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Susceptibility of chilli selected cultivars to foliar and seed infecting bacteria

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Authors' Contribution Conceived and designed the experiments: **M. U. Raja**, performed the experiments: **S, A. Jamshed**, **S. Aziz** analyzed the data: **S, A. Jamshed, M. U. Raja** contributed materials/ analysis/ tools: **T. Mukhtar and R. Mehmood**.

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ABSTRACT

Phytopathogenic bacteria are potent threat to sustainable chilli (*Capsicum annuum L.*) production all over the world. In past, bacterial infections were reported in chilli plantations of Pakistan but data is still scanty and sporadic. Therefore, this study was planned to evaluate the chilli germplasm resistance against local putative bacterial isolates. During 2021-22, chilli disease samples were collected from Mianwali, Taxila, Chakwal, Islamabad and Rawalpindi. In addition, seeds of different chilli cultivars ((Apollo, Golden Hot, California Wonder, ATX Green, Hot Pepper, Skyline) were also investigated for bacterial infection. In total, six putative isolates were obtained viz., GP, ISL, TAX, CM, and AAR from infected leaf samples while 1 (APO) from infected seeds. On the basis of biochemical and pathogenicity assays, two putative isolates; GP (*Xanthomonas vesicatoria*) and APO (*Pseudomonas syringae*) were screened for further analysis. Detached leaf and seedling inoculations assays showed that hot pepper and Gola Peshawari were found resistant against GP and APO respectively. Out of three cultivars (TAX, Sky Star, and Golden Hot), TAX cultivar showed resistance against GP isolate and Sky star show resistance against APO isolate in seedling inoculation assay. GP and APO isolates sensitivity against copper sulphate and antibiotics viz., erythromycin, penicillin, streptomycin and tetracycline was also assessed by gel diffusion and plate poisoning method respectively. It was observed that tetracycline and streptomycin were effective against GP and APO respectively while bacterial strains APO and GP showed resistance against copper sulphate. Our data showed that *Xanthomonas vesicatoria* and *Pseudomonas syringae* are prevailing in chilli field plantations and seeds. Chilli germplasm must be screened against these bacteria before cultivation to avoid future disease spread.

Keywords: *Xanthomonas vesicatoria*, *Pseudomonas syringae,* Chilli, Punjab, Pakistan

INTRODUCTION: Chilli along tomato, potato and eggplant is member of solanaceae family. Chilli crop originated in central and South America and later European spice dealers introduced it to rest of the world (Perry *et al.*, 2007). Since medieval times, chilli is known for its nutritional, medicinal and cuisine value. Capsaicin is the major bioactive substance while chilli also contains carbohydrates, fats, and proteins (Salam, 2015; Kantar *et al.*, 2016). In Pakistan, chilli is grown on area of 31 thousand acres with production of 82 thousand tons. Sindh province contributes 85% of the national production and Kunri chilli market of Sindh is considered as the biggest chilli market in Asia (Mangan and Ruthbah, 2018). Chilli crop is prone to both abiotic and biotic stresses. Among biotic stresses, bacterial diseases of chilli have worldwide distribution which cause minor losses to complete crop failure. The major bacterial diseases of chilli include wilt (*Ralstonia solanacaearum*), bacterial speck (*Pseudomonas syringae*) and bacterial leaf spot (*Xanthomonas campestris pv vesicatoria*) (Oerke, 2006; Shin and Yun, 2010). Bacterial diseases are reported from different chilli growing areas of Pakistan and responsible for significant crop losses (Begum *et al.*, 2012). In chilli like any other crop, different management strategies are followed to counter diseases which include quarantine, crop rotation, physical, chemical, and biological control (Abbasi *et al.*, 2002; Algam *et al.*, 2010; Le *et al.*, 2020; Dowarah *et al.*, 2021). However, evaluation of innate immunity in germplasm is considered as most robust, economical, long lasting, eco-friendly control measure against plant diseases. The natural ability of *X. campestris pv. vesicatoria* to cause the significant losses in chilli crop is dependent upon virulence factors (Kim *et al.*, 2008). The distribution of virulence factors in pathogenic population not only varies but continuously changing due to mutations, genetic recombination, gene and genotype flow etc. Additionally, abiotic factors such as circadian clock, temperature, humidity play their part (Ahmad and Ahmad, 2022). Similarly, varietal resistance against local population also varies and needed to be assessed regularly against prevailing pathogenic population for sustainable crop production. Subsequently, screened *R*-plant and virulent pathogen could be used for future germplasm screening programs. In spite of the fact that chilli crop is regularly attacked by bacteria in Pakistan but the information regarding indigenous resistant source against prevailing bacterial ingresses is hardly available. Therefore, this study was planned to evaluate the inherent immunity of chilli germplasm against field and seed borne bacterial pathogens.

OBJECTIVES: The research was conducted with following objectives (1) characterization of putative pathogenic bacterial isolates from chilli based upon biochemical traits. (2) unraveling of innate resistance source in chilli germplasm against local putative bacterial isolates.

MATERIALS AND METHODS: Collection and isolation of putative bacterial isolates from planting material: Symptomatic chilli plant samples were collected from Mianwali, Taxila, Chakwal, Islamabad and Rawalpindi during 2021-22. Additionally, seeds of chilli germplasm (Apollo, Golden Hot, California Wonder, ATX Green, Hot Pepper, Skyline) were also investigated for the presence of bacterial pathogens (Schaad *et al.*, 2001). Forty surface sterilized seeds of each cultivar (10seeds/plate) were subjected to count infection percentage. The seed infection percentage was calculated by formula (Boukaew *et al.*, 2017).

$$\text{Seed Infection Percentage} = \frac{\text{Number of infected seeds}}{\text{Total number of seeds}} \times 100$$

Phenotypic and biochemical characterization: Colonies of putative bacterial isolates were observed for colony colour and shape on nutrient agar (NA) and on yeast extract-dextrose-Calcium Carbonate (YDC) media. Different biochemical tests such as KOH test (Suslow *et al.*, 1982), catalase test (Schaad et al., 2001), oxidase, urease production (Goszczynska *et al.*, 2000), sulphide or motility test were performed by using sulfide indole motility (SIM media). Oxygen requirement of putative isolates were also assessed (Akbar *et al.*, 2015).

Pathogenicity assays: Detached asymptomatic or healthy leaves of chilli cultivars (Apollo, Skyline, Hot pepper, Gola Peshawari) and for seedling inoculation (TAX, Sky Star, Golden hot) methods were used to study inbred resistance and susceptibility against *X. vesicatoria and P. syringae isolates*. Fresh cultures of *X. vesicatoria* and *P. syringae* were prepared in sterilized distilled water. The OD_{600} nm was adjusted at 0.4 (10^5cfu/ml). Asymptomatic or healthy leaves of chilli were collected and placed on moist sterilized filter paper in petri plates. Leaves were pin pricked and subsequently inoculated separately with *X. vesicatoria* and *P. syringae* (Sahin and Miller, 1998). Sterilized water treated leaves were considered as negative control. Leaves were regularly monitored for 7 days post inoculation (dpi) for the appearance of disease symptoms. Three chilli cultivars (Tax, Sky star and Golden Hot) were evaluated against *X. vesicatoria* and *P. syringae* by foliar spray method while plants treated with sterilized distilled water were considered as mock treatment (Aslam *et al.*, 2017). Inoculated chilli plants were kept in green house and regularly observed for 7 dpi for the appearance of symptoms. Disease severity was assessed by using Vallejos *et al.* (2010) rating scale; 1 = no disease symptoms, 2 = slight to moderate yellowing and slight necrosis, 3 = extensive yellowing and moderate necrosis, and 4 = complete necrosis.

***Invitro* sensitivity assays:** Sensitivity of *X. vesicatoria* and *P. syringae* against antibiotics such as penicillin (10 ug), streptomycin (10ug), tetracycline (30 ug), erythromycin (15ug) was assessed by plate poison technique (Bauer *et al.*, 1966). Commercially available antibiotic (Oxoid®) discs were used. Fresh bacterial suspensions were swabbed on NA plates. Four discs were placed on plates and

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zone of inhibition around individual disc was measured. Plates swabbed with bacterial suspension but without discs were used as a control. Four replicates of each antibiotic were measured. Well diffusion method (Valgas *et al.*, 2007) was used to evaluate different concentrations (50ppm, 100ppm 200ppm and 1000ppm) of copper sulphate against putative bacterial strains. Plates were placed in incubator at 28°C for 24 hours.

RESULTS: Isolation of putative bacteria from chilli: In total, 6 putative bacterial isolates were isolated. Five putative isolates (GP, TAX, AAR, CM, ISL) were collected from chilli infected leaf segments while 1 putative isolate (APO) from seeds of different cultivars (figure 1). Out of 6 chilli cultivars investigated for seed borne infection, only two cultivars (Skyline, Hot pepper) carried no seed infection while the maximum seed infection was found in Apollo and ATX green (20%).

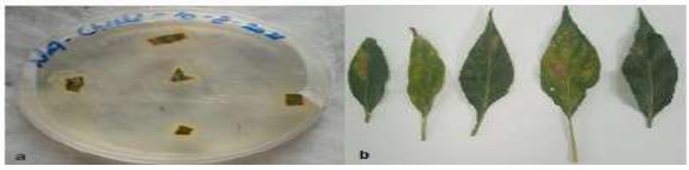

Figure 1: Bacterial growth (a) and sympatomatic chilli leaves (b).

Characterization based on biochemical traits: All the putative bacterial isolates were grown on NA and YDC culture media. Putative isolates collected from leaves appeared yellow on YDC media while bacteria isolated from seeds made cream-colored colonies. All six isolates (GP, TAX, AAR, CM, ISL, APO) formed loop and found strictly aerobic and catalase positive. However, putative isolates were unable to produce hydrogen sulphide, oxidase and arginine dihydrolase (figure 2, table 1).

Sr. No	Isolates	YDC	Loop Test	Oxygen	H_2S	Catalase	Oxidase	Arginine dihydrolase Test
1	GP	Yellow	+ve	Aerobic	-ve	+ve	-ve	-ve
2	Tax	Yellow	+ve	Aerobic	-ve	+ve	-ve	-ve
3	AAR	Yellow	+ve	Aerobic	-ve	+ve	-ve	-ve
4	CM	Yellow	+ve	Aerobic	-ve	+ve	-ve	-ve
5	ISL	Yellow	+ve	Aerobic	-ve	+ve	-ve	-ve
6	APO	Cream	+ve	Aerobic	-ve	+ve	-ve	-ve

Table 1: Characterization of bacterial isolates based on biochemical assays.

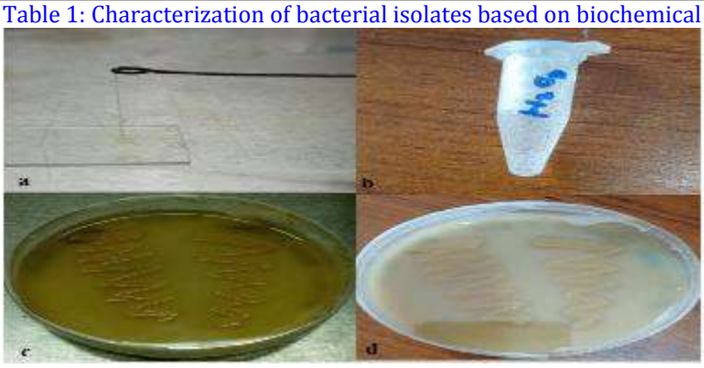

Figure 2: Biochemical assays of bacterial isolates a) Gram negative bacteria forming the loop when treated with 3% KOH. b) Catalase test indicating bubbles production on treatment with 10% H_2O_2 c) Yellow bacterial growth on YDC medium. d) Creamy bacterial growth on YDC medium.

Screening of chilli germplasm: Detached leaf method depicted that cultivars (Apollo, Skyline, Hot pepper) showed susceptibility against APO (*Pseudomonas syringae*) while no symptoms were appeared on Gola Peshawari. It is pertinent to mention that APO isolate was initially isolated from chilli cultivar Apollo. Three cultivars (Gola Peshawari, Skyline and Apollo) showed susceptibility while Hot pepper showed resistance against GP (*Xanthomonas vesicatoria*) isolate in comparison to water treated leaves.

In seed inoculation method, chilli cultivars (TAX, Sky Star and Golden Hot) tested against APO and GP isolates. Our data showed that TAX cultivar of chilli was resistant while Sky star and Golden hot were moderately resistant against GP. Sky star was found resistant while TAX and Golden hot were found susceptible against APO after 10 dpi (figure 3).

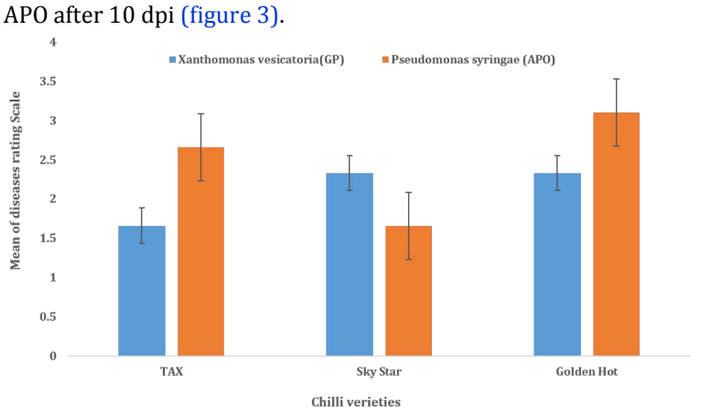

Figure 3: Statistical measurement of mean disease rating scale against *X. vesicatoria* and *P. syringae*.

***Invitro* sensitivity assays:** All four antibiotics (erythromycin. penicillin, streptomycin, tetracycline) formed zone of inhibition in plates containing *Xanthomonas vesicatoria* and *Pseudomonas syringae* by poison plate method after 24 hours. . Maximum zone of inhibition against GP (22.5) and APO (22.5) was formed by tetracycline and streptomycin respectively (figure 4, 5).

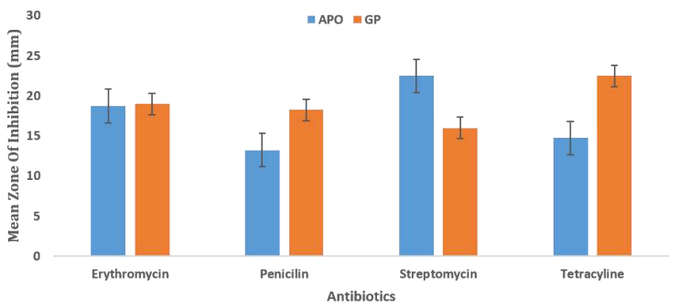

Figure 4: Zone of inhibition of antibiotic against *Pseudomonas syringae* (APO) and *Xanthomonas vesicatoria* (GP).

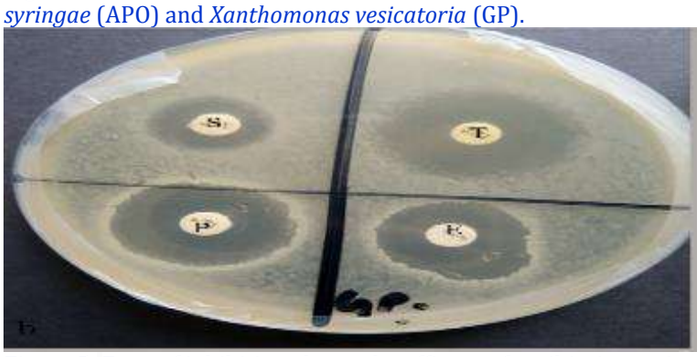

Figure 5: Zone of inhibition shows the efficacy of antibiotics against *Xanthomonas vesicatoria*. E; erythromycin, P; penicillin, S; streptomycin, T; Tetracycline.

Despite, bacterial isolates tested against different concentrations of copper sulphates but no zone of inhibition was observed on media plates (figure 6).

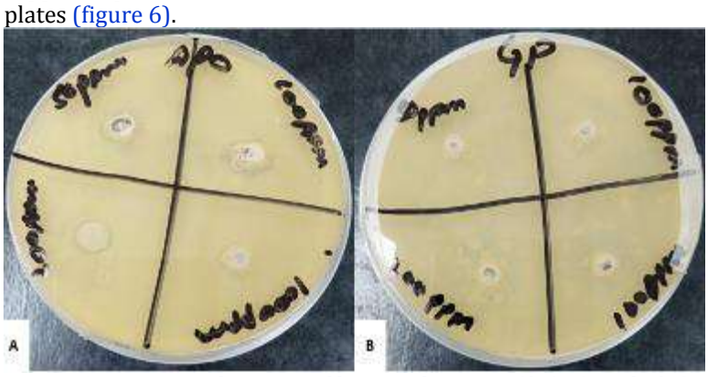

Figure 6: *Invitro* evaluations of bacterial strains A) APO and B) GP against copper sulphate (50ppm,100ppm,1000 ppm).

DISCUSSION: Pakistan is ranked 7th most vulnerable country to climate change which causes many abiotic and biotic stresses. Pakistan is also at cross road of world economies due to China Pakistan economic corridor (CPEC) which increase chances of introduction of invasive pathogen species. Likewise, chilli crop in Pakistan is also under stress due to bacterial diseases which already blighted the future of growers, traders and exporters. The current study was planned to evaluate the potential of chilli germplasm against prevailing seedborne and foliar diseases of

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bacterial origin. Pathogen virulence analysis and subsequent host resistance are two key areas which needed a consistent surveillance and evaluation. In current study, chilli seed infection up to 20% was found with *Pseudomonas syringae*. Seed is always considered as major vehicle of pathogen dispersal in new geographical areas where it previously not found. Studies showed that even a small friction of seed infection could lead to crop epidemics (Shenge *et al.*, 2010; Umesha, 2020). Therefore, *Pseudomonas syringae* presence at such a high percentage is looming threat to sustainable chilli production in Pakistan. It is imperative to mention that *Xanthomonas vesicatoria* was also isolated from symptomatic leaf segments collected from different geographical areas. In Pakistan, chilli is grown in warm areas which increases its vulnerability against *Xanthomonas vesicatoria*. *Xanthomonas vesicatoria* is pathogen who has the potential to cause losses of great magnitude in solanaceous crops. Recently, Ahmad and Ahmad (2022) reported high disease incidence and severity in tomato crop infected by bacterial leaf spot caused by *Xanthomonas vesicatoria* in different districts of KPK province of Pakistan. Studies showed that the pathogen under favourable conditions causes losses up to 50 % in different parts of Pakistan. It is worth to mention that European Cooperation in Science and Technology (COST) has actually run a program EuroXanth COST Action (2017-21) to counter the threat posed by members of family *Xanthomonadacea* which includes genus *Xanthomonas* and *Xylella* to food security (Costa *et al.*, 2021; Utami *et al.*, 2022). Detached leaf and seedling inoculation assays showed varietal variation when it comes to resistance against *Pseudomonas syringae* and *Xanthomonas vesicatoria*. Licciardello *et al.* (2022) also determined the susceptibility of citrus against *Xanthomonas citri* pv. *citri* and *X. citri* pv. *aurantifolii* by detached leaf assays. Our results showed that there is variation among plant cultivars to pathogen due to presence of different set of resistant genes to fend off hemibiotroph (*P. syringae*) and biotroph (*X. vesicatoria*). Copper based assays showed bacterial resistance which also reported by Basim *et al.* (2005) while working on Taiwanese isolates of *Xanthomonas campestris* pv. vesicatoria. However, bacteria showed susceptibility against different antibiotics (erythromycin. penicillin, streptomycin, tetracycline). McManus and Stockwell (2000) discussed the success story of streptomycin and oxytetracycline in control phytobacteria especially *Erwinia amylovora* causal organism apple and pear blight. It is imperative to mention that regulators such as environment protection agency (EPA) also allowed both streptomycin and oxytetracycline for the control of phytobacteria. Contrarily, antibiotics are not promoted for the control of bacterial diseases in country like Pakistan

CONCLUSION: Our preliminary study revealed the prevalence of *Xanthomonas vesicatoria* and *Pseudomonas syringae* both in seed and field plantations of chilli. However, chilli germplasm showed variations when it comes to resistance to both bacteria. It is worth to mention that local bacterial isolates were sensitive to antibiotics but resistant to copper sulphate. Therefore, it is recommended that chilli germplasm not only regularly evaluated against prevailing pathogens but use of antibiotics must be promoted in future disease control program.

CONFLICT OF INTEREST: Authors have no conflict of interest.

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