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RESISTANCE AND MORPHOLOGICAL RESPONSE OF CHILLI CULTIVARS AGAINST BACTERIAL WILT DISEASE AND ITS MANAGEMENT THROUGH PLANT ACTIVATORS

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Abstract

The contemporary study was designed to find out resistant and potential yield source of available chilli germplasm against bacterial wilt disease and management of bacterial wilt through plant defense activators. The findings of current study revealed that among 25 tested germplasm four cultivars named, Comega CH 403, HHP-090F, HHP-086H and Sanam showed resistant reaction having disease incidence between 1 to 20% respectively, whereas Sitara-80, Maxi and Sitara-83 were found highly susceptible with more than 80% disease incidence percentage. Current study found that, Comega CH 403, Sanam and HHP-086H were the leading varieties in terms of growth and yield contributing attributes, with maximum values of all investigated parameters in healthier conditions, however a small variation among all factors were recorded under disease stress. On the other hand minimum values for all growth parameters and yield were recorded among Maxi, Sitara-80 and Sitara-83 under both, healthy and infected environments with minimum log values. The results from management trials revealed that acibenzolar-S-methyl (ASM) was found highly effective against bacterial wilt with lowest disease incidence (38.63%), followed by chitosan (40.38%), citric acid (41.66%), salicylic acid (42.15%), benzoic acid (43.31%), ASA (45.29%), KH_2PO_4 (45.40%), K_2HPO_4 (48.30%), dichloro-isonicotinic acid (50.01%) and hydroquinone (52.43%) under greenhouse conditions. Whereas, treatment of combination of ASM+CS+CA showed least disease incidence (34.16%) followed by ASM+CS, ASM+CA, CS+CA, ASM, CS and CA against bacterial wilt of chilli under natural field conditions. Thus, the Comega CH 403, Sanam and HHP-086H are the potential varieties with high yield and resistance response against bacterial wilt.

Keywords: Acibenzolar-S-Methyl, Chilli, Bacterial Wilt, Chitosan, Disease Incidence, Resistance, Morphological Response.

INTRODUCTION

Chilli crop is subject to various impediments including viruses, fungus, bacteria and nematodes (Ali et al., 2024). Among all bacterial wilt (BW) caused by *Ralstonia solanacearum* is the most devastating element in yield losses due to its complex nature (Yabuuchi et al., 1995). *R. solanacearum* has been reported to cause a billion-dollar yield loss among solanaceous crops from more than 80 countries (Hong et al., 2012). It is a

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widely spreading pathogen in all ecological zones of Pakistan and causing a serious threat to various economical important solanaceous crops of country (Shahbaz et al., 2015; Aslam et al. 2017). Aslam et al. (2009) reported 85% of disease prevalence and 11% incidence of bacterial wilt in Punjab province of Pakistan. *Ralstonia solanacearum* belongs to class Proteobacteria, it is a gram negative, aerobic, rod shaped and motile with polar flagellum bacterium, having 0.5-0.7 × 1.5-2.0 µm dimensions (Sneath et al., 1986). Around 450 plant species from 54 botanic families are reported to be infected by *R. solanacearum*, among all solanaceous crops are highly vulnerable (Manda et al., 2020). It has been reported from tropical, subtropical and some areas of temperate climatic zones (Du et al., 2017). *R. solanacearum* frequently spread by contaminated soil, infected seeds and roots and irrigation water (Thakur et al., 2021), it invades into roots of host plants through natural wounds, where it starts to colonize root cortex and vascular parenchyma. After successful penetration and colonization of xylem vessels, it produces exopolysaccharides (EPSs) to block vascular bundle of xylem, which leads to wilting of host plant (Sahu et al., 2020).

The well-known symptoms of bacterial wilt disease are gradual yellowing and sagging of fresh plant leaves, which leads to undersized growth and death of the plant. The symptomatic appearance of bacterial wilt makes it known as 'Green wilting' as the plant remains green until the host plant wilts (Jiang et al., 2017). In case of serious attack of the disease, many adventitious root buds can emerge from the lower part of the stem close to the root zone, which signifies the infection of vascular bundle of the plant. Severe attack of cortex can cause the induction of water-soaked lesions on the outer surface of the stem (Mamphogoro et al., 2020). By dissecting the infected stem, tiny viscous yellowish, dirty white to milky colored bacterial ooze appears, which clearly indicates infection of vascular bundle (Karim et al., 2018; Champoiseau et al., 2009).

There are several ways are being implemented to overcome bacterial wilt, which includes chemical, cultural, physical and biological management approaches (Kurabachew and Ayana, 2016; Mbega et al., 2013). Among all, cultivation of resistant cultivars is the most prominent, effective, ecofriendly and economical strategy to combat bacterial wilt of chilli (Yuliar et al., 2015). Assessment of germplasm against pathogenic diseases is a chain process which is highly affected by climatic changes and diversity of pathogens, Not only a resistant variety but an area wise commercially developed cultivar with maximum yield potential is the requirement that needs immediate attention. That’s why it is a need of hour to identify the resistant sources among available germplasm with high yielding potential and to process them further in breeding programs, keeping in view the whole scenario, contemporary study was designed to screen out resistant and susceptible reaction of available chilli germplasm with better yielding capability against bacterial wilt disease. Advancement in plant disease management are decisive on account of escalating need of sustainability in agricultural products, as the pesticides are hazardous for consumer (animals and humans) and environment due to their enduring effects in food, feed and water bodies (Tudi et al., 2021). Considering these consequences, application of agrochemicals i.e. plants defense activators is comparatively novel

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approach in plant disease management. Plant activators are nontoxic to environment and potential inducer of systemic acquired resistance (SAR) in plants to boost their immunity against pathogens (Iriti and Vitalini, 2020). Application of Acibenzolar-S-methyl (ASM) has been reported to increase resistance among tomato plants against *Ralstonia solanacearum* (Sanju et al., 2017), defense activators are key factors in systemic acquired resistance which directly stimulates the production of pathogen related proteins and oxidative response (Amaral et al., 2019). That's why the current study was aimed to assess the potency of different plant activators in combating bacterial wilt of chilli caused by *R. solanacearum*.

MATERIALS AND METHODS

Surveys of Fields and Isolation of *Ralstonia Solanacearum*

The comprehensive surveys of fields were carried out in different chilli growing localities of Punjab, Pakistan. Where, infected root samples were collected based on morphological characterization of bacterial wilt disease and brought to Phyto-bacteriology Laboratory, department of Plant Pathology, UAF. The samples were firstly washed thoroughly to remove dust, then cut into small pieces (3-5 mm) and surface sterilized by dipping in 70% ethanol for 30 seconds followed by rinsing with distilled water. After drying on filter paper, sample pieces were placed on solidified nutrient agar (NA) media for the isolation of bacteria. Finally, the petri plates were wrapped, labeled and incubated (Robus Tech. RTI-150) at ±28 ºC temperature for 24-48 hours. Bacterial colony growth was observed regularly. Purification of bacterium was performed through streaking of a single colony on fresh NA plates using sterilized wire loop (Aslam and Mukhtar, 2018). Triphenyl tetrazolium chloride (TTC) media was used to grow purified bacterium culture for morphological identification of *R. solanacearum* as described by Kelman, (1954). Purified bacterial culture was preserved in 50% glycerol solution for further study.

Field Experiment for Screening of Chilli Germplasm

Chilli germplasm comprising 25 cultivars were collected from Ayub Agriculture Research Institute (AARI) Faisalabad and Chilli Research Station (CRS), Kunri. Nursery was established in plastic trays of 200 holes containing peat moss. Seedlings of 45 days age were transferred to the open field using Randomized Complete Bock Design (RCBD) having P×P 30 cm and R×R 45 cm distance. Each cultivar contained five replications and 10 plants per replication. All the agronomical practices were followed to ensure the healthy crop. After 15 days of nursery transplantation, cultivars were artificially inoculated by applying 50 mL of bacterial suspension (1×10^7 CFU/mL) through soil drenching method as stated by Aslam et al. (2017). The study was conducted for two consecutive years (2021 and 2022) at Department of Plant pathology, University of Agriculture, Faisalabad. Data for disease incidence was noted using formula given by Croxal, (1952) and after statistical analysis the data was sorted by using disease rating scale (Table 1) (Winstead and Kelman, 1952).

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$$\text{Disease incidence } \% = \frac{\text{Number of infected plants}}{\text{Total number of observed plants}} \times 100$$

Table 1: Disease Rating Scale for Screening of Chilli Germplasm against Bacterial Wilt Disease

Rating Scale	Disease incidence (%)	Reaction
0	0	Highly Resistant
1	1-20	Resistant
2	21-40	Moderately resistant
3	41-60	Moderately susceptible
4	61-80	Susceptible
5	>80	Highly Susceptible

Management of Bacterial Wilt of Chilli Using Plant Activators

Ten plant activators were evaluated against bacterial wilt of chilli caused by *R. solanacearum* under greenhouse conditions, where chilli seedlings of moderately susceptible variety (CBS-1293) of 45 days age were grown in earthen pots of 15×17 Cm containing soil (2 kg/pot), sterilized with formalin. Prior to nursery transplantation, seedlings were thoroughly washed with tape water, injured using sterilized blade and dipped into bacterial suspension (1×10^7 CFU/mL) for 2-3 minutes for artificial inoculation (Lucia et al., 2015). After successful transplantation of seedlings, phenolic antioxidants were applied in spray form using hand sprayer. Inoculated plants treated with distilled water were intended as control. There were 3 concentrations (0.5, 0.75 and 1%) of each treatment with three replications. Experiment was conducted under completely randomized design (CRD) and disease incidence percentage was recorded using formula given by (Croxal, 1952). Field trail was also conducted at research area of Department of plant Pathology, University of Agriculture, Faisalabad, where CBC-1293 genotype of chilli was grown on raised beds with R-R and P-P distance 45×30 cm. Artificial inoculation was done by dipping seedling roots in 1×10^8 CFU/mL bacterial suspension for 2-3 minutes at the time of transplantation (Lucia et al., 2015). After successful establishments of plants, three most effective plant activators under glasshouse were applied solely and in combinations i.e. Acibenzolar-S-Methyl (ASM), Citric acid (CA), Salicylic acid (SA), ASM+CA, ASM+SA, CA+SA and ASM+CA+SA at 1% concentration. Distilled water was applied for control treatments. Each treatment was replicated thrice to overcome the chance of error. Field trail was designed under Randomized Complete Block Design (RCBD). Disease incidence (%) was recorded for three weeks with one week of interval.

Determination of Horticultural Attributes in Healthy and Infected Chilli Cultivars

The parameters including plant height (PH), shoot length (SL), root length (RL) were determined in centimeters (cm) with the help of steel scale and dry root weight (DRW), fresh root weight (FRW) and total yield (Y) were recorded in grams (g) using digital weight balance (NBT-A200, NANBEI). Number of leaves and branches were manually counted. All the parameters were calculated from healthy and infected plants separately from 25 different varieties/advanced lines. Infected crop was artificially inoculated using bacterial

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suspension through soil drenching method. 15 plants from each cultivar of both infected and healthy type were randomly selected for data collection and by adding the values, a final average was calculated and used for data analysis. All the horticultural attributes of 25 varieties/advanced lines were recorded when crop reached at 90 days age (Yanti et al., 2024).

RESULTS

Field Observations and Isolation of Bacterium

In total four districts including Faisalabad, Gojra, Toba Tek Singh and Chiniot of Punjab province of Pakistan (Fig. 1) were thoroughly surveyed for collection of bacterial wilt affected samples. In total 10 samples, showing bacterial wilt symptoms were collected for isolation of bacterium. The isolated bacterium was identified as *Ralstonia solanacearum* having whitish, fluidal and irregular colonies with pinkish centers as described by Ji et al. (2005).

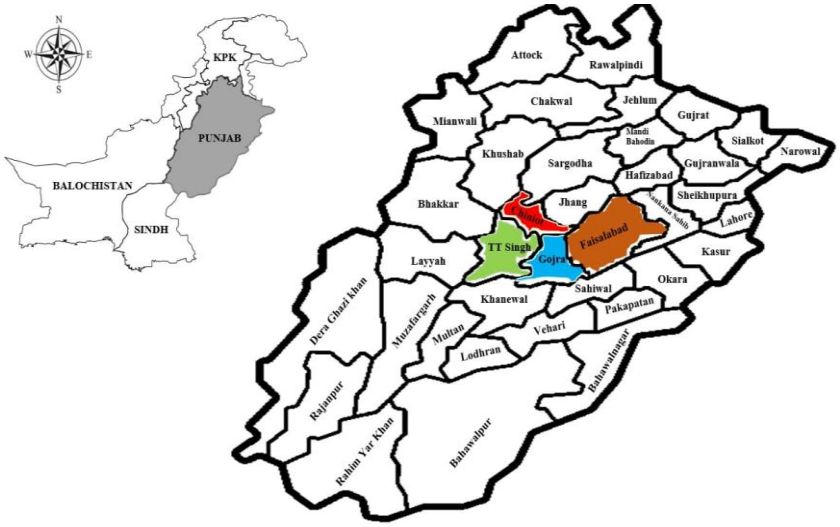

Figure 1: Geographical Map, Showing Colorful Districts as Surveyed sites for Collection of bacterial wilt affected chilli plant samples

Resistance Reaction of chilli germplasm against bacterial wilt

In the year 2021, results stated that among 25 cultivars of chilli, not a single genotype was found immune against bacterial wilt, however 4 varieties i.e. Comega CH 403, HHP 090F, HHP 086H and Sanam shown resistant reaction having disease incidence between 1 to 20%, and five cultivars (Lal Pari, Bird Eye, LG 428, Morni and HHP 102283) exhibited moderately resistant response with disease incidence range of 21-40%. According to disease rating scale five cultivars (MV 66, CBS 1202, Tikhee, HHP 041B and CBS 1293) was moderately susceptible, eight (LG Hot Red, Red Hot, Hangama, CH 203, Longi/Desi, Sitara 81, Longi hybrid and FS-1) were highly susceptible (HS) and three cultivars (Sitara

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80, Maxi and Sitara 83) was found highly susceptible response having disease incidence more than 80% (Table 2).

In 2022, all the chilli cultivars were again screened out against bacterial wilt disease, numeric indicated that four varieties (Comega CH 403, HHP 090F, HHP 086H and Sanam) were marked resistant according to disease rating against bacterial wilt with disease incidence below 20% and cultivars named 'Lal Pari, Bird Eye, LG 428, Morni and HHP 102283' were found moderately resistant ranging in 21-40% disease incidence (Rating 2). Additionally, five varieties (MV 66, CBS 1202, Tikhee, HHP 041B and CBS 1293) exhibited moderately susceptible response (Rating 3), seven genotypes (LG Hot Red, Red Hot, Hangama, CH 203, Longi/Desi, Sitara 81 and Longi hybrid) were susceptible (Rating 4) and four cultivars (FS-1, Sitara 80, Maxi and Sitara 83) were found highly susceptible with more than 80% disease incidence. The only genotype 'FS-1' exhibited dissimilar reaction in both years i.e. susceptible (S) with 79.02% in 2021 and highly susceptible response with 80.99% disease incidence in 2022 as shown in Table 2.

Table 2: Two years data of disease incidence percentage calculating from twenty five chilli varieties for assessing their resistance and susceptibility potential against bacterial wilt disease caused by *Ralstonia solanacearum*

Sr. #	Varieties/Advanced lines	Disease incidence (%) Year 2021	Disease incidence (%) Year 2021
1	Comega CH 403	7.55 r	7.89 n
2	HHP 090F	12.95 qr	14.61mn
3	HHP 086H	14.07 qr	15.55 mn
4	Sanam	18.94 pq	19.34 lm
5	Lal Pari	23.67 op	26.80 kl
6	Bird Eye	28.49 no	27.73 kl
7	LG 428	31.63 mno	31.17 jk
8	Morni	35.07 lmn	38.15 ij
9	HHP 102283	38.75 klm	38.77 ij
10	MV 66	41.90 kl	44.91 hi
11	CBS 1202	47.77 jk	49.85 gh
12	Tikhee	51.60 ij	52.45 gh
13	HHP 041B	54.32 hij	56.27 g
14	CBS 1293	57.03 ghi	57.67 fg
15	LG Hot Red	61.63 fgh	65.15 ef
16	Red Hot	65.44 efg	66.47 e
17	Hangama	67.98 ef	71.36 de
18	CH 203	70.68 def	72.23 de
19	Longi/Desi	73.38 cde	72.29 de
20	Sitara 81	74.46 cde	77.89 cd
21	Longi hybrid	78.20 cd	78.05 cd
22	FS-1	79.02 cd	80.99 3c
23	Sitara 80	81.68 bc	82.28 bc
24	Maxi	88.24 ab	90.25 ab
25	Sitara 83	91.08 a	91.97 a

*Mean values sharing similar letters in columns are not significantly different as determined by LSD ($P \leq 0.05$)

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Assessment of Phenolic Antioxidants against Bacterial wilt of Chilli under Glasshouse Conditions

Ten different plant activators were demonstrated to reveal their efficacy against bacterial wilt of chilli under glasshouse conditions. Data regarding disease incidence percentage were statistically analyzed and showed that all the treatments along with their interaction between concentrations and day's interval were found significantly effective against disease. However, among all the treatments acibenzolar-S-methyl (ASM) exhibited most effective results with lowest disease incidence (38.63%), followed by chitosan (40.38%), citric acid (41.66%), salicylic acid (42.15%), benzoic acid (43.31%), ASA (45.29%), KH_2PO_4 (45.40%), K_2HPO_4 (48.30%), dichloro-isonicotinic acid (50.01%) and hydroquinone (52.43%) (Fig. 2). When the treatments were investigated in relation with concentrations, ASM showed significant disease control at 0.5, 0.75 and 1% concentrations having disease incidence 43.46, 38.41 and 34.01%, while the highest disease incidence and minimum disease control among treatments were recorded by application of hydroquinone (49.48, 51.82 and 55.98%) (Fig. 3a).

The data was recorded for three times at different intervals i.e. 7, 14 and 21 days. The results revealed that at all three intervals the maximum disease incidence (55.41, 51.76 and 50.12%) was showed by Hydroquinone and found least effective against bacterial wilt of chilli, while, ASM expressed noticeable decrease in disease incidence (44.85, 37.68, 33.38%) at 7, 14 and 21 days. The remaining treatments exhibited minor difference in depressing disease incidence percent such as chitosan (46.85, 39.34, 34.94%), citric acid (48.65, 40.98, 35.35%), salicylic acid (48.18, 41.10, 37.18%), benzoic acid (49.83, 42.20, 37.90%), ASA (50.96, 44.60, 40.31%), KH_2PO_4 (50.73, 45.15, 40.32%), K_2HPO_4 (51.17, 48.47, 45.27%), and dichloro-isonicotinic acid (54.50, 49.39, 46.14%) (Fig. 3b).

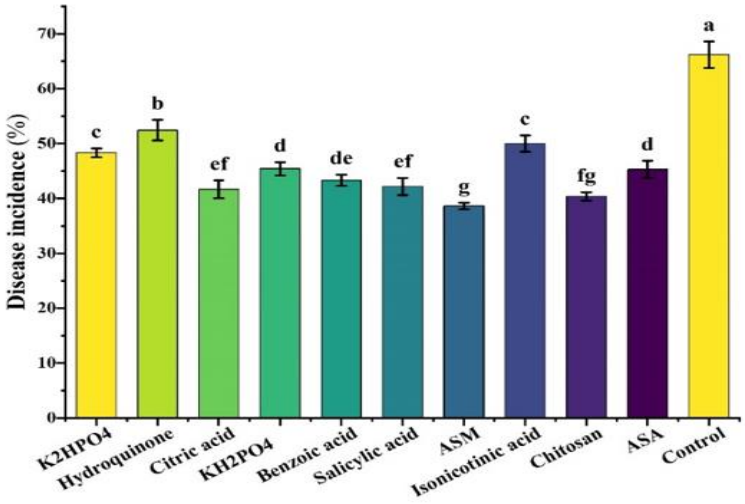

Figure 2: The mean disease incidence percentage of bacterial wilt of chilli after application of plant defense activators, calculating from 10 replications per treatment under greenhouse environment

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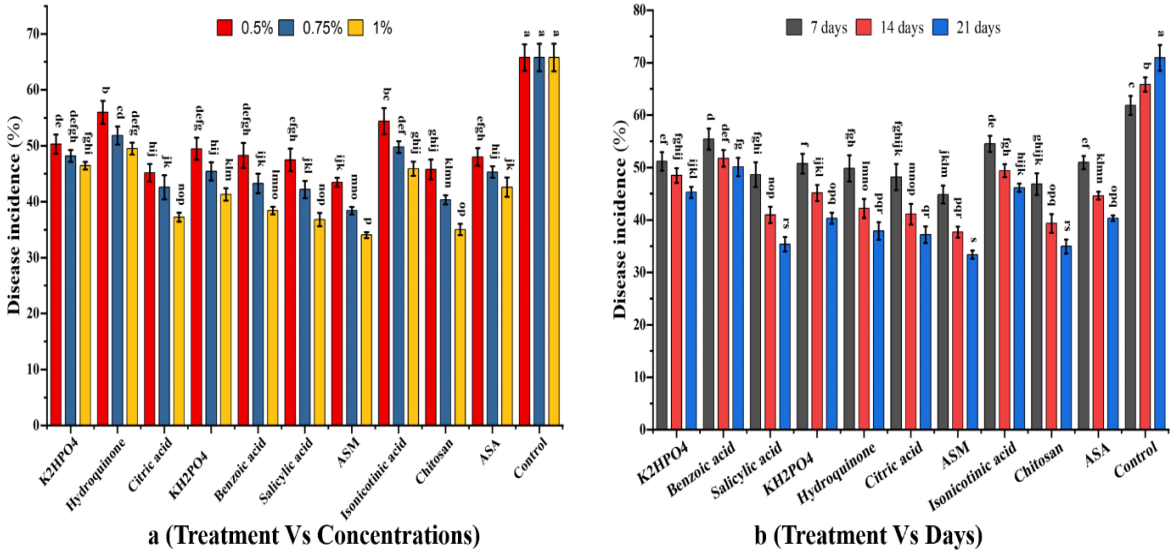

Figure 3: (a) is showing the impact of interaction between treatments and concentrations against bacterial wilt of chilli, whereas (b) is indicting the interaction of treatments and days interval on bacterial wilt incidence percentage

Evaluation of Plant Defense Activators against Bacterial Wilt of Chilli under field Conditions

Data of disease incidence percentage revealed that the combination of ASM+CS+CA was significantly effective against bacterial wilt of chilli with least disease incidence 34.16%, followed by ASM+CS (37.66%), ASM+CA (38.82%), CS+CA (41.27%), ASM (40.94%), CS (43.09%) and CA (44.64%) (Fig. 4a).

When the data was analyzed according to days interval, it showed that the disease incidence percentage was decreasing in all treatments except control. However, the maximum disease incidence was recorded by citric acid (46.47, 44.2 & 43.25%) at 7th, 14th and 21st day of application, while combination of ASM+CS+CA exhibited effective control against disease with least disease incidence i.e. 38.25, 34.17 and 30.07% at same days interval.

Furthermore, data revealed that the remaining treatments showed intermediate control against disease such as ASM+CS (40.31, 37.62 & 35.05%), ASM+CA (41.15, 39.02 & 36.31%), Acibenzolar-S-Methyl (44.16, 41.06 & 37.62%), CS+CA (42.82, 41.16 & 39.83%) and Citric acid (45.37, 42.34 & 41.57%) (Fig. 4b).

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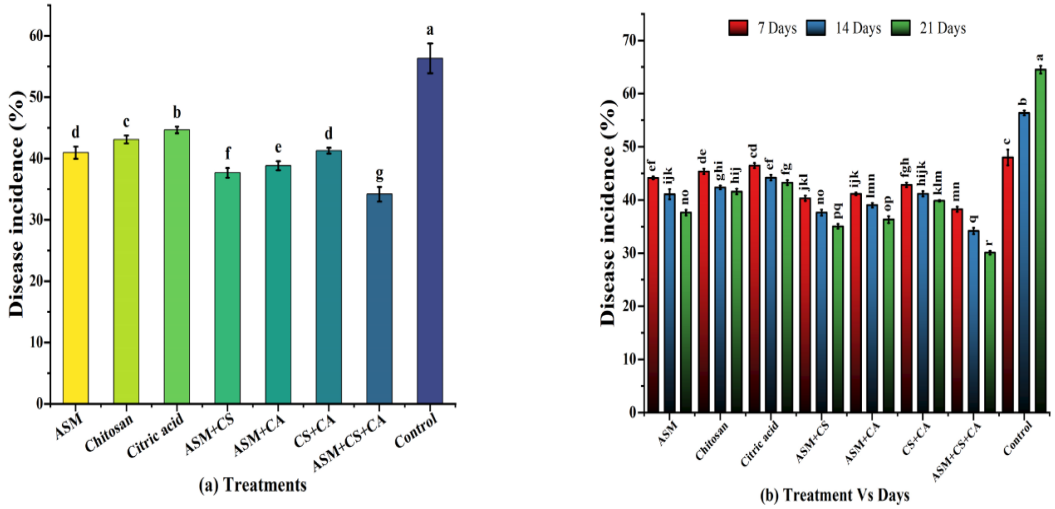

Figure 4: (a) The main impact of plant defense activators and their combinations against bacterial wilt of chilli under natural field conditions, and (b) is showing the impact of plant defense activators with passage of time.

Morphological Attributes of Chilli Germplasm under Healthy and Infected Environments

Results demonstrated that Comega CH 403, Sanam and HHP-086H are the leading potential varieties that showed maximum values for plant height (85.27, 81.447 and 79.42 cm), No. of branches (9, 11 and 12), root length (21.68, 19.36, 20.26 cm), shoot length (76.01, 76.66 and 78.97 cm), fresh weight of plant (212.13, 197.2 and 208.14 g), dry weight of plant (187.14, 172.88, 189.76 g), No. of leaves per plant (95, 93 and 89) and yield (377.69, 312.12, 354.83 g) under healthier environment, however under disease stress conditions there was a slight decline among all morphological and yield contributing attributes in same varieties such as plant height (84.81, 79.57 and 77.61cm), No. of branches (8, 11, 12), root length (19.26, 17.89 and 18.46 cm), shoot length (74.7, 75.15 and 78.35 cm), fresh weight of plant (201.14, 188.67 and 194.7 g), dry weight of plant (170.26, 147.82 and 162 g), No. of leaves per plant (92, 88 and 83) and yield (366.98, 306.83 and 344.88 g).

Whereas minimum values for growth and yield contributing factors were recorded among Maxi, Sitara-80 and Sitara-83 varieties of chilli under both, healthy and infected environments with minimum log values (Fig. 5). The interaction between healthy and infected chili varieties showed a significant variation among various growth parameters, including plant height, number of branches, number of leaves, fresh weight, dry weight, root length, shoot length and yield, which illustrate the potential of disease causing agents on various growth parameters (Fig. 6). Two-way ANOVA among healthy and infected chili varieties also demonstrated significant results for morphological and yield contributing factors (Fig. 7).

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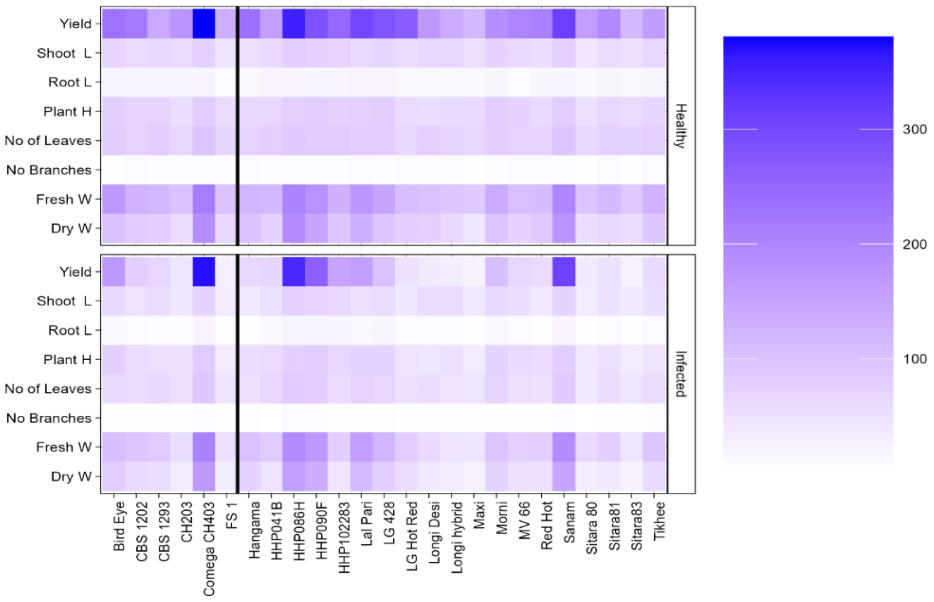

Figure 5: Heat map for the determination of growth and yield contributing factors of the investigated chili varieties against Ralstonia solanacearum under healthy and infected environments

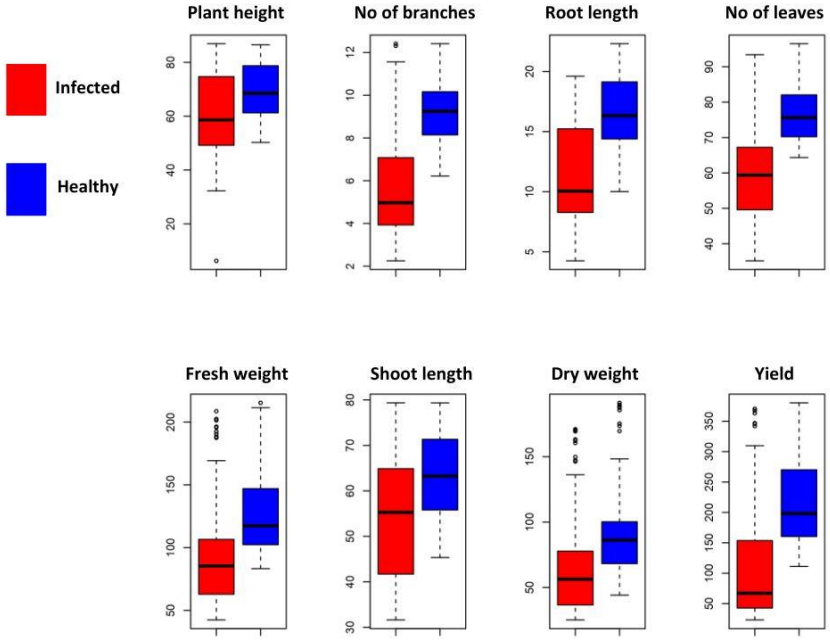

Figure 6: Mean box-plot interaction among morphological and yield contributing factors of the healthy and infected chili varieties

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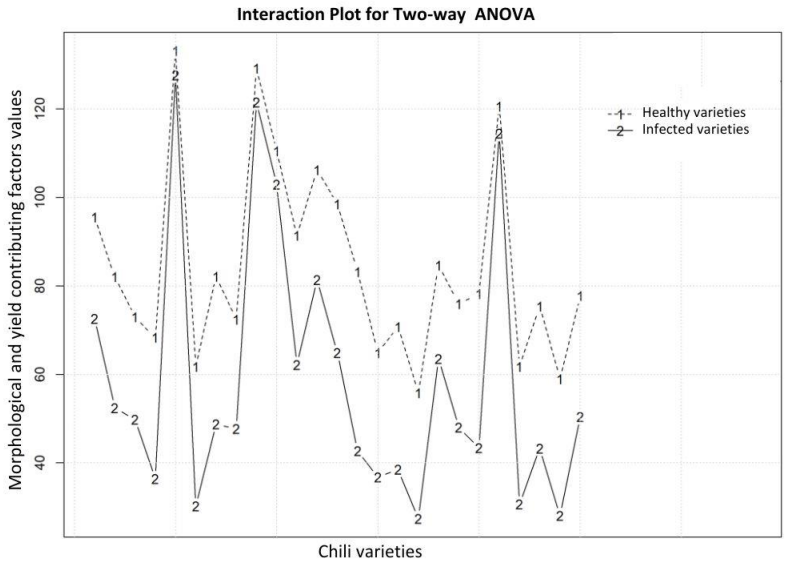

Figure 7: Interaction plot for Two-way ANOVA (Healthy and infected varieties) for morphological and yield contributing factors clearly comparing the difference between heathy and infected plants in terms of different growth promoting factors

DISCUSSION

Current study was also designed to persuade screening of different varieties of chilli to find resistant sources against BW disease. Findings of our study revealed that none of the investigated cultivar exhibited highly resistant reaction against BW in both years (2021-22), while four varieties showed resistant response and five varieties moderately resistant. All the remaining varieties exhibited susceptible or highly susceptible reaction on the basis of disease rating scale. The findings of present study are in line with Pawaskar et al. (2014) and Padalkar et al. (2012), who screened out different cultivars and found that none of the variety showed immune response against BW. Similarly, our study is also supported by the work of Aslam et al. (2017) and Pawaskar et al. (2014), who reported no any immune/highly resistant variety against BW incited by *R. solanacearum*.

Naturally plants contain different mechanisms for combating pathogenic infections. Among them, presence of resistance (R) gene in host plants is the most reliable weapon to fight against pathogens, as these genes can potentially recognize the specific effector proteins of corresponding microorganism which activates its defense system (Liu et al., 2007) and releases various enzymes including polyphenol oxidase, cytochrome oxidase, peroxidase and phenolic compounds i.e. saponins, tannins, flavonoids and coumarins in resistant genotypes. All these biochemical compounds play an effective role in certain

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metabolic processes that inhibit the growth and development of pathogens (Ochoa and Gomez, 1993).

Present study also comprises the appraisal of effectiveness of different plant activators against BW of chilli under greenhouse and field conditions. Among all the applied treatments under greenhouse conditions, ASM was found significantly effective with least disease incidence, while the interaction between treatment, days and concentration also revealed the potency of acibenzolar-S-methyl against bacterial wilt of chilli. Whereas, chitosan, citric acid and salicylic acid showed effective results after ASM. Three most effective plant activators i.e. ASM, Chitosan and Citric acid were applied as solo application and in combinations under open field conditions, where results indicated an efficient disease control with lowest incidence (34.16%) when combination of all three activators (ASM+CS+CA) were applied. Results of our study are supported by the findings of Sanju et al. (2017), who discussed the resistance enhancement in tomato plants against bacterial wilt disease by using acibenzolar-S-methyl. Chandrasekhar et al. (2017), also found significant decrease in bacterial wilt of tomato by exogenous application of salicylic acid under glasshouse conditions. Moreover, our study is also in line with the work of Muhammad et al. (2022), which evidently showed the application of plant defense activators as potential management source against fusarium wilt of chilli.

Evaluation of novel strategies for the management of plant diseases are highly essential for sustainability in agro-food chains, as the chemical pesticides are highly dangerous for humans and animals due to their toxic residual effects (Tudi et al., 2021). Phenolic antioxidants or plant activators are comparatively safest approach in crop protection due to their ecofriendly nature. Previously, it has been reported that ASM can efficiently defend the host plants from bacterial infections by activating stomatal based defense (Ishiga et al., 2020). Baysal et al. (2005) discussed that pepper leaves treated with acibenzolar-S-methyl (ASM) showed transient increase in L-phenylalanne ammonia-lyase (PAL) enzyme, total phenol content (TPC), activity of β-1-3-glucanase and chitinase, which resulted a boost in plant resistance against pathogens. Plant activators are such compounds which trigger the defense mechanism of host plants by processing of oxidative burst (Heath 1998) and increasing enzymatic activity (Oliveira et al., 2016) leads toward cell death for deceiving the pathogen in necrotic cells.

The successful improvement of any crop through breeding program hinge on genetically variable material along with high yield capability and disease resistance. Therefore, general understanding of genetic variability and heritability in gene pool of the crop cultivars for suitable traits is crucial for breeders to initiate breeding programs. Contemporary study also covers the growth parameters of chilli cultivars (resistant and susceptible) under healthy and bacterial wilt affected environments. The findings revealed that all the varieties under diseased stress expressed slight to a notable reduction in morphological attributes. On the other hand, resistant cultivars showed maximum values of recorded growth attributes under both, healthy as well as diseased environment. The same has been conferred by Sehgal and Kumar (2021), where bacterial wilt resistant

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tomato cultivars found prominent for phenotypic potentials. Our study is also in line with the findings of Sood and Thakur (2017).

Upon inoculation of *Ralstonia solanacearum* in plant roots, various alterations have been observed, among them, primary root growth inhibition is a common phenomenon (Digonnet et al., 2012; Turner et al., 2009). This growth arrest is likely instigated due to cell death at root tips and simultaneous disruption of activity of root apical meristem (Zolobowska and Gijsegem, 2006; Lu et al., 2018; McGarvey et al., 1999). Bacterial wilt infection starts by invasion of *R. solanacearum* into root zone through natural openings or wounds and moves toward vascular bundles of xylem vessels within 24 hours (Caldwell et al., 2017), where, the bacterium eventually multiplies into xylem and spreads into the shoots (Digonnet et al., 2012; Turner et al., 2009; Vasse et al., 1995).

The bacterium secrets exopolysaccharide (EPS) that directly leads to obstruction of the xylem tissues, and results in severe wilting of the host plant due to inhibition of water transportation (Denny, 2000; Denny and Baek, 1991; McGarvey et al., 1999; Saile et al., 1997; Schell, 2000). Ultimately, these changes in plant affects the physical growth and total yield of the plant. All these events are directly associated with the susceptibility and resistance of the host plants against bacterium. There are several studies showing that host resistance is correlated with the limitation of bacterial dispersion within the stem (Nakaho and Allen, 2009; Nakaho et al., 2004). Additionally, it has been reported that EPS was found prominently distributed in vascular tissues of the susceptible as compared to resistant varieties within 5 days of inoculation (McGarvey et al., 1999), however it is suggested that roots of resistant plants are directly involved in restriction of the bacterial spread which leads to vigorous plant growth, development and total yield under diseased environment.

CONCLUSION

Bacterial wilt of chilli is a devastating disease causing huge production losses under favorable environments. Our study suggest that. Due to complex nature of pathogen, it is the need of hour to screen out available germplasm for recognition of resistant sources to minimize yield losses. Based on study of disease impacts on yield contributing factors of different resistant and susceptible chilli varieties, we show the exact varietal potential against bacterial wilt disease. Moreover, the disease management through plant defense activators showed potential use of activators to maximize varietal resistance against pathogenic diseases. These findings will show a path to breeder’s community to produce and enhance the genetic resistance and yield potential among different chilli cultivars against this devastating disease

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References

1) Ali M.U., Rajput N.A., Atiq M., Atif R.M., Crandall S.G. 2024. Population dynamics and aggressiveness of fungal pathogens associated with chilli root rot. Pakistan Journal of Botany, 56(1): 377-387.

2) Amaral L.S., Debona D., Costa L.C., Silva A.L.R., Oliveira J.R., Rodrigues F.A. 2019. Biochemical insights into basal and induced resistance in cabbage to black rot. Journal of Phytopathology, 167: 390-403.

3) Aslam M.N., Mukhtar T. 2018. Distributional variability of bacterial wilt of chili incited by *Ralstonia solanacearum* in eight agro-ecological zones of Pakistan. Peer Journal Preprints, 6: 26668v1.

4) Aslam M.N., Mukhtar T., Ashfaq M., Asad M.J., Hussain M.A. 2009. Incidence and prevalence of bacterial wilt of chili in Punjab, Pakistan. Mycopathology, 13: 37-41.

5) Aslam M.N., Mukhtar T., Ashfaq M., Hussain M.A. 2017. Evaluation of chili germplasm for resistance to bacterial wilt caused by *Ralstonia solanacearum*. Australasian Plant Pathology, 46: 289-292.

6) Aslam M.N., Mukhtar T., Hussain M.A., Raheel M. 2017. Assessment of resistance to bacterial wilt incited by *Ralstonia solanacearum* in tomato germplasm. Journal of Plant Disease Protection, 6: 585-590.

7) Baysal O., Turgut C., Mao G. 2005. Acibenzolar-S-methyl induced resistance to *Phytophthora capsiciin* pepper leaves. Biologia Plantarum, 49: 599-604.

8) Caldwell D., Kim B.S., Iyer-Pascuzzi A.S. 2017. *Ralstonia solanacearum* differentially colonizes roots of resistant and susceptible tomato plants. Phytopathology, 107:528-536.

9) Champoiseau P., Jones J.B., Allen C. 2009. *Ralstonia solanacearum* race 3 biovar 2 causes tropical losses and temperate anxieties. Plant Health Progress, 1-10.

10) Chandrasekhar B., Umesha S., Kumar H.N. 2017. Proteomic analysis of salicylic acid enhanced disease resistance in bacterial wilt affected chilli (*Capsicum annuum*) crop. Physiological and Molecular Plant Pathology, 98: 85-96.

11) Croxal H. E., Gwynne D.C., Jenkins J.E.E. 1952. The rapid assessment of Apple scab on leaves. Plant Pathology, 1: 39-41.

12) Denny T.P. 2000. *Ralstonia solanacearum*-A plant pathogen in touch with its host. Trends In Microbiology, 8:486-489.

13) Denny T.P., Baek S.R. 1991. Genetic evidence that extracellular polysaccharide is a virulence factor of *Pseudomonas solanacearum*. Molecular Plant-Microbe Interaction, 4:198-206.

14) Digonnet C., Martinez Y., Denancé N., Chasseray M., Dabos P., Ranocha P., Marco Y., Jauneau A., Goner D. 2012. Deciphering the route of *Ralstonia solanacearum* colonization in Arabidopsis thaliana roots during a compatible interaction: Focus at the plant cell wall. Planta, 236:1419-1431.

15) Du H., Chen B., Zhang X., Zhang F., Miller S.A., Rajashekara G., Xu X., Geng S. 2017. Evaluation of *Ralstonia solanacearum* infection dynamics in resistant and susceptible pepper lines using bioluminescence imaging. Plant Disease, 101: 272-278.

16) Gupta S.K., Thind T.S. 2006. Disease Problems in Vegetable. In: Diseases of Cruciferous Vegetables. Scientific Publishers, India. pp. 170-185.

17) Heath M.C. 1998. Apoptosis, programmed cell death and the hyper-sensitive cell death. European Journal of Plant Pathology, 104: 117-124.

18) Iriti M., Vitalini S. 2020. Sustainable crop protection, global climate change, food security and safety plant immunity at the crossroads. Vaccines, 8: 42.

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 08 | 2024
DOI: 10.5281/zenodo.13358050

19) Ishiga T., Iida Y., Sakata N., Ugajin T., Hirata T., Taniguchi S., Hayashi K., Ishiga Y. 2020. Acibenzolar-S-methyl activates stomatal-based defense against *Pseudomonas cannabina* pv. *alisalensis* in cabbage. Journal of General Plant Pathology, 86: 48-54.

20) Jiang G., Wei Z., Xu J., Chen H., Zhang Y., She X., Macho A.P., Ding W., Liao B. 2017. Bacterial wilt in China: history, current status, and future perspectives. Frontiers in Plant Sciences, 8: 1549.

21) Karim Z., Hossain M.S., Begum N.N. 2018. *Ralstonia solanacearum*: a threat to potato production in Bangladesh. Fundamental and Applied Agriculture, 3: 407-421.

22) Kelman A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathology, 44: 693-695.

23) Kurabachew H., Ayana G. 2016. Bacterial wilt caused by *Ralstonia solanacearum* in Ethiopia: status and management approaches: a review. International Journal of Phytopathology, 5: 107-119.

24) Liu J., Liu X., Dai L., Wang G. 2007. Recent progress in elucidating the structure, function and evolution of disease resistance genes in plants. Journal of Genetics and Genomics, 34: 765-776.

25) Lu H., Lema A.S., Planas-Marquès M., Alonso-Díaz A., Valls M., Coll N.S. 2018. Type III secretion-Dependent and-independent phenotypes caused by *Ralstonia solanacearum* in Arabidopsis roots. Molecular Plant-Microbe Interactions, 31:175-184.

26) Lucia M.B., Sagarino R.M., Calamba R.B., Contioso M.A.A., Jansalin J.G.F., Calibo C.L. 2015. Potential of chitosan for the control of tomato bacterial wilt caused by *Ralstonia solanacearum* (Smith) Yabuuchi et al. Annals of Tropical Research, 37: 57-69.

27) Mamphogoro T.P., Babalola O.O., Aiyegoro O.A. 2020. Sustainable management strategies for bacterial wilt of sweet peppers (*Capsicum annuum*) and other Solanaceous crops. Journal of Applied Microbiology, 129: 496-508.

28) Manda R.R., Addanki V.A., Srivastava S. 2020. Bacterial wilt of solanaceous crops. International Journal of Chemical Studies, 8: 1048-1057.

29) Mbega E.R., Adriko J., Mortensen C.N., Wulff E.G., Lund O.S., Mabagala R.B. 2013. Improved sample preparation for PCR-based assays in the detection of *Xanthomonads* causing bacterial leaf spot of tomato. British Biotechnology Journal, 3: 556-574.

30) McGarvey J.A., Denny T.P., Schell M.A. 1999. Spatial-temporal and quantitative analysis of growth and EPSI production by *Ralstonia solanacearum* in resistant and susceptible tomato cultivars. Phytopathology, 89:1233-1239.

31) Muhammad N., Rajput N.A., Atiq M., Sahi S.T., Rehman A., Hameed A., Kachelo G.A., Ahmed S. 2022. Integrated management of fusarium wilt of chilli caused by *Fusarium oxysporum* f. sp. *capsici* through different management approaches. Pakistan Journal of Botany, 54(5): 1963-1970.

32) Nakaho K., Allen C. 2009. A pectinase-deficient *Ralstonia solanacearum* strain induces reduced and delayed structural defences in tomato xylem. Journal of Phytopathology, 157:228-234.

33) Nakaho K., Inoue H., Takayama T., Miyagawa H. 2004. Distribution and multiplication of *Ralstonia solanacearum* in tomato plants with resistance derived from different origins. Journal of General Plant Pathology, 70:115-119.

34) Ochoa A.N., Gomez J.E.P. 1993. Activity of enzymes involved in capsaicin biosynthesis in callus tissue and fruits of chili pepper (*Capsicum annuum* L.). Journal of Plant Physiology, 141: 147-152.

35) Oliveira M.D.M., Varanda C.M.R., Félix M.R.F. 2016. Induced resistance during the interaction pathogen x plant and the use of resistance inducers. Phytochemistry Letters, 15: 152-158.

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 08 | 2024
DOI: 10.5281/zenodo.13358050

36) Padalkar N.R., Devmore J.P., Thaware B.G., Nawale R.N. 2012. Reaction of chilli genotypes to *Ralstonia solanocearum*. Indian Phytopathology, 65: 220-227.

37) Pawaskar J.R., Kadam J.J., Navathe S., Kadam J.S. 2014. Response of chilli varieties and genotypes to bacterial wilt caused by *Ralstonia solanacearum* and its management. Indian Journal of Sciences, 11: 66-69.

38) Sahu P.K., Singh S., Gupta A., Singh U.B., Paul S., Paul D., Kuppusamy P., Singh H.V., Saxena A.K. 2020. A Simplified protocol for reversing phenotypic conversion of *Ralstonia solanacearum* during experimentation. International Journal of Environmental Research and Public Health, 17(12): 4274.

39) Saile E., McGarvey J.A., Schell M.A., Denny T.P. 1997. Role of extracellular polysaccharide and endoglucanase in root invasion and colonization of tomato plants by *Ralstonia solanacearum*. Phytopathology, 87:1264-1271.

40) Sanju K., Mathews L.P., Joshua H.F., Laura R., Stephen M.O., James C., Jeffrey B.J. 2017. Foliar applications of Acibenzolar-S-Methyl negatively affect the yield of grafted tomatoes in fields infested with *Ralstonia solanacearum.* Plant Disease, 101: 890-894.

41) Schell M.A. 2000. Control of virulence and pathogenicity genes of *Ralstonia Solanacearum* by an elaborate sensory network. Annual Review of Phytopathology, 38:263-292.

42) Sehgal N., Kumar S. 2021. Variability and Traits Association Analyses in Bacterial Wilt Resistant Genotypes of Tomato (*Solanum lycopersicum* L.) under Mid-Hill Conditions of Himachal Pradesh. Indian Journal of Experimental Biology, 59(09):617-625.

43) Shahbaz M.U., Mukhtar T., Haque M.I., Begum N. 2015. Biochemical and serological characterization of *Ralstonia solanacearum* associated with chilli seeds from Pakistan. International Journal of Agriculture and Biology, 17: 31-40.

44) Sneath P.H., Bread R.S., Murray E.G., Smith R.N. 1986. Bergeys Manual of Determination Bacteriology. William and Wilkins Co., London, pp. 232.

45) Sood S., Thakur M. 2017. Screening for bacterial wilt resistance of bell pepper under sick field conditions and morphological characterization. Journal of Hill Agriculture, 8(4):442-448.

46) Thakur H., Sharma A., Sharma P., Rana R.S. 2021. An insight into the problem of bacterial wilt in *Capsicum* spp. With special reference to India. Crop Protection, 140: 105420.

47) Tudi M., Ruan H.D., Wang L., Lyu J., Sadler R., Connell D., Chu C., Phung D.T. 2021. Agriculture development, pesticide application and its impact on the environment. *International Journal of Environmental Research and Public Health*, 18:1112.

48) Turner M., Jauneau A., Genin S., Tavella M.J., Vailleau F., Gentzbittel L., Jardinaud M.F. 2009. Dissection of bacterial wilt on *Medicago truncatula* revealed two type III secretion system effectors acting on root infection process and disease development. Plant Physiology, 150:1713-1722.

49) Vasse J., Frey P., Trigalet A. 1995. Microscopic studies of intercellular infection and protoxylem invasion of tomato roots by *Pseudomonas Solanacearum*. Molecular Plant-Microbe Interaction, 8:241-251.

50) Winstead N.N., Kelman A. 1952. Inoculation techniques for evaluating resistance to *Pseudomonas solanacearum*. Phytopathology, 42: 628-634.

51) Yabuuchi E., Kosako Y., Yano I., Hotta H., Nishiuchi Y. 1995. Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* genus nov.: proposal of *Ralstonia pickettii* (Ralston, Palleroni and Douderoff 1973) comb. nov., *Ralstonia solanacearum* (Smith 1896) comb. nov. and *Ralstonia eutropha* (Davis 1969) comb. nov. Microbiology and Immunology, 39: 897-904.

ISSN: 1673-064X
E-Publication: Online Open Access
Vol: 67 Issue 08 | 2024
DOI: 10.5281/zenodo.13358050

52) Yanti Y, Hamid H, Nurbailis N, Yaherwandi Y, Liswarni Y, Wibowo I, Selviana S. 2024. Exploration of *Actinobacteria indigenus* as biological control agent of bacterial leaf blight (*Xanthomonas axonopodis* pv. Allii) and increasing production of shallot. Pakistan Journal of Phytopathology, 36(1):211-24.

53) Yuliar N.A., Toyota K. 2015. Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum*. Microbes and Environments, 30: 1-11.

54) Zolobowska L., Van Gijsegem F. 2006. Induction of lateral root structure formation on Petunia roots: A novel effect of GMI1000 *Ralstonia solanacearum* infection impaired in Hrp mutants. Molecular Plant-Microbe Interactions, 19:597-606.