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Evaluation of chemical and biological management strategies against bacterial blight of French bean (*Phaseolus vulgaris*) caused by *Xanthomonas axonopodis* pv. *phaseoli*

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ABSTRACT

Bacterial blight caused by *Xanthomonas axonopodis* pv. *phaseoli* is a severe disease in French bean (*Phaseolus vulgaris* L.), leading to yield losses of about 10–40%, depending on the susceptibility of the cultivar and environmental conditions. The study was carried out during rainy (*kharif*) season of 2020 and 2021 at Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Himachal Pradesh, to evaluate the effectiveness of various management strategies, including chemical treatments and bioagents, to mitigate. Efficacy of different chemicals was evaluated under *in vitro* conditions using well diffusion method. The results showed streptocycline as the most potent inhibitor of *Xanthomonas axonopodis* pv. *phaseoli* showing maximum zone of inhibition (22.20 mm). Further evaluation of chemicals under pot culture experiment revealed that seed treatment using streptocycline (100 ppm) combined with three foliar sprays of copper oxychloride (0.3%) applied at 7 days interval was the most effective strategy, significantly reducing the disease severity. During the field trials the combination treatments (T_6 and T_7) proved most effective in reducing disease indices and increasing yield with the highest disease control and yield per hectare. Seed treatment using streptocycline (100 ppm) and spray of copper oxychloride (0.3%) (T_6) provided the most robust results followed by the combination of seed treatment with streptocycline (100 ppm) and streptocycline spray (T_7). These treatments not only controlled the disease effectively but also offered the best economic returns as indicated by the ICBR ratios. The findings emphasize the effectiveness of combining seed treatments and foliar sprays in controlling bacterial blight and improving French bean productivity.

Keywords: Bacterial blight, Bean, Management, Streptocycline

French bean (*Phaseolus vulgaris* L.) is one of the most important vegetable crop belonging to the family Leguminosae which is grown over tropical, subtropical and temperate regions all over the world. French bean is the most commonly grown legume worldwide which is consumed as vegetable when the pods are immature, delicate and tender. The green pods are quite nutritious being the potential source of protein and carbohydrates and rich in calcium, phosphorus, iron and zinc (Kanwar and Mehta 2018). Successful and economic cultivation of beans is affected by various diseases and pests. Among several diseases, common bacterial blight (CBB) caused by *Xanthomonas axonopodis* pv. *phaseoli* is one of the most devastating disease causing high yield losses (Coyne *et al.* 2003). Common bacterial blight (CBB) symptoms can affect all above ground parts of the bean plant, including seedlings, leaves, stems, pods and seeds. Initially, symptoms appear as water soaked spots that progress into necrotic lesions on leaves, pustules on pods and cankers on stems. In cases of severe infection, the plant may experience defoliation and wilting (Chen *et al*. 2021). The disease was first time recorded in New York in 1892 (Beach 1892). Later it was reported in several countries like Colombia, Chile (Schuster and Coyne 1975), Brazil and Mexico (Crispin and Campos 1976), which produce most of the beans consumed in the world. Under favourable conditions the disease may cause yield losses of >40%. The disease is very prevalent and cause yield losses of around 70% in Brazil and 30–70% in Ethiopia (Foucher *et al.* 2022). Further the disease has also been reported in different Asian countries like India, Bangladesh, Japan and China (Karavina *et al.* 2011). Whereas, in India the disease was first recorded in 1943 at Puna by Uppal *et al.* (1946). In Southern India, the disease reduced the crop yield by 40% whereas, the yield losses

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range from 25–50% in north western Himalayan regions of India (Kumar *et al.* 2006). Since, effective management of this disease is very important to help reduce the yield losses in major bean growing areas of Himachal Pradesh, so the present study was designed to study the pathogenic potential and further formulate effective management strategies for the control of disease.

MATERIALS AND METHODS

Isolation of the pathogen: The study was carried out during rainy (*kharif*) season of 2020 and 2021 at Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Himachal Pradesh. Plant samples showing disease symptoms were collected from French bean growing areas of Solan and Sirmaur districts of Himachal Pradesh. Samples were examined under the microscope and further various biochemical tests were performed to verify the presence of the related bacterium (Suchita *et al*. 2023). Leaves exhibiting typical 'V'-shaped lesions and oozing symptoms were chosen for pathogen isolation. The infected leaves were rinsed with sterile water and small sections were excised from the lesion borders, ensuring that some healthy tissue was included. These sections were surface sterilized using a 5% sodium hypochlorite solution, then rinsed three times with sterile distilled water. The sterilized tissue pieces were placed in flasks with distilled water and agitated on a mechanical shaker for 2–3 days, with at least two hours of shaking each day. A loopful having bacterial suspension was further streaked onto nutrient agar plates followed by incubation at 28 ± 1°C for 48 h. After incubation, the plates were checked for colony growth, and the isolated colonies were purified on Luria-Bertani (LB) media, following the method described by Sultana *et al.* (2018).

Inoculum preparation: To prepare the inoculum, a bacterial suspension was made using the purified culture of the pathogen grown on LB plates. The fully grown bacterial colonies were transferred into 100 ml of nutrient broth and incubated on a mechanical shaker for 2–3 days. The resulting suspension was then serially diluted (up to seven dilutions) to reach a final concentration of 10^7cfu/mL (colony forming units per ml).

Inoculation method: In field and pot experiments, inoculation was performed on three weeks old healthy bean plants. The leaf surface was gently abraded using a forefinger or cheese cloth with carborundum powder, applied against the direction of leaf hair growth, to create slight injuries. A cotton swab dipped in the bacterial suspension (10^7cfu/mL) was then used to rub the suspension across the entire leaf surface.

In vitro *evaluation*: Using the well diffusion method, the effectiveness of various chemicals against the isolated bacterium was assessed. In this technique, a sterile cotton swab was dipped in a fresh (24–48 h) bacterial suspension (10^7cfu/ml) and spread evenly over the entire nutrient agar plates until the bacterial suspension was fully absorbed on the plates. To prepare the wells, 8 mm-diameter holes were punctured aseptically using a sterile cork borer. With the use of a micropipette, approximately 10 µl of each chemical were added to each well at the desired concentrations. Each treatment was replicated four times. After 48 h of incubation at 28 ± 2°C, observations were made for the development of inhibition zone around the wells. The chemicals used for *in vitro* evaluation were Blitox (Common name: copper oxychloride 50 WP @1000, 2000, 3000 ppm), Bordeaux mixture [Common name: copper sulphate + lime (0.8%) @1000, 2000, 3000 ppm], Kocide (Common name: copper hydroxide 46 WP @000, 2000, 3000 ppm), Streptocycline (Common name: streptomycin sulphate 90% w/w + tetracycline hydrochloride 10% w/w @150, 200, 250 ppm), Agrimycin (Common name: streptomycin 17 WP @150, 200, 250 ppm), and Omycin (Common name: kasugamycin 3% SL @150, 200, 250 ppm).

Pot culture experiment: To test the effectiveness of chemicals and bioagents against common bacterial blight under pot culture, an experiment was setup using bean seeds of Contender variety sown in sterilised soil containing plastic pots (9 cm diameter). One month old plants were inoculated with 48 h old suspension (10^7cfu/ml) of the bacterial pathogen using carborundum abrasion method. Following inoculation, the pots were kept in a relative humidity and temperature control cabinet set at 90% relative humidity (RH) and a temperature of 28 °C. he experiment utilised a total of seven treatments including the control. All the treatments (T_1, Control; T_2, Seed treatment with streptocycline @150 ppm; T_3, Seed treatment with *Pseudomonas fluorescence* (10 g/kg); T_4, Spray of blitox (copper oxychloride) @0.3% applied three times at 7 days interval started after 4 days of inoculation; T_5, Spray of *Pseudomonas fluorescence* (0.1%) thrice at 7 days interval started after 4 days of inoculation; T_6, $T_2 + T_4$ and T_7, $T_3 + T_5$) were replicated four times, in which six plants were taken per replication. The data on per cent disease severity was evaluated after 10, 20 and 30 days after inoculation by as per the disease rating scale given by Dursun *et al.* (2002). The disease rating was given as 0, No disease; 1, 0.1–10%; 2, 10.1–25%; 3, 25.1–50%; 4, 50.1–75%; and 5, >75% infected leaf area.

The percent disease severity was calculated using the formula given by Mckinney (1923):

$$\text{Disease severity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings} \times \text{Maximum disease grade}} \times 100$$

Field evaluation: A field trial with a plot size of 2.25 m × 2.25 m and three replications was set up at Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, during the *kharif* season of 2020 and 2021 to assess the effectiveness of various chemicals and bioagent against bacterial blight of bean. Nine treatments including the control, were used altogether, viz. T_1, Seed treatment with streptocycline (100 ppm) before sowing; T_2, Seed treatment with *Pseudomonas fluorescence* @10 g/kg seed; T_3, Spray of blitox (copper oxychloride) @0.3% applied three times at 10 days interval started after appearance of disease; T_4, Spray of streptocycline (100 ppm) three times at 10 days intervals started after appearance of disease; T_5, Spray of *Pseudomonas fluorescence* @0.1% three times at 10 days interval started after appearance of the disease; T_6, T_1+T_3; T_7, T_1+T_4; T_8, T_2+T_5 and T_9, Control. Periodic observations were taken for disease severity, green pod yield and incremental cost benefit ratio (ICBR):

$$\text{ICBR} = \frac{\text{Additional funds obtained by treatment}}{\text{Amount spent for disease management}}$$

The additional funds obtained = The income obtained on incremental pod yield in the treatment plot compared to control treatment plot

Extra amount spent for disease management in respective treatment = Labour cost + Cost of fungicide/ bioagent + Depreciation cost of appliance

Statistical analysis: Following the methodology outlined by Gomez and Gomez (1984), analysis of variance (ANOVA) was conducted for experiments performed under controlled conditions, organized using completely randomized design (CRD) and randomized block design (RBD). The 'F' test was applied at a significance level of 0.05, with degrees of freedom (t-1), (t) (r-1) for CRD and (r-1), (r-1) (t-1) for RBD. The sum of squares for replication and treatment means were compared to the error mean squares. F-values calculated were further compared with the tabulated F-values, and if the F-test showed significant results, the critical difference was determined to assess whether any treatment was superior. Data analysis was performed using OPSTAT software, and box plots were generated with the help of R software (3.6.1.version).

RESULTS AND DISCUSSION

In vitro *evaluation*: The study showed that different fungicides and antibiotics had varying degrees of success in inhibiting *Xanthomonas axonopodis* pv. *phaseoli* (Table 1, Fig. 1). Streptocycline demonstrated the highest level of inhibition (22.20 mm) among all the chemicals tested, followed by copper oxychloride (18.10 mm) and copper hydroxide (16.80 mm). Copper sulphate + lime (11.80 mm) and agrimycin (11.30 mm) caused somewhat less inhibition, and their results were statistically comparable. The least inhibitory agent, omycin, was found to have a minimum inhibition zone of 9.80 mm. In general, as chemical concentration levels increased, the inhibitory response to all of the chemicals tested against *Xanthomonas axonopodis* pv. *phaseoli* increased.

These findings were in agreement with those of other researchers who found that various chemicals, including streptocycline and copper oxychloride, were effective against under *in vitro* experiments (Raju *et al*. 2012, Antre *et al*. 2016). Copper based antibacterial chemicals like Blitox, Kocide, and Bordeaux mixture were significantly inhibitory against the pathogen, according to *in vitro* evaluation of chemicals (Kumar *et al.* 2018). Similar findings were observed by Jadhav *et al.* (2018) while evaluating several antibiotics and botanicals against *Xanthomonas axonopodis* pv. *citri* where streptocycline showed maximum inhibitory effect followed by copper oxychloride.

Pot culture experiment: Under the pot culture experiment the most effective treatment against the bacterial blight, reflecting the lowest disease severity (32.4%) after 30 days of inoculation, was treatment combination (T_6) consisting of seed treatment with streptocycline @100 ppm followed by three periodic sprays of copper oxychloride (0.3%) at seven days intervals started after appearance of disease (Table 2, Fig. 2).

In contrast to the use of *P. flourescens* as a seed dresser, which was ineffective against bean bacterial blight, streptocycline based seed treatment significantly reduced the development of the disease..

The findings of the study were similar to those of Jagtap

Table 1 *In vitro* evaluation of different chemicals against *Xanthomonas axonopodis* pv. *phaseoli*

Treatment	Trade name	Common name	Zone of inhibition (mm) at concentrations			Mean
			C_1	C_2	C_3	
T_1	Blitox*	copper oxychloride 50 WP	14.80	18.20	21.30	18.10
T_2	Bordeaux mixture*	copper sulphate 0.8% + lime	9.70	11.40	14.50	11.80
T_3	Kocide*	copper hydroxide 46 WP	14.50	16.30	19.70	16.80
T_4	Streptocycline**	streptomycin sulphate 90% w/w + tetracycline hydrochloride 10% w/w	16.60	22.70	27.20	22.20
T_5	Agrimycin**	streptomycin 17 WP	8.80	11.60	13.30	11.30
T_6	Omycin**	kasugamycin 3% SL	7.80	9.80	11.70	9.80
	Mean		12.10	15.10	18.00	
	CD (p=0.05)		Chemical, 0.78; Concentration, 0.55 Chemical × Concentration =1.35			

*Concentration C_1, C_2, C_3 used were 1000, 2000 and 3000 ppm, respectively; ** Concentration C_1, C_2, C_3 used were 150, 200 and 300 ppm, respectively. Treatment details are given under Materials and Methods.

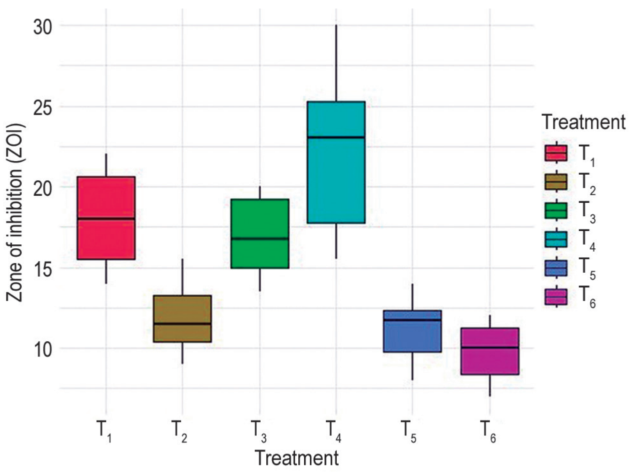

Fig. 1 Box plot represents the zone of inhibition (ZOI) due to the effect of various chemicals.
Treatment details are given under Materials and Methods.

et al. (2012), who claimed that periodic sprays of copper oxychloride (0.25%) + streptocycline (100 ppm) were successful in preventing bacterial blight of cotton. Similar findings were reported by various researchers (Silva *et al.* 2008, Lokesh *et al.* 2014).

Field evaluation: The results of field evaluation study for the year 2020 and 2021 (Table 3) indicated variable response of the chemical and bioagent treatments against bacterial blight of bean. For the year 2020, the study revealed that treatment (T_7) consisting of seed treatment using streptocycline @100 ppm followed by three foliar sprays of streptocycline @100 ppm started after the initiation of disease proved to be the most effective in limiting the disease (72.28%) and enhancing the pod yield (106.06 q/ha). However, the treatment (T_6), which involved seed treatment with streptocycline at a concentration of 100 ppm, followed by three sprays of copper oxychloride at a concentration of 0.3%, was found to be the most cost-effective in terms of disease control (67.60%) and improving the pod yield (by 100.79 q/ha), with an ICBR ratio of 1:16.38. Streptocycline @100 ppm and copper oxychloride @0.3% applied on a regular basis significantly reduced the disease (55.17% and 44.86%, respectively). Seed treatment with Streptocycline @100 ppm also contributed to some disease control (37.64%) while seed treatment with *P. flourescens* had the lowest disease control rates (23.60%).

In 2021, the treatment T_7 (T_1+T_4) exhibited the highest disease control at 78.03%, along with the maximum pod yield of 110.67 q/ha, while seed treatment with *P. fluorescens* @10 g/kg (T_2) showed the lowest disease control at 24.79% and a yield of 54.67 q/ha. In terms of the ICBR ratio, T_6 (T_1+T_3) had the highest ratio of 1:10.39, indicating strong economic returns. These results underscore the superior efficacy of combined treatments like T_7 in managing disease and boosting productivity compared to other treatments. Overall, integrated approaches yielded the best outcomes in terms of disease management and productivity across both years.

The field evaluation studies conducted by Fininsa (2003) were in agreement with the current study as they reported copper sulphate and copper hydroxide applications significantly reduced CBB caused *by Xanthomonas axonopodis* pv. *phaseoli* on bean plants. Pathak and Godika (2006) also conducted studies for bacterial blight of cotton caused by *Xanthomonas axonopodis* pv. *malvacearum* and

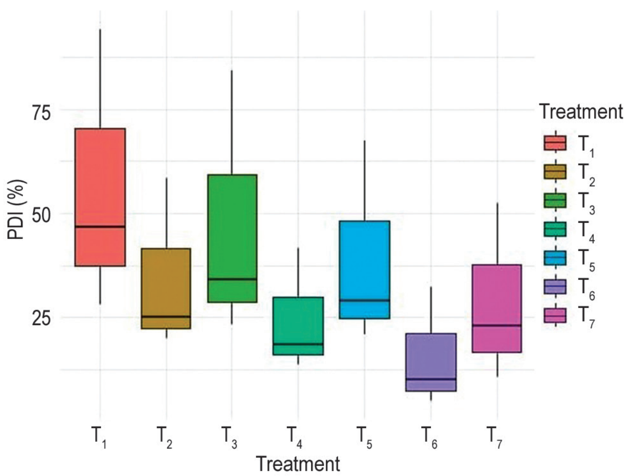

Fig. 2 Box plot represents the average per cent disease index (PDI %) recorded at three intervals [10, 20, 30 days after inoculation (DAI)] after the use of different treatments.
Treatment details are given under Materials and Methods.

Table 2 Evaluation of various chemicals and bioagents against bacterial blight of bean in pot culture

Treatments	Per cent disease severity after days of inoculation			Mean
	10	20	30	
T_1, Control	27.92 (31.88)	46.62 (43.04)	94.12 (76.29)	56.22 (50.40)
T_2, ST Streptocycline @100 ppm	19.92 (26.49)	25.07 (30.01)	58.27 (49.77)	34.42 (35.42)
T_3, ST *P. flourescens* @10 g/kg	23.30 (28.82)	34.15 (35.70)	84.35 (66.82)	47.26 (43.78)
T_4, Spray copper oxychloride @0.3%	13.65 (21.56)	18.3 (25.30)	41.62 (40.14)	24.52 (29.00)
T_5, Spray *P. flourescens* @0.1%	20.75 (27.06)	29.05 (32.57)	67.47 (55.22)	39.09 (38.29)
T_6, T_2 + T_4	4.95 (12.67)	9.92 (18.23)	32.4 (34.66)	15.75 (21.85)
T_7, T_3 + T_5	10.72 (18.95)	22.82 (28.49)	52.45 (46.38)	28.66 (31.27)
Mean	17.31 (23.92)	26.56 (30.48)	61.52 (52.75)	
CD (p=0.05)	Treatment, 1.98; Interval, 1.29; Treatment × Interval = 3.43			

The figures in parentheses represent values that have been transformed using arc sine transformation.

Treatment details are given under Materials and Methods.

Table 3 Evaluation of various chemicals and bioagents against bacterial blight of bean under field conditions

Treatment	Disease index (%) 2020	Disease index (%) 2021	Disease control (%) 2020	Disease control (%) 2021	Yield (kg/plot) 2020	Yield (kg/plot) 2021	Yield (q/ha) 2020	Yield (q/ha) 2021	ICBR ratio 2020	ICBR ratio 2021
T_1, ST Streptocycline @100 ppm	30.30 (33.37)	32.06 (34.45)	37.64 (37.81)	41.63 (40.15)	3.23	3.06	63.90	60.60	11.58	7.71
T_2, ST *P. flourescens* @10 g/kg	37.30 (37.60)	38.70 (38.44)	23.60 (29.05)	24.79 (29.84)	2.83	2.76	55.99	54.67	5.55	3.21
T_3, Spray copper oxychloride @0.3%	26.93 (31.21)	28.43 (32.18)	44.86 (42.03)	45.80 (42.56)	3.70	3.86	73.12	76.41	9.92	5.05
T_4, Spray Streptocycline 100 ppm	21.76 (27.75)	19.93 (26.42)	55.17 (47.97)	63.20 (52.72)	4.56	4.83	90.25	95.52	6.91	2.38
T_5, Spray *P. fluorescence* @0.1%	33.40 (35.22)	35.03 (36.24)	32.00 (34.40)	35.64 (36.53)	3.16	3.23	62.58	63.90	9.66	7.50
T_6, T_1+T_3	16.00 (23.46)	15.76 (23.29)	67.60 (55.30)	71.11 (52.72)	5.10	5.53	100.79	109.35	16.38	10.39
T_7, T_1+T_4	13.43 (21.42)	11.93 (20.19)	72.28 (58.29)	78.03 (62.03)	5.36	5.60	106.06	110.67	8.57	3.25
T_8, T_2+T_5	23.20 (28.70)	23.56 (28.94)	52.80 (46.58)	56.49 (48.77)	4.03	3.80	79.71	75.09	13.80	8.85
T_9, Control	48.86 (44.32)	54.40 (47.50)	-	-	2.36	2.46	46.77	48.74	-	
CD (p=0.05)	3.01	5.608	5.07	6.10	1.16	0.67	24.01	13.36		

The figures in parentheses represent values that have been transformed using arc sine transformation.

concluded that foliar spray with streptocycline (100 ppm) and copper oxychloride (0.3%) was the most effective treatment. Various studies conducted by Bala *et al*. (2017), Madavi *et al*. (2020) and Jat *et al.* (2022) also revealed foliar spray of streptocycline (250 ppm) + copper oxychloride (2000 ppm) as the best management practice for bacterial blight disease.

The study conducted at Dr. Yashwant Singh Parmar University of Horticulture and Forestry highlighted the effectiveness of various chemical treatments and bioagents in managing bacterial blight in French bean. Among the treatments evaluated, a combination of seed treatment with streptocycline and foliar sprays of copper oxychloride emerged as the most effective strategy for reducing disease severity and enhancing yield. Field trials confirmed that these combination treatments not only controlled the disease effectively but also provided the best economic returns. The findings suggested that integrating seed treatments with foliar sprays is a promising approach to managing bacterial blight and boosting French bean productivity.

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