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RESEARCH ARTICLE OPEN ACCESS

In vitro and *In vivo* Management of Bacterial Blight (*Xanthomonas axonopodis* pv. *cyamopsidis*) of Clusterbean

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Abstract

Bacterial Blight of clusterbean(BLB), which is brought on by *Xanthomonas axonopodis* pv. *cyamopsis*, is a significant disease that causes severe infestation, resulting in losses of 58-68% of seed production. In order to determine the best BLB management measures, two-year field experiments and laboratory investigations were carried out in Bikaner, Rajasthan. The experiment was carried out on the BLB-susceptible cv. RGC-986. Garlic clove extract showed the most efficacy (12.80 mm) when evaluated in lab trials against *Xanthomonas axonopodis* pv. *cyamopsis*, whereas *P. fluorescens* bioagents revealed the highest efficacy (18.10 mm). Streptocycline, an antibiotic with strong antimicrobial activity that inhibited the pathogen and exhibited maximum growth of inhibition, 21.34 mm at 200 ppm, while Copper Oxy Chloride, a fungicide, had a maximum growth inhibiting activity as 19.60 mm at a dose of 1000 ppm. The field experiment's findings revealed that all treatments significantly reduced disease severity and net return while also increasing seed yield when compared to the control treatment. The maximum per cent disease reduction 92.11% and 88.77% was recorded after 45 and 60 days, respectively with T_7 (T_{1+}One spray of Streptocycline (150 ppm + Copper oxy chloride 0.2%). The treatment that was least effective was seed soaking with validamycin (200 ppm), followed by streptocycline at 150 ppm and then by COC at 0.2%.

Keywords: Clusterbean, Bacterial Blight, Evaluated, Susceptible, Management, *In vitro*, *In vivo*

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INTRODUCTION

Clusterbean, also called "guar" or "cow chow," is a dry and semi-arid legume crop that belongs to leguminaceae family. Its scientific name is *Cyamopsis tetragonoloba* (L.) Taub. Because of its extensive tap roots system and great capacity to recover from water stress, it is a leguminous crop that endures drought. It is a drought-tolerant crop which is suitable for growing under rainfed conditions during India's *kharif* season. By fixing the nitrogen in the atmosphere, it improves the soil's fertility. The crop matches the arid farmer's livelihood requirements with limited input requirements and aftercare. In India, between 80 and 85 percent of the clusterbean gum is exported, while the remaining 15 percent is being used for domestic purpose. A total of 0.17 million tons of crop and its derivative products are exported from India for a total value of Rs 500 crore. The status of an industrialized crop is having a high concentration of glactomannan in its endosperm, which has a variety of industrial uses. The green pods contain 82.5% water, 3.7% protein, 9.9% carbohydrates, 0.2% fat, 2.3% fiber and 1.4% other minerals, including 0.13% calcium, 0.25% phosphorus, 5.8 mg/100 g iron and 49 mg/100 g vitamin. Approximately 80% of the world's production of clusterbeans is produced in India. It is grown on more than 4 million acres, however only around 80% of the country's production is concentrated in Rajasthan. Due to a lack of availability in the global market, it has expanded into less traditional growing regions such as Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra and Chhattisgarh. The total area of clusterbean in India is 31.40 lakh ha., which is having production of 16.24 million tons, with a yield of 428 kg per ha. In Rajasthan, clusterbean production covers an area of 28.41 lakh ha., yielding 452 kg per ha. and producing 12.84 lakh tonnes annually.[1] Total area which is used for the cultivation of clusterbeans is about 60%, which is primarily limited to the north-western hot and dry region, which includes the districts Bikaner, Churu, Barmer, Jaisalmer, Hanumangarh, and Jodhpur. About one-fourth of Rajasthan's clusterbean production and area was produced in the Bikaner district.

Xanthomonas axonopodis pv. *cyamopsis* (Patel): Bacterial blight is one of the most severe illnesses affecting clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.] is vauterin. The pathogen is primarily internal in nature and acts as a primary inoculum for secondary dissemination. The pathogen has been implicated in the occurrence of BLB of clusterbean in US by Orellana et al.,[2] Mihail and Alcorn[3] in Arizona, Madison by Undersander et al.[4] In India, Rajasthan by Patel et al.,[5] Haryana[6] and Karnataka[7], Chakravarthy et al.[8] have all documented many cases of illness. Guar bacterial blight *Xanthomonas axonopodis* pv. *cyamopsis* - initiated According to reports, it can reduce grain output by 58-68 percent in cases of severe illness. The goal of the current inquiry was to find the best course of action for managing BLB in a hot, arid climate.

MATERIALS AND METHODS

The experiment work was accomplished on the laboratory experiments like control of *Xanthomonas axonopodis* pv. *cyamopsidis* by antibiotics, bactericides, plant extracts and bio-agents were carried out *in vitro* in advance laboratory on bio-agents in Plant Pathology Department from 2018 to 2020. While, field trials for the management of pathogen with antibiotics and bacteriocides, were laid out during *Kharif* seasons 2018 and 2019 at Instructional farm, COA, SKRAU, Bikaner. Bikaner is situated in Rajasthan at latitude of 28° 01' N, longitude 73° 22' E and an elevation of 234.7 M above mean sea level. Bikaner is located in Argo ecological region No. 2 (M9E1), which is classified as a desert environment, according to the "Agro ecological region map" published by the National Bureau of Soil Survey and Land Use Planning (NBSS & LUP).

Bikaner falls in tropical climate conditions and the average annual rainfall in months of July to September was 260 mm that are more favourable for the perpetuation and development of *Xanthomonas axonopodis* pv. *cyamopsidis.*

In vitro evaluation of plant extracts, bio agents, antibiotics and fungicides against (*Xanthomonas axonopodis* pv. *cyamopsidis*)

Seven plant extracts, two bacterial bio-control agents three antibiotics and three

Table 1. Details of plant extracts and bio-agents using *in vitro* conditions against *Xanthomonas axonopodis* pv. *cyamopsidis*

Plant extract	Part used	Dose (%)
T_1 - Neem leaf extract	Leaves	5, 10, 15, 20
T_2 - Garlic cloves extract	Cloves	5, 10, 15, 20
T_3 - Tulsi Leaf extract	Leaves	5, 10, 15, 20
T_4 - Ginger extract	Rhizome	5, 10, 15, 20
T_5 - Datura Leaf extract	Leaves	5, 10, 15, 20
T_6 - Neem oil	Oil	5, 10, 15, 20
T_7 - Turmeric powder	Powder	5, 10, 15, 20
Bio-agents		Cfu
T_8 - *Pseudomonas fluorescens*	Pf suspension	10^5, 10^6, 10^7, 10^8
T_9 - *Bacillus subtilis*	Bs suspension	10^5, 10^6, 10^7, 10^8
T_{10} - Control (Without botanicals and bio-agents)		

Table 2. Details of antibiotics and bacteriocides using *in vitro* conditions against *Xanthomonas axonopodis* pv. *cyamopsidis*

Common name	Trade name	Dose % (ppm)
Validomycin	Validacin	50, 100, 150, 200
Kasugamycin	Kasu-B	50, 100, 150, 200
Streptocycline	Streptocycline	50, 100, 150, 200
Bacterianashak	Bacterinashak	250, 500, 750, 1000
Copper oxy chloride	Blitox -50, 50% WP	250, 500, 750, 1000
Copper hydroxide	Kocide	250, 500, 750, 1000

fungicides were evaluated to test their antagonistic action against the bacterium on the basis of zone inhibition method. The cultures of these bio-control agents isolated from soil rhizosphere and samples for plant extracts were collected from Instructional farm, College of Agriculture, Bikaner. Botanicals and bio-control agent which were relatively safe, economical and non-hazardous to the health, used successfully for the management of bacterial disease.

The efficacy of each plant extracts, bacterial bio-control agents, antibiotics and fungicides were evaluated at four different concentrations against the growth of *Xanthomonas axonopodis* pv. *cyamopsidis* by paper disc method.[9] The bacterium culture was multiplied by inoculating the culture into 20 ml nutrient broth taken in Erleyenmayer's flask. The inoculated flasks were incubated at 28 °C for 72 hours and the bacterial suspension was then seeded to the nutrient agar medium and mixed thoroughly. Then the seeded medium was poured into the sterilized Petri plates and plates were allowed to solidify (Table 1 and Table 2).

Different concentrations solution of botanicals (5%, 10%, 15% and 20%) were prepared by mixing the stock solutions of the respective concentrations with 95, 90, 85 and 80 ml of sterilized distilled water, respectively and bio-control agent (10^5, 10^6, 10^7, 10^8 cfu) were prepared for each chemical. The solutions of antibiotics/fungicides were prepared at different concentrations accordingly. The 5 mm-diameter filter paper discs (Whatman No. 42) were placed one disc per plate on the surface of the poured medium after being soaked in the corresponding prepared solution for 10 minutes. For four hours, the infected plates were refrigerated at 5 °C to for the chemicals to diffuse into the medium. Following a 72 hour incubation period at 28 ± 2 °C, observations were made about the formation of inhibitory zones surrounding the filter paper discs.

The present investigation was aimed to screen out antibacterial properties of different plant extracts against *Xanthomonas axonopodis* pv. *cyamopsidis*.

The percent zone inhibition was calculated by the formula given by Vincent.[10]

Percent Zone Inhibition % (I) = C-T / C x 100

Where,

I = Percent Zone inhibition of pathogen

C = Growth (mm) of tested bacteria in untreated control plates

T = Growth (mm) of tested bacteria in treated plates

The experimental details are given below:

Experimental details of botanicals, bio-agents, antibiotics and bacteriocides

In the experiment of botanicals and bio agents, a total of ten treatments and three replications were used. In antibiotics and bactericides experiment, total of seven treatments with three replication for each treatment were used and CRD design was in all the experiments.

Management of bacterial blight of clusterbean through different antibacterial chemicals under field condition

The present experiment was conducted in field at instructional Research Farm, COA, SKRAU, Bikaner during *kharif* season of 2018 and 2019. The seeds were artificially inoculated with pathogen by soaking the seed in bacterial suspension (2.5 x 10^8 cfu/ml) for 30 minutes and then dried under shade.[11] The inoculated seeds were used for sowing in field experiments. Variety grown was RGC-986 susceptible to bacterial blight. When characteristic bacterial blight symptoms appeared

Table 3. Rating scale used for disease observation

Rating	PDI	Category
0	0 or less than 1.0	Free from disease (Immune)
1	1-10%	Resistant (R)
2	10-25%	Moderately resistant (MR)
3	25-50%	Moderately susceptible (MS)
4	50-75%	Susceptible (S)
5	More than 75%	Highly susceptible (HS)

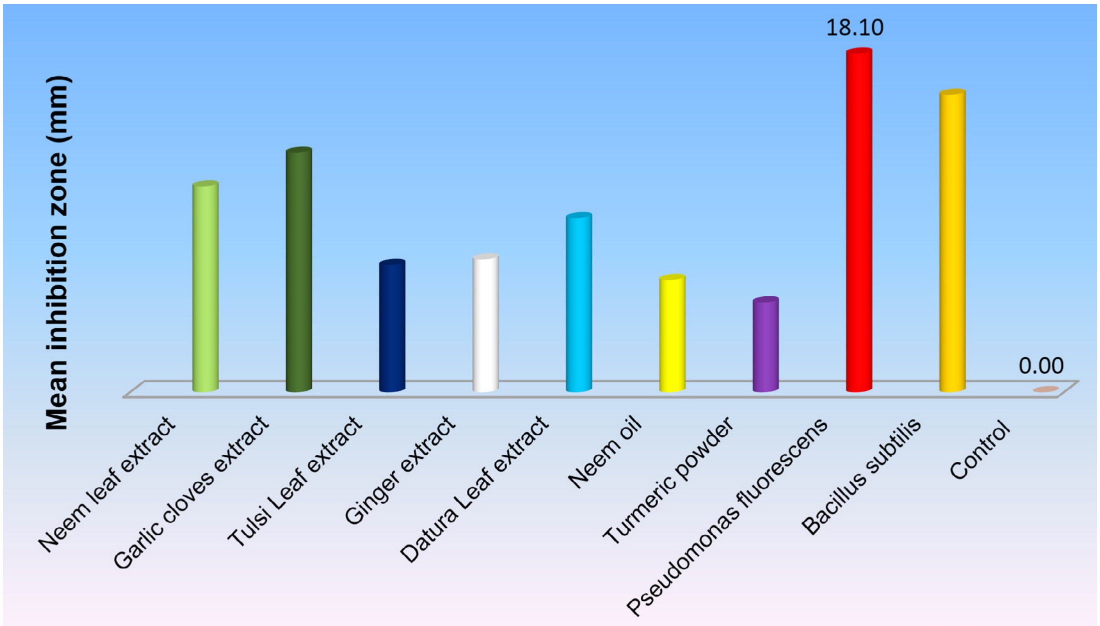

Figure 1. *In vitro* evaluation of plant extracts and bio-agents against *X. axonopodis* pv. *cyamopsidis*

Table 4. Details of antibacterial chemicals using *in vivo* conditions against *Xanthomonas axonopodis* pv. *cyamopsidis*

No.	Antibiotics and fungicides
1.	Seed soaking with Streptocycline (200 ppm)
2.	Seed soaking with Kasugamycin (200 ppm)
3.	Seed soaking with Validamycin (200 ppm)
4.	T_1+ Two spray of Streptocycline (150 ppm)
5.	T_2 + Two spray of Kasugamycin (150 ppm)
6.	T_3 + Two spray of Validamycin (150 ppm)
7.	T_1 + One spray of Streptocycline (150 ppm) + COC (0.2%)
8.	T_1 + One spray of Streptocycline (150 ppm) + Copper hydroxide (0.2%)
9.	T_1 + One spray of Streptocycline (150 ppm) + Bacterianashak (0.2%)
10.	T_2 + One spray of Kasugamycin (150 ppm) + COC (0.2%)
11.	T_2 + One spray of Kasugamycin (150 ppm) + Copper hydroxide (0.2%)
12.	T_2 + One spray of Kasugamycin (150 ppm) + Bacterianashak (0.2%)
13	T_3 + One spray of Validamycin (150 ppm) + Copper oxychloride (0.2%)
14	T_3 + One spray of Validamycin (150 ppm) + Copper hydroxide (0.2%)
15	T_3 + One spray of Validamycin (150 ppm) + Bacterianashak (0.2%)
16	T_{16-} Control

on clusterbean plants, first foliar spray of each treatment was done just after the appearance of disease and subsequently second spray was followed at 15 days interval. The disease intensity was recorded after the 15 days of second spray from 15 randomly selected plants from each plot by using 0-5-point scale (Table 3), after grain yield and net return per plot was recorded. In field experiment, the following treatments were set up using randomized block design (RBD) with three replications, respectively.

The observations on bacterial blight were recorded as per cent disease index and per cent disease reduction over control was worked out. The disease reduction (%) over control was calculated as follows:

$$\text{PDI} = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves observed x Maximum rating scale}} \text{ x } 100$$

$$\text{Disease reduction (\%)} = \frac{\text{PDI (Control) - PDI (Treatment)}}{\text{PDI (Control)}} \text{x}100$$

Where, PDI (Percent disease index)

Seed yield was recorded for each treatment at harvest and percent increase in yield was calculated by using following formula:

$$\text{Percent increase in yield} = \frac{\text{Yield in treatment-Yield in control}}{\text{Yield in control}} \text{x}100$$

Experiment details of field trial

A total of 16 treatments were used for field trials with three replication for each treatment, plot size was 4 × 3 m^2 with 25 × 10 cm spacing and RBD (Randomized Block Design) was used. RGC-986 variety of clusterbean was taken for field experiments.

The data of percent disease intensity in all the experiments were transformed to their Arcsine values.[12] The statistical analysis was done by using Completely Randomized Design. The data of field experiments were analyzed by Randomized Block Design.[13]

RESULT AND DISCUSSION

In vitro evaluation of botanicals and bio-agents against bacterial blight in clusterbean

Total nine plant extracts and bio-agents were evaluated against *Xanthomonas axonopodis* pv. *cyamopsidis* under *in vitro* condition at four different concentrations (5%, 10%, 15% and 20%) and the data are summarized in Table 4. The results showed that the efficacy increased with increasing the concentration of the botanicals extract. All four concentrations of botanicals inhibited bacterial growth but maximum efficacy was reported at 20% concentration.

Out of the seven botanicals treatments, Garlic cloves extract revealed the maximum efficacy in terms of zone of inhibition 12.80 mm at 20% concentration followed by 10.60 mm at 15%, 7.90 mm at 10%. Minimum inhibition zone 6.10 mm was found at 5% concentration. Neem extracts showed the maximum zone of inhibition 11.00 mm at 20% concentration followed by 9.8 mm at 15%, 7.60 mm at 10%. Minimum inhibition zone 5.40 mm was found at 5% concentration. Datura leaf extract exhibited the maximum zone of inhibition 9.30 mm at 20% concentration followed by 8.20 mm at 15%, 6.40 mm at 10%. Minimum inhibition zone 4.20 mm was found at 5% concentration. Ginger extract revealed the highest activity 7.10 mm at 20% concentration followed by 5.60 mm at 15%, 4.80 mm at 10%. Minimum inhibition zone 3.30 mm was found at 5% concentration. Tulsi leaf extract showed the maximum effect (6.80 mm zone of inhibition) at 20% concentration and minimum (3.10 mm) at 5% concentration. Neem oil showed the less activity in comparison to neem leaf extract. It showed 6.00 mm inhibition zone at 20% concentration and 2.90 mm at 5% concentration. Turmeric powder showed the least activity among all tested seven botanicals. It revealed 4.80 mm zone of inhibition at 20% concentration followed by 4.30 mm at 15%, 3.20 mm at 10% and the lowest zone of inhibition 2.10 mm was found at 5% concentration. Similar findings were made by Mali *et al*.[14] and Kumar *et al*.,[15] who showed that the greatest

Table 5. *In vitro* efficacy of plant extract and bio-agents against *Xanthomonas axonopodis* pv. *cyamopsidis*

Treatments	Mean inhibition zone (mm)			
	I	II	III	IV
T_{1-} Neem leaf extract	5.40	7.60	9.8	11.00
T_{2-} Garlic cloves extract	6.10	7.90	10.60	12.80
T_{3-} Tulsi Leaf extract	3.10	4.40	5.20	6.80
T_{4-} Ginger extract	3.30	4.80	5.60	7.10
T_{5-} Datura Leaf extract	4.20	6.40	8.20	9.30
T_{6-} Neem oil	2.90	4.10	5.00	6.00
T_{7-} Turmeric powder	2.10	3.20	4.30	4.80
T_{8-} *P. fluorescens*	13.67	15.10	16.87	18.10
T_{9-} *Bacillus subtilis*	11.0	12.80	14.2	15.90
T_{10-} Control	0.0	0.00	0.00	0.00
CD (P = 0.05)	0.69	1.05	1.05	1.46
S.Em (±)	0.23	0.35	0.35	0.49
CV (%)	7.74	9.22	7.71	9.29

Table 6. *In vitro* efficacy of antibiotics and fungicides against *Xanthomonas axonopodis* pv. *cyamopsidis* (5 days)

Treatments	Mean inhibition zone (mm)			
	I	II	III	IV
T_1 Validamycin	8.67	10.30	12.33	13.30
T_2 Kasugamycin	9.33	11.67	13.33	14.67
T_3 Streptocycline	13.67	16.10	18.63	21.34
T_4 Bacterinashak	9.67	11.33	13.20	15.67
T_5 Copper oxychloride	12.33	15.10	17.33	19.60
T_6 Copper hydroxide	10.40	12.67	14.67	16.34
T_7 Control	0.00	0.00	0.00	0.00
CD (P = 0.05)	0.97	0.81	0.60	0.76
S.Em (±)	0.31	0.26	0.19	0.25
CV (%)	6.01	4.15	2.66	2.99

antibacterial activity against the pathogen *in vitro* was demonstrated by water and ethyl acetate extract of garlic clove. The food poisoning and paper disc diffusion to assess the effectiveness of leaf extracts from fourteen different plants against *X. oryzae* pv.[16] *oryzae* was used. Datura metel concentrations of 20% and 15% showed the greatest inhibition of bacterial growth, followed by *Azadirachata indica* concentrations of 20% and 15% (Table 5 & Figure 1 and 2).

Two bio-agents, *Pseudomonas fluorescens* and *Bacillus subtilis* were also evaluated against *Xanthomonas axonopodis* pv. *cyamopsidis* at four different concentrations (10^5, 10^6, 10^7, 10^8 cfu). The efficacy of bio-agents increased with increasing the concentration. *P. fluorescens* showed the highest inhibition activity 18.10 mm at 10^8 cfu and minimum 13.67 mm at 10^5 cfu concentration. *B. subtilis* inhibited the plant pathogen 15.90 mm at 10^8 cfu while at 10^5 cfu, the bio-agents inhibited 11.00 mm growth of the pathogen. Similar results also showed conformity with the present findings.[17] They observed that among the eight bio-agents, the maximum growth inhibition was showed by *Pseudomonas fluorescens* followed by *Bacillus subtilis*. These findings are similar to the results reported by Jindal *et al.,* who tested the efficacy of *Bacillus* spp. and found that *Bacillus subtilis* was effective in controlling bacterial blight of cowpea.[18] The inhibitory action of *Bacillus subtilis* against the bacterium which is similar to the observations recorded in the present study Massomo et al.[19] (Table 5).

In vitro evaluation of antibiotics and bactericides against _Xanthomonas axonopodis_ pv. _cyamopsidis_

Three antibiotics Validamycin, Kasugamycin and Streptocycline were evaluated against the plant pathogen at four different concentrations 50, 100, 150 and 200 ppm (Table 6 and Figure 3).

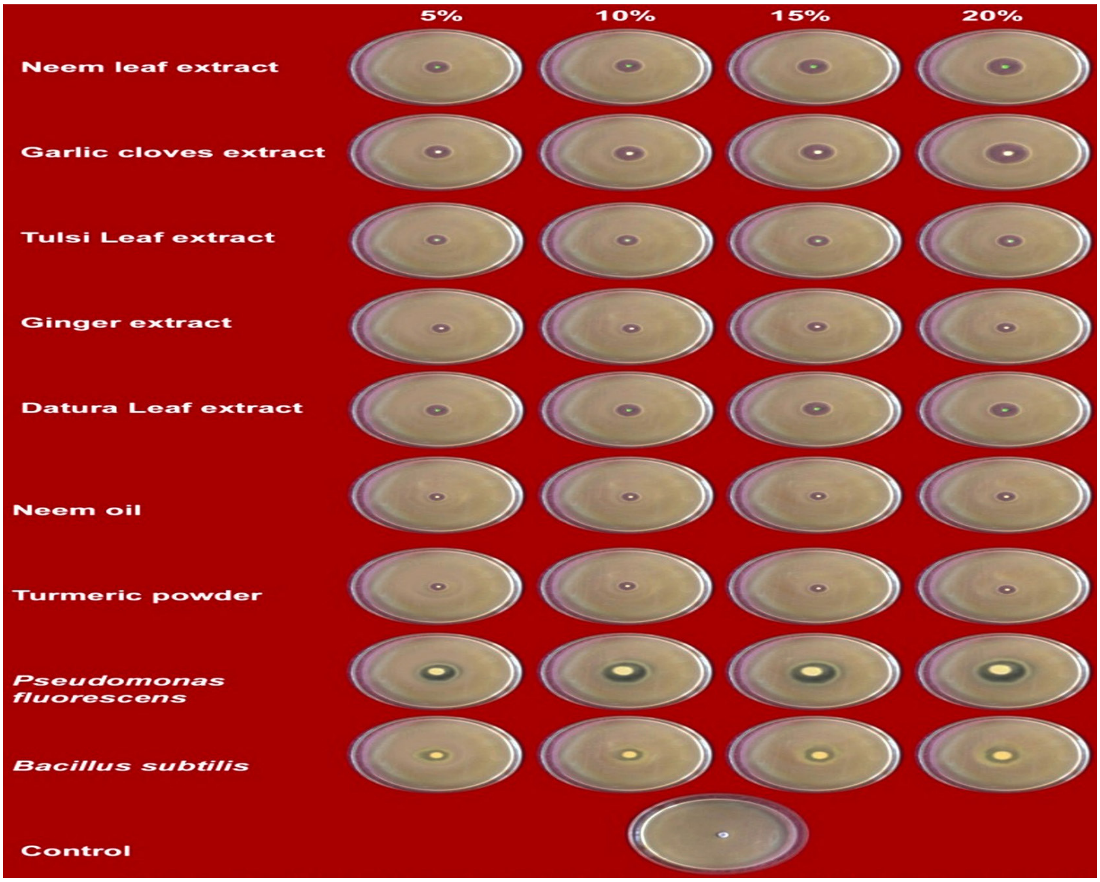

Figure 2. Evaluation of botanicals and bio-agents against *Xanthomonas axonopodis* pv. *cyamopsidis*

Among all the tested antibiotics, Streptocycline was found strong antibiotic activity which inhibited the pathogen and showed the maximum zone of inhibition 21.34 mm at 200 ppm concentration and minimum 13.67 mm at 50 ppm. Kasugamycin revealed the maximum activity 14.67 mm at 200 ppm which followed by 13.33 mm, 11.67 mm at 150 ppm and 100 ppm, respectively. The minimum zone of inhibition 9.33 mm was found at 50 ppm concentration. Validamycin showed the highest efficacy 13.30 mm at 200 ppm concentration against this pathogen. Similar results reported by Bhure *et al.*,[20] who evaluated antibiotics at four different concentrations (100 ppm, 250 ppm and 500 ppm conc.) under *in vitro* against pathogen, where, streptomycin sulphate (500 ppm) showed maximum inhibition zone as 24.3 mm. The lowest zone 8.67 mm was found at 50 ppm concentration.

Three bactericides, Bacterianashak, copper oxychloride and copper hydroxide were evaluated against *Xanthomonas* at four different concentrations (250, 500, 750 and 1000 ppm). The results showed that the efficacy was also increased with increasing the concentration of fungicides. Among all the fungicides, Copper oxychloride showed the maximum inhibition zone of 19.60 mm at 1000 ppm concentration, followed by 17.33 mm, 15.10 mm at 750 ppm and 500 ppm, respectively. The lowest activity 12.33 mm was recorded at 250 ppm concentration. Copper hydroxide showed the maximum zone of inhibition 16.34 mm at 1000 ppm concentration and lowest activity of this bactericide 10.40 mm was recorded at 250 ppm concentration. Bacterinashak revealed the maximum zone of inhibition 15.67 mm at 1000 ppm concentration and followed by 13.20 mm, 11.33 mm at 750 ppm and 500 ppm concentration, respectively. The lowest activity 9.67 mm was recorded at 250 ppm concentration. Similarly results also reported by Raju *et al*.[21] who tested many chemicals against the pathogen and revealed that Streptocycline + COC with an inhibition zone of 3.3 cm exhibited superior efficacy followed by Streptocycline (2.80 cm) and COC (2.65 cm). Antre *et al*.[22] studied efficacy of total 18 chemicals, botanicals and bio-agents against the bacterium by using disc diffusion method. Maximum zone of inhibition were recorded in chemical treatments. Copper oxychloride (0.3%) + Streptomycin

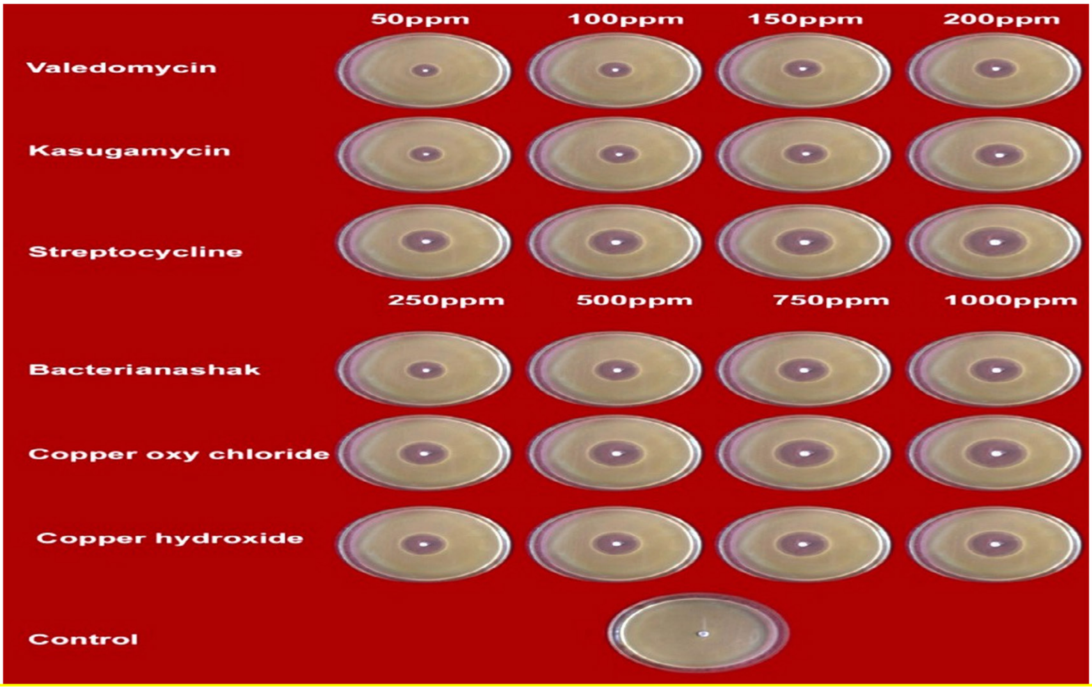

Figure 3. Evaluation of antibiotics and bactericides against *Xanthomonas axonopodis* pv. *cyamopsidis*

sulphate (500 ppm) was found significantly superior in inhibiting the growth of bacteria with 14.33 mm zone of inhibition.

Efficacy of antibiotics, bactericides and combination product against (*X. axonopodis* pv. *cyamopsidis*) under field conditions

Total sixteen effective combinations were studied against the pathogen in clusterbean under artificial inoculated field conditions with different concentrations (Table 7 and Figure 4).

In field management study, percent disease incidence was monitored after 45 and 60 days during *Kharif* season 2018 and 2019. Among different treatments, T_7 (T_{1+} One spray of Streptocycline (150 ppm) + COC (0.2%), was found most effective treatment against bacterial blight disease which showed 2.95 and 3.33 per

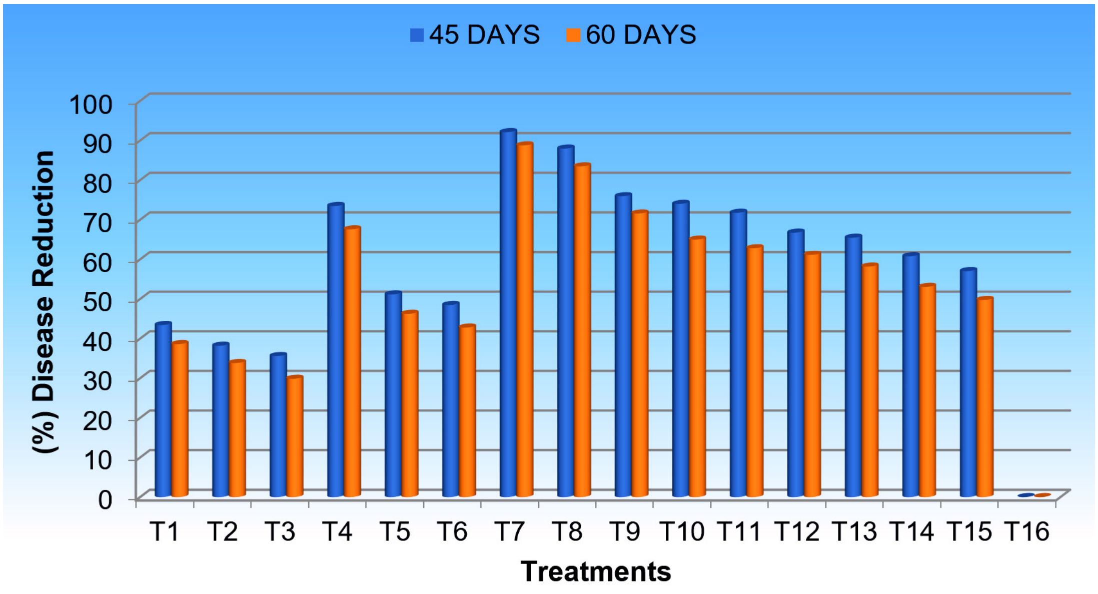

Figure 4. Percent disease reduction of bacterial blight of clusterbean through different antibacterial chemicals under field condition

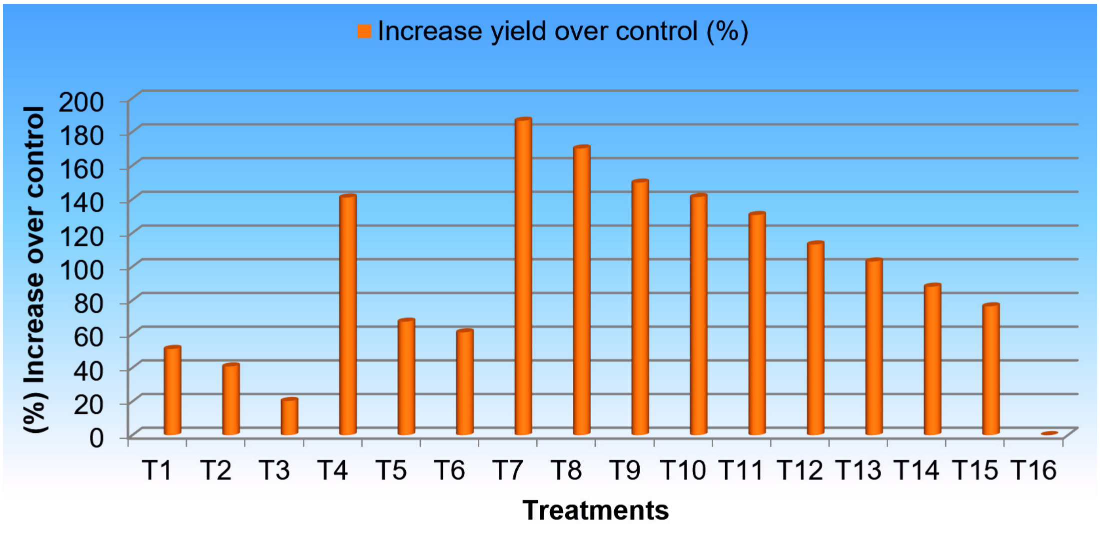

Figure 5. Effect of different antibacterial chemicals on grain yield of clusterbean under field conditions

Table 7. Management of bacterial blight of clusterbean through different antibacterial chemicals under field condition

Treatments	45 Days		Pooled	60 Days		Pooled	(%) Disease Reduction	
	2018	2019		2018	2019		45 Days	60 Days
T_1	21.37	23.67	22.52	25.85	28.86	27.35	43.43	38.60
T_2	22.87	26.33	24.6	27.27	31.67	29.47	38.20	33.84
T_3	24.33	26.95	25.64	29.61	32.88	31.24	35.59	29.87
T_4	9.67	11.45	10.56	13.55	15.33	14.44	73.47	67.58
T_5	17.37	21.56	19.46	22.33	25.55	23.94	51.17	46.26
T_6	18.15	22.87	20.51	23.83	27.16	25.49	48.48	42.78
T_7	2.95	3.33	3.14	4.33	5.67	5.00	92.11	88.77
T_8	4.33	5.25	4.79	6.43	8.30	7.36	87.96	83.47
T_9	8.27	10.90	9.58	11.87	13.46	12.66	75.93	71.58
T_{10}	8.87	11.83	10.35	14.33	16.88	15.60	74.00	64.98
T_{11}	9.33	13.16	11.24	14.87	18.27	16.57	71.76	62.80
T_{12}	11.66	14.83	13.24	15.13	19.55	17.34	66.74	61.14
T_{13}	12.34	15.16	13.75	16.49	20.78	18.63	65.46	58.18
T_{14}	13.87	17.36	15.61	19.50	22.33	20.91	60.78	53.06
T_{15}	15.70	18.50	17.1	20.67	24.10	22.38	57.04	49.76
T_{16}	38.30	41.33	39.81	42.73	46.38	44.55	0.0	0.0
CD (P = 0.05)	3.29	3.32	4.54	3.59	3.77	4.52		
S.Em (±)	1.13	1.14	1.49	1.24	1.30	1.49		
CV (%)	13.11	11.14	12.66	11.10	10.09	10.10		

cent disease incidence whereas the least effective treatment was T_3 (Seed soaking with Validamycin (200 ppm), which revealed 24.33 and 26.95 per cent disease intensity in *kharif* 2018 and 2019 after 45 days of foliar spray, After 60 days of foliar spray of T_7 (T_{1+} First spray of Streptocycline (150 ppm) + COC (0.2%), treatment showed 4.33 and 5.67 per cent disease intensity whereas T_3 (Seed soaking with Validamycin (200 ppm), showed the less effect among all the treatments during *kharif* both season, respectively. The pooled data analysis revealed that T_7 treatment was highly effective and showed less PDI while T_3 was found lowest effective treatment which showed highest PDI 31.24 after 60 days of foliar spray. Similar finding were reported by Amin and Patel[23] they conducted the experiments having five different spray schedule along with untreated control with randomised block design (RBD) along with four replications. Seed soaking in streptocycline (250 ppm) for 30 min plus first spray of streptocycline (250 ppm) at just appearance of the disease and second spray at fifteen days after first spray showed the maximum yield gross and net realisation and minimum disease intensity. These findings corroborate with those of Lodha,[24] who revealed that seed treatment with Streptocycline + foliar spray of Streptocycline at 35 and 49 DAS helped in disease reduction and increased seed production in a field trial in Jodhpur, Rajasthan, India during the cropping seasons of 1991 and 1992. Rathore[25] noted that hot water, Bavistin [carbendazim], captan, thiram, and topsin M [thiophanate-methyl] were all used in the seed treatment process. The results showed that dipping seeds in an aqueous solution containing 0.02% streptocycline for three hours was very helpful in reducing illness.

Results of pooled analysis of 45 and 60 days of foliar spray, revealed that maximum per cent disease reduction 92.11% and 88.77% was recorded after 45 and 60 days, respectively with T_7 (T_{1+} Onespray of Streptocycline (150 ppm) + Copper oxy chloride (0.2%). T_3 (Seed soaking with Validamycin (200 ppm) was found least effective due to show minimum per cent disease reduction 35.59% and 29.87% after 45 and 60 days, respectively. The grain yield per plot was recorded from each plot in this field experiment. Data revealed that maximum yield (11.32 q/ha) was (Table 8 and Figure 5) obtained with

Table 8. Effect of different antibacterial chemicals on grain yield of clusterbean under field conditions

Treatments	Grain yield (q ha^{-1})		Pooled	Increase yield over control (%)
	2018	2019		
T_1 Seed soaking with Streptocycline (200 ppm)	6.96	4.97	5.97	51.14
T_{2-} Seed soaking with Kasugamycin (200 ppm)	6.45	4.67	5.56	40.76
T_{3-} Seed soaking with Validamycin (200 ppm)	5.33	4.17	4.75	20.25
T_{4-} T_1 + Two spray of Streptocycline (150 ppm)	11.33	7.71	9.52	141.01
T_{5-} T_2 + Two spray of Kasugamycin (150 ppm)	7.96	5.25	6.61	67.34
T_{6-} T_3 + Two spray of Validamycin (150 ppm)	7.54	5.17	6.36	61.01
T_{7-} T_1 + One spray of Streptocycline (150 ppm) + COC (0.2%)	12.97	9.67	11.32	186.58
T_8 T_1 + One spray of Streptocycline (150 ppm) + Copper hydroxide (0.2%)	12.45	8.89	10.67	170.13
T_{9-} T_1 + One spray of Streptocycline (150 ppm) + Bacterionashak (0.2%)	11.57	8.17	9.87	149.87
T_{10-} T_2 + One spray of Kasugamycin (150 ppm) + COC (0.2%)	11.23	7.82	9.53	141.27
T_{11-} T_2 + One spray of Kasugamycin (150 ppm) + Copper hydroxide (0.2%)	10.79	7.43	9.11	130.63
T_{12-} T_2+ One spray of Kasugamycin (150 ppm) + Bacterionashak (0.2%)	9.96	6.87	8.42	113.16
T_{13-} T_3 + One spray of Validamycin (150 ppm) + COC (0.2%)	9.69	6.34	8.02	103.04
T_{14-} T_3 + One spray of Validamycin (150 ppm) + Copper hydroxide (0.2%)	8.93	5.93	7.43	88.10
T_{15-} T_3 + One spray of Validamycin (150 ppm) + Bacterionashak (0.2%)	8.47	5.46	6.97	76.45
T_{16-} Control	4.79	3.11	3.95	00
CD (P = 0.05)	2.64	2.02	3.07	
S.Em (±)	0.91	0.69	1.01	
CV (%)	17.19	18.99	18.39	

T_7(T_{1+}Onespray of Streptocycline (150 ppm) + Copper oxy chloride (0.2%). The minimum yield (4.75 q/ha) was found with T_3(Seed soaking with Validamycin (200 ppm). All the treatments were significantly differing with each other and found superior over control. Kumhar and Meena[26] have observed similar findings after examining thirteen distinct treatments both in isolation and in conjunction with one another to combat the infection. The treatments with the lowest bacterial blight PDI (8.5, 7.5, and 5.83) were Streptocycline (250 ppm) + Blitox (0.2%), which were sprayed twice at a 15 day interval. This was followed by Treatment (Streptocycline 150 ppm + Carbendazim 12% + Mancozeb 63% (0.2%), sprayed twice at a 15-day interval) with PDI of 11.0, 11.83, and 8.16.

Observations of the all present findings revealed that all the treatments were significantly superior over the control. On the basis of economically cost and benefits analysis T_{7-} (T_{1+} One spray of Streptocycline (150 ppm) + COC (02%) was found superior with highest grain yield (11.32 q/ha) and net gain 27280 Rs/ha over to control, While minimum grain yield and net gain were recorded in control as well as treatment T_3 (Seed soaking with Validamycin (200 ppm) 4.75 q/ha and net gain 3097 Rs/ha (Table 9). Data presented in table 7 of all treatments and cost of treatments as well as net gain over to control. Similar results were noted by Jagtap et al.[27] who claimed that the combination of carbendazim 0.1% + streptocycline 100 ppm was effective against bacterial blight

Table 9. Economics of different treatments against bacterial blight of clusterbean (pooled kharif 2018 and 2019)

Treatments	Quantity of treatment (kg/ha)	Cost of treatment (Rs/ha)	Labour cost (Rs/ha)	Total cost of treatment (Rs/ha)	Seed yield (q/ha)	Gross realization (Rs/ha)	Net realization over control (Rs/ha)	Net gain (Rs/ha)
T_1 Seed soaking with Streptocycline (200 ppm)	Streptocycline 0.006	50	100	150	5.97	23,880	8080	7930
T_{2-} Seed soaking with Kasugamycin (200 ppm)	Kasugamycin 0.006	6.48	100	106.48	5.56	22,240	6440	6,333.52
T_{3-} Seed soaking with Validamycin (200 ppm)	Validamycin 0.006	3	100	103	4.75	19,000	3200	3097
$T_{4-}T_1$ + Two spray of Streptocycline (150 ppm)	Streptocycline 0.126	1050	1300	2350	9.52	38,080	22,280	19,930
$T_{5-}T_2$ + Two spray of Kasugamycin (150 ppm)	Kasugamycin 0.126	136.08	1300	1436.08	6.61	26,440	10,640	9203.92
$T_{6-}T_3$ + Two spray of Validamycin (150 ppm)	Validamycin 0.126	63	1300	1363	6.36	25,440	9640	8277
$T_{7-}T_1$ + One spray of Streptocycline (150 ppm) + COC (0.2%)	Streptocycline 0.066, CoC 1.0	900	1300	2200	11.32	45,280	29,480	27280
T_8T_1 + One spray of Streptocycline (150 ppm) + Copper hydroxide (0.2%)	Streptocycline 0.066,+Copper hydroxide 1.0	1080	1300	2380	10.67	42,680	26,880	24500
$T_{9-}T_1$ + One spray of Streptocycline (150 ppm) + Bacterinashak (0.2%)	Streptocycline 0.066, +Bacterinashak 1.0	7220	1300	8520	9.87	39,480	23,680	15,160
$T_{10-}T_2$ + One spray of Kasugamycin (150 ppm) + COC (0.2%)	Kasugamycin 0.066+COC - 1.0	421.28	1300	1721.28	9.53	38,120	22,320	20,598.72
$T_{11-}T_2$ + One spray of Kasugamycin (150 ppm) + Copper hydroxide (0.2%)	Kasugamycin 0.066+Copper hydroxide - 1.0	601.28	1300	1901.28	9.11	36,440	20,640	18,738.72
$T_{12-}T_2$ + One spray of Kasugamycin (150 ppm) + Bacterinashak (0.2%)	Kasugamycin 0.066 +Bacterinashak -1.0	6741.28	1300	8041.28	8.42	33,680	17,880	9838.72
$T_{13-}T_3$ + One spray of Validamycin (150 ppm) + Copper oxy chloride (0.2%)	Validamycin 0.066+COC - 1.0	383	1300	1683	8.02	32,080	16,280	14,597

Table 9. Cont...

Treatments	Quantity of treatment (kg/ha)	Cost of treatment (Rs/ha)	Labour cost (Rs/ha)	Total cost of treatment (Rs/ha)	Seed yield (q/ha)	Gross realization (Rs/ha)	Net realization over control (Rs/ha)	Net gain (Rs/ha)
T_{14}- T_3 + One spray of Validamycin (150 ppm) + Copper hydroxide (0.2%) +Copper hydroxide-1.0	Validamycin 0.066	563	1300	1863	7.43	29,720	13,920	12,057
T_{15}- T_3 + One spray of Validamycin (150 ppm) + Bacterinashak (0.2%)	Validamycin 0.066 +Bacterinashak - 1.0	6703	1300	8003	6.97	27,880	12,080	4077
T_{16}- Control	-	-	-	-	3.95	15,800	-	-

Clusterbean price Rs. 4000/q. Streptocycline = Rs. 50/6 gm, Kasugamycin = Rs.1080/lit, Validamycin =Rs.500/lit,
Copper hydroxide = Rs. 530/kg, Copper oxychloride = Rs. 350, /kg ,Labour cost of seed soaking/ha = Rs. 100, Labour cost of sprays/ha = 1200

of clusterbean and that the minimal disease severity (8.35%) of bacterial blight of cotton was discovered in that therapy. Yenjerappa *et al.,*[28] applied bactericides, bio-agents and botanicals for their efficacy against leaf spot of pomegranate, lowest disease index of 22.3% in streptocycline (0.05%) + copper oxychloride (0.2%) treated plots followed by bacterinol (0.05%) + copper oxychloride (0.2%) and bacteriomycin (0.05%) + copper oxychloride (0.2). Raju *et al.*, [21] investigated different bactericides to inhibit the pathogen *Xanthomonas axonopodis.* pv. *punicae.* Among the different chemicals, Streptocycline + COC with an inhibition zone of 3.3 cm exhibited superior efficacy followed by Streptocycline (2.80 cm) and COC (2.65 cm). Copper oxychloride (0.3%) + Streptomycin sulphate (500 ppm) was found significantly superior in inhibiting the growth of bacteria with 14.33 mm zone of inhibition.[22]

CONCLUSION

In an *in vitro* study, seven plant treatments of Garlic clove extract revealed the maximum efficacy at 20% and minimum at 5% concentration while minimum efficacy was observed in case of turmeric powder. Out of two bio-agents, *P. fluorescens* showed the highest activity as compared to *B. subtilis.* Out of three antibiotics, the maximum inhibition was shown by Streptocycline while Validamycin shown the minimum inhibition activity. Among three fungicides the maximum inhibition was recorded with Copper oxychloride followed by copper hydroxide while Bacterianashak revealed the lowest activity against the pathogen *X. a.* pv. *cyamopsidis.* In the field study, T_7 treatment (Seed soaking with Streptocycline (200 ppm) + One spray of Streptocycline (150 ppm) + Copper oxychloride (0.2%) was found significantly superior in all the treatments. T_3 treatment (Seed soaking with Validamycin (200 ppm) was found least effective. Maximum grain yield was also obtained with T_7 treatment, whereas minimum yield was obtained with T_3 treatment.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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