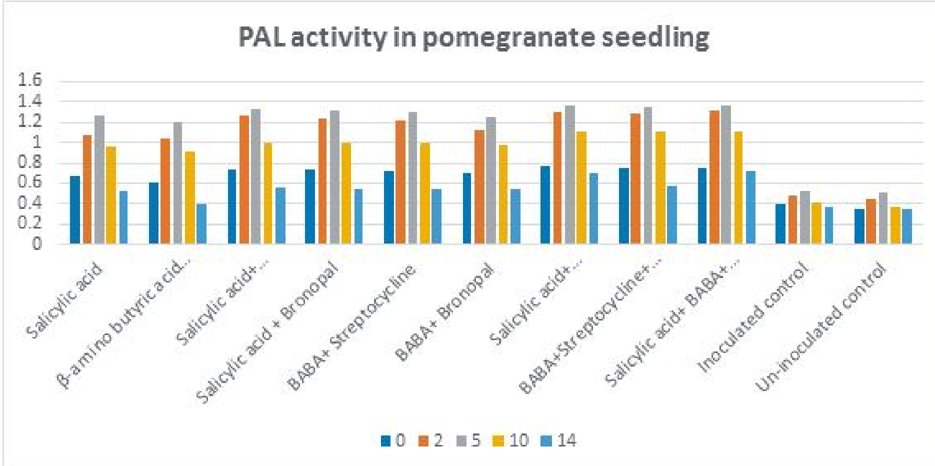

Fig. 5 : Effect of individual and conjoint application of Resistance inducers and chemicals on phenylalanine ammonia lyase (PAL) activity in pomegranate seedlings cv. Kandhari under pot conditions.

Table 5 : Effect of individual and conjoint application of Resistance inducers and chemicals on phenylalanine ammonia lyase (PAL) activity in pomegranate seedlings cv. Kandhari under pot conditions.

T. no.	Treatments	Conc. (ppm)	PAL activity (µmol trans-cinnamic acid $min^{-1}g^{-1}$) Sampling interval after pathogen inoculation (days)					Mean
			0	2	5	10	14	
1.	Salicylic acid	300	0.670	1.069	1.268	0.954	0.525	0.897
2.	β-amino butyric acid (BABA)	300	0.599	1.039	1.209	0.917	0.399	0.832
3.	Salicylic acid+ Streptocycline	300+ 500	0.743	1.266	1.336	0.998	0.565	0.982
4.	Salicylic acid + Bronopal	300+ 500	0.736	1.241	1.319	0.990	0.549	0.967
5.	BABA+ Streptocycline	300+ 500	0.725	1.212	1.299	0.987	0.543	0.953
6.	BABA+ Bronopal	300+ 500	0.701	1.119	1.254	0.975	0.537	0.917
7.	Salicylic acid+ Streptocycline + Bronopal	300+ 500 + 500	0.761	1.298	1.365	1.110	0.710	1.049
8.	BABA+Streptocycline+ Bronopal	300 + 500 + 500	0.755	1.278	1.354	1.102	0.580	1.014
9.	Salicylic acid+ BABA+ Streptocycline + Bronopal	300 + 300 + 500 + 500	0.745	1.316	1.369	1.115	0.715	1.052
10.	Inoculated control	-	0.391	0.479	0.534	0.414	0.370	0.438
11.	Un-inoculated control	-	0.347	0.445	0.514	0.369	0.345	0.404
	Mean		0.652	1.069	1.165	0.903	0.531	
	$CD_{0.05}$		Treatment= 0.017, Interval= 0.011 Treatment × Interval= 0.037					

with both individual and simultaneous applications of resistance inducers and chemicals. Pomegranate seedling leaves treated with salicylic acid + BABA + Streptocycline + Bronopal exhibited the highest phenylalanine ammonia lyase (PAL) activity (1.369 ìmol trans-cinnamic acid $min^{-1}g^{-1}$), followed by subsequent treatment with salicylic acid + Streptocycline + Bronopal (1.365). In contrast, BABA-treated pomegranate seedlings displayed the lowest phenylalanine ammonia lyase (PAL) activity (1.209 µmol trans-cinnamic acid $min^{-1}g^{-1}$). Over time, phenylalanine ammonia lyase (PAL) activity also increased in the water-treated control (0.514 µmol trans-cinnamic acid $min^{-1}g^{-1}$) and pathogen-treated control (0.534), though to a lesser extent than in the other treatments. Li *et al.* (2020) observed a significant phenylalanine ammonia lyase (PAL) activity increase at 4-, 16-, 24- and 72-hours post-inoculation with BABA against *Botrytis cinerea*, compared to the control. These findings align with Mandal *et al.* (2009), who reported a 3.5-fold increase in phenylalanine ammonia lyase (PAL) activity in tomato plants treated with salicylic acid 72 hours post-inoculation, compared to the control. They further noted that after seven days (168 hours) of salicylic acid feeding to the roots, enzyme activity was 5.9 times higher than in control plants. In plant metabolism, phenylalanine ammonia lyase (PAL) plays a vital role in phenylpropanoid metabolism, converting the amino acid phenylalanine to trans-cinnamic acid—a precursor for the synthesis of lignin, flavonoids, and phytoalexins (Hahlbrock and Sheel, 1989). Kalaivanan *et al.* (2020) conducted experiments to evaluate the efficacy of various chemical inducers, including salicylic acid, potassium silicate, potassium sulfate, fosetyl aluminum, humic acid at 1000 ppm, and COC 3g/lit + streptocycline 300ppm, against *Pectobacterium carotovorum* subsp. *carotovorum* which were found to stimulate defense enzymes such as peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), superoxide dismutase (SOD) and phenols by the 5th day after inoculation.

Conclusion

In conclusion, the study aimed to evaluate the impact of various resistance inducers and chemicals on the defense mechanisms of pomegranate seedlings against bacterial blight caused by *Xanthomonas citri* pv. *punicae*. The results indicated that the foliar application of resistance inducers such as salicylic acid, β-amino butyric acid (BABA) and their combinations, along with chemicals like Streptocycline and Bronopal, significantly influenced biochemical parameters related to plant defense. The study observed an increase in salicylic acid content, polyphenol oxidase (PPO) activity, peroxidase activity, total phenol content, and phenylalanine ammonia lyase (PAL) activity in pomegranate seedlings treated with the mentioned compounds. These biochemical changes were particularly notable in treatments combining multiple resistance inducers. The findings align with previous research indicating that salicylic acid, a crucial signaling molecule, plays a significant role in triggering defense responses and sensitizing plant cells for a robust reaction to pathogen attacks. PPO and peroxidase activities were also elevated, contributing to enhanced resistance against pathogens. The rise in phenol content was associated with plant defense mechanisms, strengthening cell walls and creating barriers against pathogen entry. The study contributes valuable insights into environmentally friendly and sustainable approaches for managing bacterial blight in pomegranate cultivation. The adoption of resistance inducers offers a promising avenue to reduce reliance on chemical interventions, addressing concerns related to toxicity and ecological impacts. The research encourages a shift toward holistic and eco-friendly agricultural practices, promoting plant health and productivity.

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