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Induced systemic resistance against *X. axonopodis* pv. *citri.* through chemical elicitors

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Abstract
ISR elicitors *viz.*, Salicylic acid, B-aminobutyric acid, Jasmonic acid, Isonicotinic acid, L-arginine, L-methionine and L-ornithine were evaluated *in vitro* (Pot culture), observations were recorded on Per cent disease intensity and lesion diameter of acid lime bacterial canker. The ISR elicitors (chemical) applied plants of acid lime revealed that, the mean per cent disease intensity, in all the treatments it was decreased over control. However, minimum mean per cent disease intensity was recorded with B-aminobutyric acid (08.00) followed by Salicylic acid (08.66), L-methionine (09.89), Jasmonic acid (13.11), Isonicotinic acid (17.78) and L-ornithine (19.66); whereas it was recorded maximum in L-arginine (19.88). After 15 days of challenge inoculation with *X. axonopodis* pv. *citri*, the lesion diameter decreased over control. However, no lesson was recorded with Salicylic acid and B-aminobutyric acid which were found significantly superior amongst the rest all; whereas, minimum lesion diameter was recorded with L-methionine (01.33). Decreasing trend in Lesion diameter after 30 days and 45 days of challenge inoculation with *X. axonopodis* pv. *citri*, over control was observed. However, minimum lesion diameter after 30 days and 45 days was recorded same with Salicylic and B-aminobutyric acid (01.33 and 02.67, respectively) and found superior over the rest all; whereas, it was found maximum with L-arginine (05.33 and 07.00, respectively) and L-ornithine (05.33 and 07.67, respectively).

Keywords: Bacterial canker, acid lime, chemicals, induced systemic resistance

Introduction
Copper (Cu) based bactericides are a standard control measure for citrus canker worldwide (Kuhara, 1978., Stall *et al.* 1982., Leite and Mohan 1990) [27, 29, 28]. Cu reduces bacterial populations on leaf surfaces, but multiple applications are needed to achieve adequate control on susceptible citrus hosts (Graham, 2001., Stall *et al.*, 1982 & 1990) [30, 29]. Long-term use of Cu bactericides have other possible disadvantages, including resistance in xanthomonad populations (Marco, and Stall 1983., Rinaldi and Leite 2000) [32, 30, 33]. Systemic acquired resistance (SAR) is a mechanism of induced defense that may confer long-lasting protection against a broad spectrum of microorganisms (van Loon *et al*. 2006) [22]. Induced resistance requires the signal molecule salicylic acid and is associated with the accumulation of pathogenicity-related (PR) proteins, and induction of defense enzymes. The latter two are thought to contribute to resistance. (Ojha and Chaterjee, 2012) [23]. β-Aminobutyric acid (BABA) is a non-protein amino acid which induces resistance against a broad range of disease-causing organisms including fungi, bacteria, viruses, and nematodes (Jakab *et al*. 2001; Francis *et al*. 2009) [24, 25]. Pretreatment with BABA can also be effective against bacterial diseases by inducing systemic resistance against *X. citri* pv. *citri* (Graham and Leite 2007; Francis *et al*. 2009) [25]. Resistance to diseases in plants was induced by BABA, either through the activation of a signaling pathway that depends on SA or through the activation of a novel signaling cascade not dependent on SA but on jasmonic acid or ethylene (Zimmerli *et al*. 2000) [26].

Induced resistance can also be provoked by some chemicals such as salicylic acid (SA), jasmonic acid (JA), Iso-nicotinic acid (NA) (Esmailzadeh *et al*. 2008., Wang and Liu 2012., Li and Wang 2013) [12, 13, 14]. Salicylic acid plays an important role in induction of plant defense against a variety of biotic and abiotic stresses through morphological, physiological and biochemical mechanisms (Yao and Tian, 2005., Hayat *et al*. 2010) [15, 16]. It is involved in endogenous signaling, mediating in plant defense against pathogens. Jasmonic acid is a class of lipidic plant hormones, not only involved in plant-microbe's interactions in defense and symbiosis (Ballaré, 2011) [17] but also induced secondary plant metabolites, like alkaloids, terpenoids, flavonoids, coumarins and so forth (Faurie *et al.* 2009) [18]. In addition, NA is involved in metabolic functions such as biosynthesis of nucleic acids, amino acids and pantothenate, lignin formation and synthesis of pathogenesis-related (PR) proteins and regulated redox process (Hanson and Roje 2001) [19]. So far, some studies have provided the evidence that phytohormones like SA, JA and NA have the potentiality for mitigating plant disease by modulating redox balance activating enzymes (Cossins., 2000., Wen *et al*. 2008) [20, 21]. Above information reveals that chemicals like salicylic acid, β-aminobutyric acid (BABA), jasmonic acid, Isonicotinic acid and L-arginine, L-methionine, L-ornithine create a favorable condition for plant to resist pathogen by modulating physiological and molecular process.

However, the research on the effect of ISR chemicals on the induction of resistance in plants is very limited. So, we tested the effect of the salicylic acid (SA), β-aminobutyric acid (BABA), jasmonic acid, Isonicotinic acid and L-arginine, L-methionine, L-ornithine on induced resistance against bacterial canker disease in acid lime plants. The term ISR used here to include ISR and SAR inducers with activity against pathogen.

Material and methods

ISR Chemicals

ISR Elicitors chemicals *viz.*, salicylic acid (SA), β-aminobutyric acid (BABA), jasmonic acid, Isonicotinic acid and L-arginine, L-methionine, L-ornithine were used during present study.

Plant material and treatments

One year acid lime (*Citrus aurantifoliia*) healthy seedlings were collected from Central nursery VNMKV, Parbhani and was transplanted in polyethylene bags/ earthen pots (one seedling/bag or pot) containing sterilized potting mixture of sterile loamy soil and sand (2:1). Potted acid lime seedlings were maintained under shed/ in screen house and watered as and when required. Acid lime plants were foliar spry-treated with ISR @ 50 ppm concentration of each. Distilled water spry-treated plants were also maintained as control. This experiment was designed in screen house (Pot culture) by using CRD with three replications and total eight treatments including control.

Bacterial culture and inoculation

For the preparation of the bacterial suspension, the bacterial strain was cultured in Nutrient agar (NA) and grown at 28ºC for 24 h. Then, a single colony was transferred to Yeast Extract Peptone medium and grown at 28^0C for 24 h to log phase. The final bacterial suspension was pelleleted at 10,000 rpm for 20 min and again suspended in distilled water to reach to a 7×10^8 colony forming unit (CFU/ml).

Immature leaves were inoculated using a tuberculin syringe to produce a zone of water-soaked tissue. The infiltrated areas of the leaf were approximately 6 mm diameter and contain approximately 5 µl of bacterial suspension. Two injection infiltrations were performed on each side of the mid-vein and eight leaves were inoculated per plant.

Observations of per cent disease intensity and lesion diameter were recorded at 15, 30 and 45 days after challenge inoculation of bacterial suspension. The results of experiments are mentioned in table

Table 1: *In vitro* efficacy of ISR elicitors (Chemicals) on per cent disease intensity (pot culture)

Tr. No.	Treatments	PDI			Mean PDI	Mean PDC
		15DAI	30DAI	45DAI		
T_1	Jasmonic acid	3.00 (9.87)*	13.67 (21.67)*	22.67 (28.41)*	13.11	47.09
T_2	Salicylic acid	0.00 (0.00)	8.33 (16.76)	17.67 (24.83)	08.66	65.05
T_3	Isonicotinic acid	9.00 (17.43)	17.67 (24.83)	26.67 (31.07)	17.78	28.24
T_4	B-aminobutyric acid (BABA)	0.00 (0.00)	8.67 (17.10)	15.33 (23.27)	08.00	67.71
T_5	L-arginine	9.33 (17.77)	21.00 (27.24)	29.33 (32.77)	19.88	19.77
T_6	L-methionine	2.67 (9.26)	8.33 (16.76)	18.67 (25.57)	09.89	60.08
T_7	L-ornithine	9.00 (17.43)	21.00 (27.23)	29.00 (32.56)	19.66	20.66
T_8	Control	12.67 (20.82)	25.67 (30.41)	36.00 (36.85)	24.78	00.00
	S.E. ±	0.64	0.66	0.58	--	--
	C.D. (P=0.01)	1.95	2.00	1.76	--	--

*Mean of three replications Fingers in parenthesis are angular transformed values

Results (Table 1 and Fig. 1) indicated that in ISR elicitor applied plants of acid lime after 15 days of challenge inoculation with *X. axonopodis* pv. *citri,* percent disease intensity was significantly decreased over control and it was ranged from 0.00 to 09.33. However, disease intensity was recorded with Salicylic acid and B-aminobutyric acid which were found superior amongst the rest all; whereas minimum per cent disease intensity was recorded in L-methionine (02.67) followed by Jasmonic acid (03.00), both were at par with each other, while L-arginine recorded maximum per cent disease intensity which was at par with remaining two treatments *viz.*, Isonicotinic acid (09.00) and L-ornithine (09.00). After 30 days of challenge inoculation with *X. axonopodis* pv. *citri*, in all the treatments percent disease intensity significantly decreased over control and it was ranged from 08.33 to 25.67. However, minimum per cent disease intensity was recorded with Salicylic acid (08.33) followed by L-methionine, B-aminobutyric acid (08.67), but all three were at par with each other and found significantly superior treatments, Isonicotinic acid (17.67), Jasmonic acid (13.67); whereas least superior treatments were L-arginine (21.00) and L-ornithine (21.00) which recoded maximum per cent disease intensity but both were at par with each other. The percent disease intensity after 45 days of challenge inoculation with *X. axonopodis* pv. *citri*, also significantly decreased over control and it was ranged from 15.33 to 29.33. However, significantly superior treatment was found B-aminobutyric acid (15.33) in which minimum per cent disease intensity was recorded, followed by Salicylic acid (17.67), L-methionine (18.67) but both were at par with each other, Jasmonic acid (22.67), Isonicotinic acid (26.67), L-ornithine (29.00) and L-arginine (29.33), but Isonicotinic acid, L-ornithine and L-arginine were at par with each other and found least superior.

In case of mean per cent disease intensity, in all the treatments it was decreased over control (24.78) and varied from 08.00 to

19.88. However, minimum mean per cent disease intensity was recorded with B-aminobutyric acid (08.00), followed by Salicylic acid (08.66), L-methionine (09.89), Jasmonic acid (13.11), Isonicotinic acid (17.78) and L-ornithine (19.66) while maximum mean per cent disease intensity was recorded in L-arginine (19.88). Mean per cent disease control was ranged from 19.77 to 67.71. However, maximum mean per cent disease control was recorded with B-aminobutyric acid (67.71), followed by Salicylic acid (65.05), L-methionine (60.08), Jasmonic acid (47.09), Isonicotinic acid (28.24) and L-ornithine (20.66). While, minimum mean per cent disease control was recorded with L-arginine (19.77).

Table 2: Effect of ISR elicitors on lesion diameter of bacterial canker disease

Tr. No.	Treatments	Lesion diameter (mm)*			Mean lesion diameter (mm)	Per cent reduction of lesion diameter over control
		15 DAI	30 DAI	45 DAI		
T_1	Jasmonic acid	1.67 (1.62)*	2.67 (1.91)*	4.33 (2.30)*	2.89	52.70
T_2	Salicylic acid	0.00 (1.00)	1.33 (1.52)	2.67 (1.91)	1.33	78.23
T_3	Isonicotinic acid	1.67 (1.62)	3.00 (2.00)	4.67 (2.37)	2.55	58.26
T_4	B-aminobutyric acid (BABA)	0.00 (1.00)	1.33 (1.52)	2.67 (1.91)	1.66	72.83
T_5	L-arginine	2.67 (1.91)	5.33 (2.50)	7.00 (2.82)	5.00	18.00
T_6	L-methionine	1.33 (1.52)	2.00 (1.71)	3.00 (1.98)	2.11	65.45
T_7	L-ornithine	2.33 (1.82)	5.33 (2.51)	7.67 (2.94)	5.11	16.36
T_8	Control	3.67 (2.15)	6.00 (2.64)	8.67 (3.10)	6.11	00.00
S.E. ±		0.08	0.10	0.08	--	--
C.D. (P=0.01)		0.25	0.30	0.27	--	--

*Mean of three replications
Fingers in parenthesis are square root transformed values

Results (Table 2, Fig. 2) revealed that, after 15 days of challenge inoculation with *X. axonopodis* pv. *citri*, the lesion diameter significantly decreased over control (03.67) and it was ranged from 0.00 to 02.67. However, no lesson was recorded with Salicylic acid and B-aminobutyric acid which were found significantly superior amongst the rest all; whereas, minimum lesion diameter was recorded with L-methionine (01.33), followed by Jasmonic acid (01.67), Isonicotinic acid (01.67) but were at par with each other, while with L-ornithine (02.33) and L-arginine (02.67) recorded maximum lesion diameter which were at par with each other. Lesion diameter after 30 days of challenge inoculation with *X. axonopodis* pv. *citri*, also significantly decreased over control (06.00) and it was ranged from 1.33 to 5.33. However, minimum lesion diameter was recorded with Salicylic (01.33) acid and B-aminobutyric acid (0.133), but were at par with each other and found significantly superior over the rest all followed by L-methionine (02.0), Jasmonic acid (01.67), Isonicotinic acid (03.00), but were at par with each other; whereas, maximum lesion diameter was found with L-arginine (05.33) and L-ornithine (05.33), but were at par. Lesion diameter after 45 days of challenge inoculation with *X. axonopodis* pv. *citri*, also significantly decreased over control (08.67) and it was ranged from 02.67 to 07.67. However, minimum lesion diameter was recorded with Salicylic (02.67) acid and B-aminobutyric acid (02.67), but were at par with each other and found significantly superior over the rest all; followed by L-methionine (03.00), Jasmonic acid (04.33), Isonicotinic acid (04.67), but were at par with each other; whereas, maximum lesion diameter was found with L-arginine (07.00) and L-ornithine (07.67).

Mean lesion diameter, in all the treatments was decreased over control (06.11) and varied from 01.33 to 05.11. However, minimum mean lesion diameter was recorded with Salicylic acid (01.33), followed by B-aminobutyric acid (01.66), L-methionine (02.11), Isonicotinic acid (02.55), Jasmonic acid (02.89), and L-arginine (05.00) while, maximum mean lesion diameter was recorded with L-ornithine (05.11); whereas mean per cent reduction of lesion diameter over control (0.00) was ranged from 16.36 to78.23. However, maximum mean per cent reduction of lesion diameter over control was recorded with Salicylic acid (78.23), followed by B-aminobutyric acid (72.83), L-methionine (65.45), Isonicotinic acid (58.26), Jasmonic acid (52.70), and L-arginine (18.00) while, minimum mean per cent reduction of lesion diameter over control was recorded in L-ornithine (13.36).

Similar findings were recorded earlier by many workers. Beheshti *et al*. (2011) treated ISR chemicals, by spray-treatment on the lime (*Citrus aurantifolia*) plants challenge inoculated with *X. axonopodis* pv *citri.* with β-Aminobutyric Acid (BABA) @ 250 ppm, salicylic acid @ 2 mM. Lesion diameters of inoculated leaves were evaluated twenty days after treatment inoculated citrus leaves with *Xcc.* Results revealed that, spray treatment with BABA reduced lesion size by about 50% as compared to the water control. Thus while water treated leaves showed lesion sizes of about 1.2 cm, BABA and treated plants had lesions of only about 0.6 cm. While, significant reduction in lesion size was observed following treatment with salicylic acid. Hasabi *et al.* (2014) [9] studied effect of three amino acids *viz.*, L-arginine, L-methionine, L-ornithine, and distilled water application on induced resistance against citrus canker caused by *X. axonopodis* pv. *citri.* in lime plants. Plants were inoculated with the suspension of the test bacterium and canker lesion diameter was measured four weeks after inoculation. They reported that plants treated L-methionine expressed significantly increased induced resistance and decreased disease severity, by reducing necrotic lesion size by about 78.5% as compared to untreated control. No significant reduction in lesion size was observed following treatment with L-arginine or L-ornithine.

Conclusion

All the ISR elicitors (chemicals) significantly reduced disease intensity as well as lesion diameter on acid lime. Of all the chemicals evaluated, B-aminobutyric acid (BABA) was found most superior; second best was Salicylic acid.

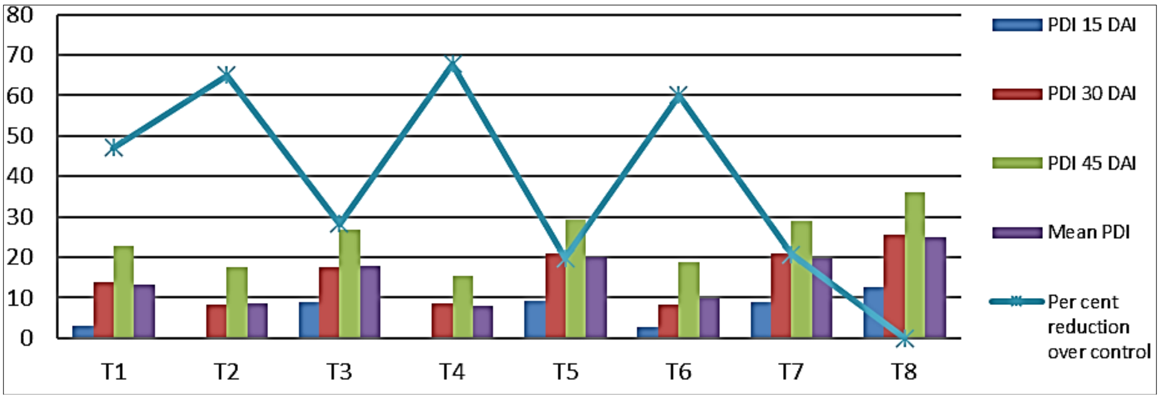

Fig 1: *In vitro* efficacy of ISR elicitors (Chemicals) on per cent disease intensity (pot culture)

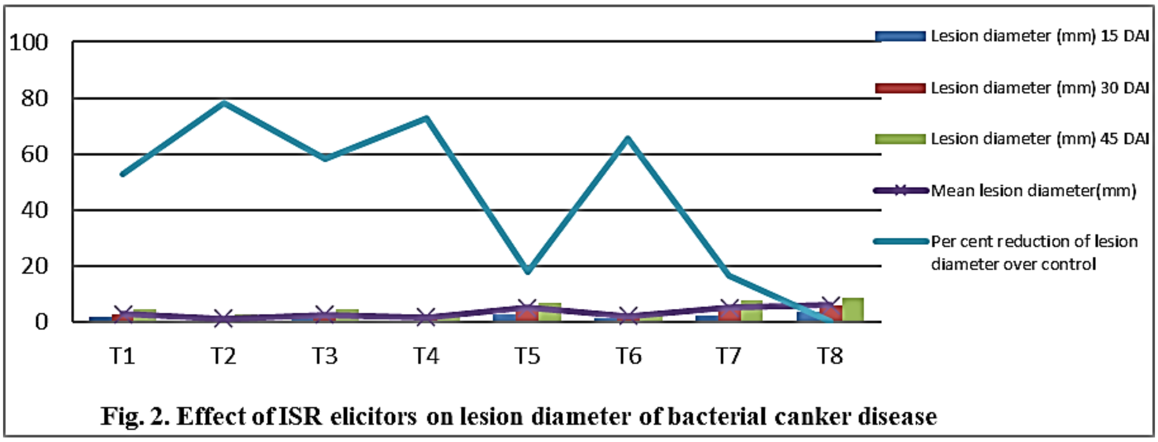

Fig 2: Effect of ISR elicitors on lesion diameter of bacterial canker disease

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