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Management of bacterial wilt of potato and tomato caused by *R. solanacearum* through resistance inducer chemicals

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Abstract
Bacterial wilt of potato and tomato caused by *Ralstonia solanacearum* Yabuuchi *et al.* (1995) is a highly destructive disease of solanaceous crops worldwide. Now, the disease is known to occur in all the states of India. The high percentage of plant mortality and lack of effective control methods make *R. solanacearum* one of the world's most destructive plant pathogens. Therefore, management of bacterial wilt of potato and tomato by using resistance inducer chemicals were studied in the present investigation to manage this destructive disease and prevent the further spread of the disease. One month old tomato (Pusa Ruby) and potato (Kufri Jyoti) seedlings were drenched with 50 ml, 3mM concentration of resistance inducers chemicals like Acibenzalor – s- methyl (ASM), β - Aminobutyric acid (BABA), Benzoic acid (BA) and Salicylic acid (SA). Control pots were drenched with sterilized water. After 2 days of drenching with resistance inducer chemicals half of tomato and potato plants were inoculated with *R. solanacearum*. The observations were recorded after 7, 14 & 21 days after inoculation. The results of tomato inoculated plant revealed that the minimum population of *R. solanacearum* was found (3.00 cfu log/g tissue) in Benzoic acid treatment followed by Salicylic acid (3.221 cfu log/g), Beta aminobutyric acid (3.221 cfu log/g) and Acibenzalor s methyl (3.36 cfu log/g tissue) after 21 days of inoculation. The maximum population of *R. solanacearum* was found (4.65 cfu log/g tissue) in control. In case of potato, the results revealed that the minimum population of *R. solanacearum* was found (2.752 cfu log/g tissue) in Acibenzalor s methyl treatment followed by Benzoic acid (3.164 cfu log/g), Salicylic acid (3.318 cfu log/g) and β - Aminobutyric acid (3.762 cfu log/g tissue) after 21 days of inoculation. The maximum population of *R. solanacearum* was found (4.591 cfu log/g tissue) in control.

Keywords: Bacterial wilt, *Ralstonia solanacearum*, resistance inducer chemicals etc.

1. Introduction
Bacterial wilt caused by *Ralstonia solanacearum* Yabuuchi *et al.* (1995) [28] is a highly destructive disease of solanaceous crops worldwide (Hayward, 2005) [10]. The *Ralstonia solanacearum* infects more than 450 plant species, distributed in 54 families (Wicker *et al.*, 2007) [27]. Because, of its broad geographical distribution and wide host range. The high percentage of plant mortality and lack of effective control methods make *R. solanacearum* one of the world's most destructive plant pathogens (Prior *et al.*, 1998) [20]. The disease is predominant in a warm humid tropical, subtropical and temperate region of the world (Hayward, 1991) [8]. It is the first bacterial disease recorded in India from Pune district of Maharashtra (Cappel, 1892) [5]. Now the disease is known to occur in all the states of India. This disease has been reported from the former Bombay, Mysore and Madras States (Butler, 1918) [4] from Uttar Pradesh, Bihar and Bengal (Mann and Nagpurkar, 1920; Patel *et al*., 1952) [15, 19] from Assam (Sen, 1930) [25] and from Hyderabad (Nath *et al*., 1958). In India, bacterial wilts inflicted yield losses up to 90% in tomato during one summer season (Kishun, 1987). Bacterial wilt is endemic to nearly 32% of total potato growing areas of India. Loss in yield due to disease in egg plant and tomato has been reported up to 81% (Rao, 1976) and 90.62% (Kishun, 1987) respectively. Control is difficult due to high variability of the pathogen, limited possibility for chemical control, high capacity of the pathogen to survive in diverse environments and it's extremely wide host range (Anonymous, 2004) [1].
Application of synthetic bactericides is still the most effective management strategy on account of unavailability of suitable disease-resistant cultivars for commercial use. However, the use of potentially hazardous bactericides in agriculture has been the subject of growing concern because of their possible adverse effects on the environment and emergence of bactericide-resistant pathogens (Pal *et al*. 2001) [18].

Moreover, frequent application of bactericides leads to the development of tolerance in pathogens. Therefore, alternative control method for management of bacterial wilt of potato and tomato by using resistance inducer chemicals were studied in the present investigation.

Materials Methods

Isolation and purification of *R. solanacearum*

Diseased samples of potato & tomato showing typical symptoms of bacterial wilt were collected from different locations of Samastipur districts of Bihar (India). Isolation of bacteria was done by following standard procedure on TTC medium as described by Kelman (1954) [13]. The single colony of bacterium showing fluidal, irregular and creamy white with pink at the centre was picked, and culture were maintained on the CPG slants and stored at 4ºC for further study.

Races & Biovar determination

The races of *R. solanacearum* were identified by inoculating the *R. solanacearum* on a wide host range (Buddenhagen & Kelman, 1964) [3]. Potato (Solanum tuberosum), Tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*) and chili (*Capsicum annuum*) were used as a test plant to determine the host range of *R. solanacearum*. The stem puncture technique was made for tomato, potato, brinjal and chili for inoculation of *R. solanacearum*. In this technique, bacterial suspension of potato and tomato isolate was prepared and adjusted to inoculum density of 10^8CFU/ml and wilt symptoms were observed daily.

Determination of biovars

Isolates of *R. solanacearum* were differentiated into biovar based on their ability to utilize disaccharides (sucrose, lactose, maltose) and sugar alcohols (manitol, sorbitol and dulcitol) using KB009 HiCarbohydrateTM Kit (HiMedia Laboratories Pvt. Limited), which contains above mentioned disaccharide and sugar alcohols as described previously Hayward (1964) [9] and He *et al.* (1983) [11]. 50 µl of bacterial suspension prepared from 48 h old culture of *R. solanacearum* strains was inoculated into each well by surface inoculation method and incubated at 35±1°C. The observations of changing colour were recorded after 18h of inoculation of culture up to 21 days.

Potato & Tomato plants grown in glasshouse

Diseased free potato tubers were collected and sown in the pot and local tomato seedlings were taken and transplanted in pot in glasshouse. 30 days old healthy potato & tomato plants were used for inoculation.

R. solanacearum inoculums preparation and method of inoculation

Ralstonia solanacearum, isolate R3bv2, isolated from naturally infected potato plants and R1bv3 isolates isolated from tomato plant were used in these studies. After 48 h from culturing on CPG media, a suspension of strain was prepared in sterile distilled water and adjusted to approximately 10^8 CFU/ml used for 30 days old potato & tomato plants inoculation by soil drenching method. For tomato & potato root inoculations, an alcohol-flamed knife was inserted 5–10 cm deep into the soil of each pot to cut the roots along two sides and inoculation was performed by soil drenching with 50 ml of the bacterial suspension, which was added to each pot around the basis of each plant. Control plants were treated with the same volume of distilled water. Prior to inoculation, plants were not watered for 24 h. The inoculated and non-inoculated tomato plants were kept in the same greenhouse as before and watered regularly with tap water.

Preparation of solution of resistance inducer chemical and application

Different resistance inducer chemicals i.e. ASM (Acibenzalor – s- methyl), BABA (β- amino butyric acid), BA (Benzoic acid) & SA (Salicylic acid) were prepared at 3 Mm concentration
and used for irrigating the tested plants. Potato & tomato seedlings were drenched with 50 ml, 3mM concentration of resistance inducers chemicals like ASM, BABA, BA and SA. Control pots were drenched with sterilized water. After 2 days of drenching with resistance inducer chemicals half of tomato and potato plants were inoculated with *R. solanacearum* R1bv3 and R3bv2 respectively. The observations were recorded after 7, 14 & 21 days after inoculation. From each treatment 3 plants were uprooted and 1 gm of basal stem portion was taken and macerated in mortar pestle and grinded with 10 ml of sterilized water. Then serial dilution were prepared and plated on CPG medium.

Results and Discussion

Isolation of bacterial wilt pathogen, *Ralstonia solanacearum*

Those samples confirmed the ooze test, the ooze were collected and plated on Triphenyl terazolium chloride (TTC) medium by following the standard procedure streaking and serial dilution method and kept for incubation at 27±1 ºC, to isolate bacterial wilt pathogen caused by *R. solanacearum*. After 48 hrs of incubation bacterial colonies observed for appearance of fluidal, irregular and creamy white with pink at the centre, were selected. These colonies were further streaked on TTC medium for purification and finally single purified colonies were taken on casamino acid peptone glucose (CPG) medium for further use.

Pathogenicity test

The colonies selected on the basis fluidal, irregular and creamy white with pink at the centre were further tested for pathogenicity. Pathogenicity test was done through stem inoculation, potato isolates on potato plant cv. Kufri Jyoti and tomato isolates on tomato plant (local). All the potato and tomato isolates were able to produce wilt symptom on potato and tomato plants after 10 days and 7 days of inoculation respectively. Those isolates confirm the pathogenicity test, were maintained on CPG test tube for further biovar characterization and race determination.

Characterization of *R. solanacearum* isolates of potato & tomato

Determination of biovars

Isolates of *R. solanacearum* (Potato) and isolates of *R. solanacearum* (Tomato) were taken to differentiate biovar based on their ability to utilize disaccharides (sucrose, lactose, maltose) and sugar alcohols (manitol, sorbitol and dulcitol) by using KB009 HiCarbohydrate™ Kit (HiMedia Laboratories Pvt. Limited). Out of 6 biovars present in the world, two biovars, *i.e.,* bv2 and bv2T were found in Bihar to infect potato plants. Isolates of tomato, was found belong to biovar 3.

Race Identification

The races of *R. solanacearum* were identified by pathogenicity test on differential hosts, such as Potato (Kufri Jyoti), Tomato (local), Brinjal (Plaster local) and Chilli (Sadabahar local). The result of pathogenicity test showed that *R. solanacearum* isolates collected from wilted potato plant indicating narrow host range belong to race 3. On the other hand, isolates obtained from wilted tomato plant produced wilt symptom on tomato, chilli, potato and brinjal belong to race 1 due to wide host range.

Ritu and Ranjan (2019) [24], also reported that based on the isolation study (colony characteristics on TZC media), ooze test, pathogenicity test, and different biochemical tests, the identity of potato wilt pathogen was established as *R. solanacearum*. Race characterization showed that strains of *R. solanacearum*, causing bacterial wilt disease in potato in Bihar, belong to race 3 and also reported that the strain of *R. solanacearum*, causing bacterial wilt of potato, collected from different locations of Samastipur districts of Bihar belong to bv2 & bv2T, which is also confirmed in our findings. Ranjan and Singh, (2015) [21] also confirmed the bv1, bv2, bv2T, and bv3 were present in India, cause bacterial wilt in potato plant.

Effect of resistance inducer chemical on *R. solanacearum* population inoculated in Potato and tomato plant:

The *R. Solanacearum* isolates R3bv2 used for potato inoculation and R1bv3 used for inoculation of tomato plants by soil drenching method. One month old tomato (Pusa ruby) seedlings were drenched with 50 ml, 3mM concentration of resistance inducers chemicals like ASM, BABA, BA and SA (Fig.1). Control pot were drenched with sterilized water. After 2 days of drenching with resistance inducer chemicals half of tomato plants were inoculated with *R. solanacearum* R1bv3. The observations were recorded after 7, 14 & 21 days after inoculation. From each treatment 3 plants were uprooted and 1 gm of basal stem portion were taken and macerated in mortar pestle and grinded with 10 ml of sterilized water. Then serial dilution were prepared and plated on CPG medium. The population of *R. solanacearum* were counted and converted into log value which is presented in table 1.

The results revealed that the minimum population of *R. solanacearum* was found (3.00 cfu log/g tissue) in Benzoic acid treatment followed by Salicylic acid (3.221 cfu log/g), Beta aminobutyric acid (3.221 cfu log/g) and Acibenzalor s methyl (3.36 cfu log/g tissue) after 21 days of inoculation. The maximum population of *R. solanacearum* was found (4.65 cfu log/g tissue) in control.

Table 1: Effect of resistance inducer chemical on *R. solanacearum* population inoculated in tomato plant.

S.N.	Treatment	Population of *R. solanacearum* (cfu log value / g of stem tissue)**		
		7 DAI*	14 DAI*	21 DAI*
1	ASM+ *R. solanacearum*	4.238	3.823	3.367
2	BABA+ *R. solanacearum*	4.213	3.602	3.221
3	BA+ *R. solanacearum*	3.668	3.425	3.00
4	SA+ *R. solanacearum*	3.884	3.522	3.221
5	Control+ *R. solanacearum*	4.425	4.556	4.649
6	Control- *R. solanacearum*	0	0	0
	SE	0.111	0.071	0.070
	CD at 5%	0.352	0.223	0.222
	CV	10.452	16.621	14.253

** (Average of 3 samples)

* (Days after inoculation)

One month old potato seedlings were drenched with 50 ml, 3mM concentration of resistance inducers chemicals like ASM, BABA, BA and SA. Control pots were drenched with sterilized water (Fig.2). After 2 days of drenching with resistance inducer chemicals half of potato plants were inoculated with *R. solanacearum* R3bv2. The observations were recorded after 7, 14 & 21 days after inoculation. From each treatment 3 plants were uprooted and 1 gm of basal stem portion was taken and macerated in mortar pestle and grinded with 10 ml of sterilized water. Then serial dilution were prepared and plated on CPG medium. The population of *R. solanacearum* were counted and converted into log value which is presented in table 2.

The results revealed that the minimum population of *R. solanacearum* was found (2.752 cfu log/g tissue) in Acibenzalor s methyl treatment followed by Benzoic acid (3.164 cfu log/g), Salicylic acid (3.318 cfu log/g) and Beta aminobutyric acid (3.762 cfu log/g tissue) after 21 days of inoculation. The maximum population of *R. solanacearum* was found (4.591 cfu log/g tissue) in control (Fig.3).

Table 2: Effect of resistance inducer chemical on *R. solanacearum* population inoculated in potato plant.

S.N.	Treatment	Population of *R. solanacearum* (cfu log value / g of stem tissue)**		
		7 DAI*	14 DAI*	21 DAI*
1	ASM+ *R. solanacearum*	4.257	3.499	2.752
2	BABA+ *R. solanacearum*	4.293	4.028	3.762
3	BA+ *R. solanacearum*	4.113	3.613	3.164
4	SA+ *R. solanacearum*	4.146	3.887	3.318
5	Control+ *R. solanacearum*	4.329	4.452	4.591
6	Control- *R. solanacearum*	0	0	0
	SE	0.326	0.470	0.347
	CD at 5%	1.029	1.483	1.093
	CV	13.828	23.172	18.390

** (Average of 3 samples)

* (Days after inoculation)

These results show that Benzoic acid (BA), Salicylic acid (SA), Acibenzalor-s-methyl (ASM) and β-Aminobutyric acid (BABA) played an effective role in reducing population of *R. solanacearum* in tomato and potato plants as compare to control.

In the present study, soil amended with ASM, SA & BA influenced the presence of *R. solanacearum* population density in soil rhizosphere of both tomato and potato plants. This may be because SA played an effective role in the induction signals in the mechanism of SAR leading to the reduction of both the percentage of infection and disease severity in tomato and potato plants as well as the bacterial counts in their roots (Raskin 1992; Sticher *et al*. 1997) [23, 26]. SA is initially proposed to bind to catalase and ascorbate peroxidase, which might lead to the formation of free radicals involved in lipid peroxidation, which can activate defense gene expression. SA known to inhibit jasmonic acid and auxin-mediated responses enhancing the disease-resistance mechanism (Fujita *et al*. 2006; Ndamukong *et al*. 2007) [6, 17]. These responses allow plants not only to survive pathogen infection, but also to acquire a long-lasting SAR responsible for the protection from further infections by a broad range of pathogens (Grant and Lamb 2006) [7]. BABA induction causes the accumulation of pathogenesis related (PR) protein before challenge (Hwang *et al*. 1997) [12]. BABA is thought to have the ability to break or cut chemical signals between tomato and potato roots by changing the chemical composition of root exudates secreted by both plants and increasing the amount of phenolic compounds secreted in plant root exudates (Bais *et al*. 2004) [2]. Therefore, resistance inducers chemical may be the good option to manage the deadly disease bacterial wilt of potato and tomato caused by *R. solanacearum*.

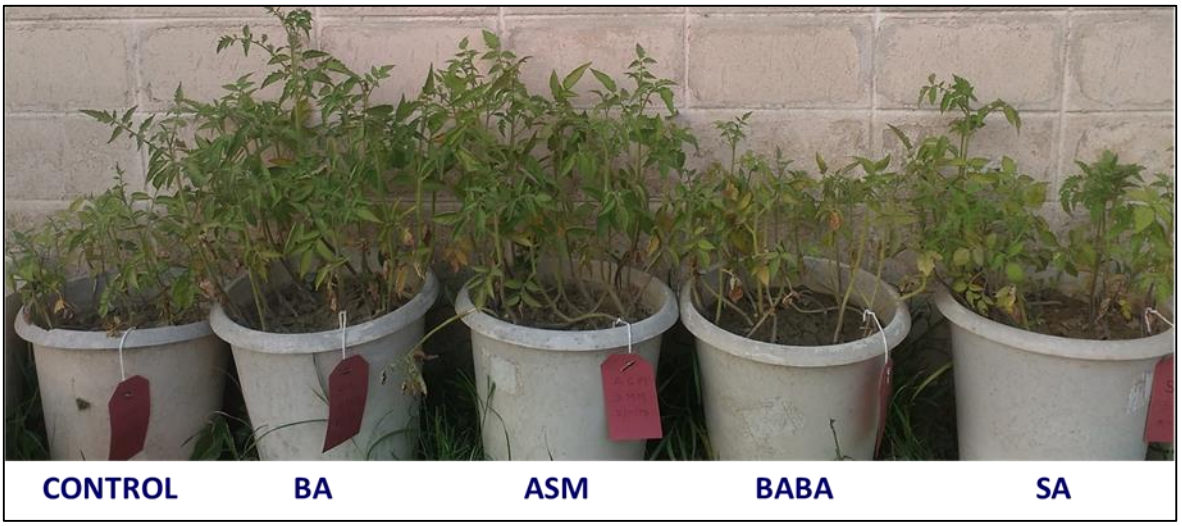

Fig 1: Effect of resistance inducer chemical on population of *R. solanacearum* inoculated in tomato plant.

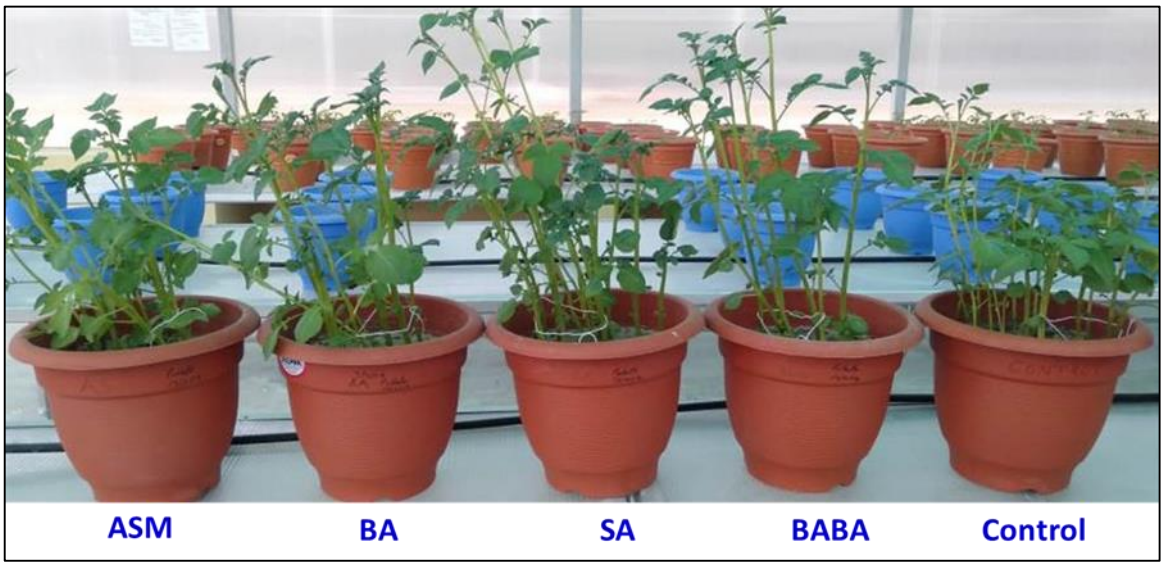

Fig 2: Effect of resistance inducer chemical on population of *R. solanacearum* inoculated in potato plant

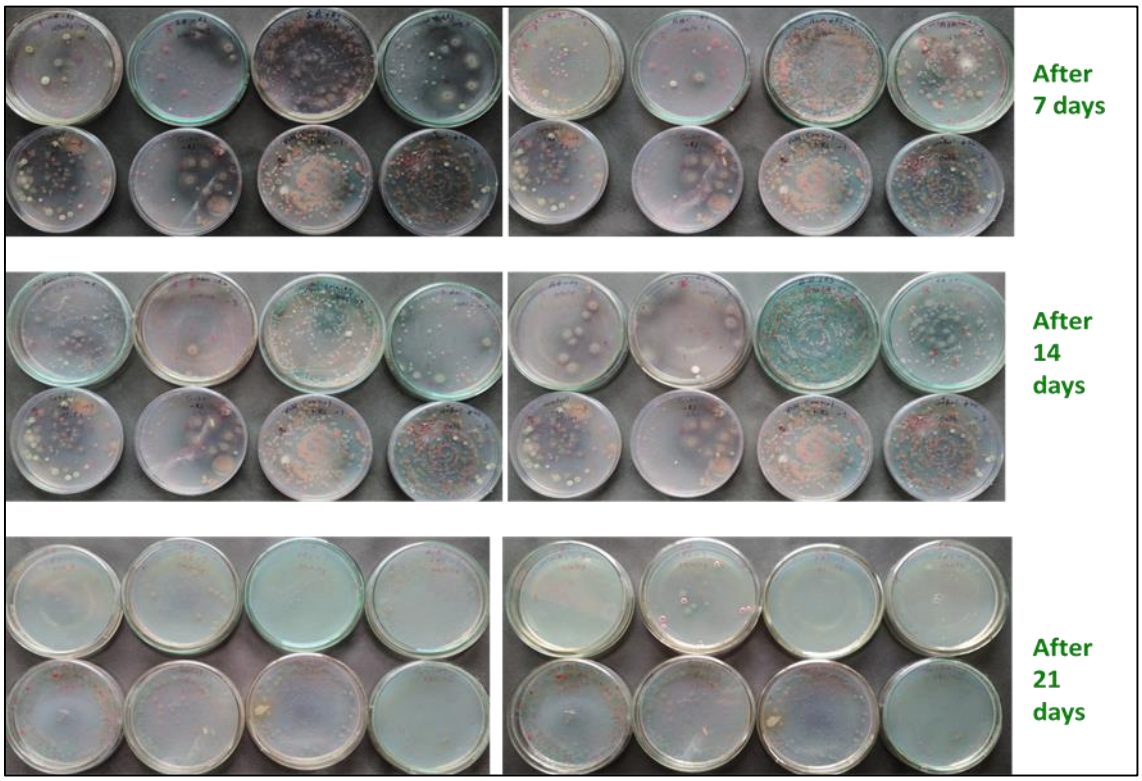

Fig 3: Effect of resistance inducer chemical on population of *R. solanacearum* at 7, 14 &21 days interval after inoculation in potato & tomato plant.

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