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In-vitro evaluation of different chemicals, botanicals and bio agents against *Xanthomonas campestris* pv. *arecae*

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Abstract
Bacterial leaf stripe of arecanut caused by *Xanthomonas campestris* pv. *arecae* is menace in arecanut cultivation. Some of the chemicals are effective against disease but they have not proved economical and they leave harmful residues in soil and plants. Therefore, present investigation was taken up to manage the disease with help of botanicals, bio agents and chemicals which have bactericidal action. Hence, nine botanicals, three bio agents and six antibacterial chemicals were evaluated against pathogen and the results of chemicals indicated that, Kasugamycin showed maximum inhibition zone and significantly superior over all the other bactericides tested. However, among the different plant extracts revealed that, Neem recorded maximum inhibition zone of 13.00 mm that significantly differed with all other botanicals, followed by Pongamia (11.20 mm). Among biocontrol agents, *Trichoderma harzianum* was found significantly superior in inhibiting the growth of *X. campestris* pv. *arecae* (24.10 mm) followed by *Pseudomonas flourescens* (15.70 mm).

Keywords: *Xanthomonas campestris* pv. *arecae*, bio agents, botanicals, chemicals

Introduction
Arecanut (*Areca catechu* L.) belongs to the family arecaceae is a palm is grown in most part of the tropical Asia and East Africa regions. Arecanut production in India is the highest in the world, accounting for 49.74 per cent of its world output and is exported to many countries. Within India, as of 2013-14, Karnataka produces 62.69 per cent of the crop followed by Kerala and Assam; all three states together account for 88.59 per cent of its production. In Karnataka, Shivamoga district the crop is grown extensively, and is considered by the plantation owners as a prestige of symbol [1].Yong arecanut seedlings were severally affected by bacterial leaf stripe disease caused by *Xanthomonas campestris* pv. *arecae* leading up to 60% seedlings mortality**.** The initial symptoms include dark green water soaked stripes running alongside and parallel to the mid rib of the leaf lets. The lesions were covered with abundant quantity of bacterial exudates and all the leaflets of a leaf may be affected resulting in complete or partial blighting of the leaf [2]. Some of the chemicals are effective against this disease but they have not proved economical and leave harmful residues in soil and plants. In recent years emphasis has been given on eco-friendly management practices. Therefore present investigation was planned to manage the disease with the help of botanicals, bio agents and some chemicals which have bactericidal action. The effort has been made to evaluate the efficacy of botanicals, bio agents and antibacterial chemicals against the pathogen under laboratory condition.

Materials and Methods
Toxicity of chemicals @ 1000ppm, 2000ppm, 3000ppm concentration against *Xanthomonas campestris* pv. *arecae* were studied by inhibition zone assay method [3]. Antibacterial chemicals used for the study are Streptocycline + Copper oxychloride, Copper oxychloride, Kasugamycin, Streptocycline, Copper hydroxide, Streptocycline + Copper hydroxide. In this method, sterilized whatman filter paper (5mm) was used for study. These discs were impregnated with requisite quantity of chemicals for 30 minutes. Impregnated paper discs were aseptically placed on nutrient agar plates, containing 24 hours old bacterial culture. The discs moistened with sterilized distilled water served as control. All plates were kept incubation @ 28 ºC for 48 to 72 hrs. After incubation period observations were recorded based on zone of inhibition.

Nine botanicals *viz.*, Neem (*Azadirachta indica*), Glyricidia (*Glyricidia Spp*), Honge (*Pongamia pinnata*), Lantana (*Lantana camara*), Nilgiri (*Eucalyptus Spp*), Noni (*Morinda citrifolia*), Garlic (*Allium sativum*), Onion (*Allium cepa*) and Tridax (*Tridox procumbens*) were tested for their bactericidal properties against *Xanthomonas campestris* pv. *arecae.* Plant extracts were prepared from freshly collected plant parts and these were assessed @ 5%, 10 %, 15% concentration by paper disc method. A heavy suspension of the *X. campestris* pv. *arecae* was prepared and poured into sterile Petri plates and allowed to solidify. Sterilized filter paper disc measuring 5 mm in diameter was soaked in different concentrations of the plant extracts, were dried and placed on solidified medium. The plates were then incubated at 28 ºC for 48 to 72 hrs. Observations on inhibition zone were recorded after the incubation period with three replications.

To determine the inhibitory effect of certain bio agent's *viz.*, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Trichoderma harzianum* were evaluated under *in vitro* condition. Antagonistic activity of bio agents were judged against promising isolate of *Xanthomonas campestris* pv. *arecae* by paper disc method. In this method culture filtrate were poured in 5mm diameter paper formed in each petriplates containing nutrient agar seeded with pathogen. The plates were kept incubation @ 28 ºC for 48 to 72 hrs. The potential antibacterial property was calculated by comparing zone of inhibition with that of control after 3 days incubation. Collected data were analyzed statistically.

Results and Discussion

Sensitivity of antibacterial chemicals against pathogen was conducted through paper disc or inhibition zone method. Data pertaining to inhibition zone (mm) are depicted in Table 1 and Plate 1. Differences among treatments and concentrations were found to be statistically significant. Among six chemicals tested, Kasugamycin showed maximum inhibition zone of 22.00 mm at 3000 ppm and significantly superior over all the other bactericides tested. Followed by, Streptocycline and Copper hydroxide has shown the inhibition zone of 11.30 and 11.00 mm respectively. This may be due to the ability of Kasugamycin to inhibit proliferation of bacteria by tampering with their ability to make new proteins as it inhibits the ribosome there by inhibiting protein synthesis at the step of translation initiation by direct competition with initiator transfer RNA there by going to kill the pathogen. While least inhibition zone of 5.70 mm was recorded in Streptocycline + Copper oxy chloride at 1000 ppm.

This findings were in accordance with [4] reported that streptocycline (streptomycin sulphate + tetracycline hydrochloride, 9:1) + copper sulphate ($CuSo_4$) at 1000 ppm showed the maximum inhibition zone of 25.0 mm, followed by streptomycin sulphate + copper oxychloride (24.33mm), streptomycin sulphate alone (22.66mm) and agrimycin 100 (16.25mm).

The data regarding the per cent inhibition over control were presented in Table 2 and Plate 2. Among the nine botanicals tested, Inhibition zone of 18.00 mm was recorded by *Azadirachta indica* at 15 per cent concentration which was superior over all other botanicals and concentrations followed by Pongamia extract (15.30 mm) and least inhibition zone of 6.00 mm was recorded by Glyricidia at 5 per cent concentration, similar results were observed by [5]. The antibacterial activity of Neem has been known for a long time. Neem leaf extracts contains quercetin (flavonoid) and nimbosterol (ß- sitosterol) as well as number of liminoids (nimbin and its derivatives). Quercetin (a polyphenolic flavonoid) is known to have antibacterial and antifungal properties. Pongamia leaves contain furanoflavones, furanoflavonols and chromenoflavones compounds that have antibacterial property [6].

Among the three antagonistic agents tested *Trichoderma harzianum* was found significantly superior in inhibiting the growth of *X. campestris* pv. *arecae* (24.10 mm) followed by *Pseudomonas flourescens* (15.70 mm). However, the least inhibition zone of 12.40 mm was observed in *Bascilus subtilis* against the growth of *Xanthomonas campestris* pv. *arecae* represented in Table 3 and Plate 3. These findings were supported by [7] who evaluated *P. fluorescens in vitro* by dual culture method, revealed that it produced inhibition zones of 4.24 mm against *X. oryzae* pv. *oryzae*, whereas evaluation by culture filtrate method produced inhibition zones of 14.75mm.

Table 1: *In Vitro* Evaluation of Bactericides against *Xanthomonas Campestris pv. Arecae.*

Sl. No.	Bactericides	Mean inhibition zone (mm) Concentration (ppm)			Mean
		1000	2000	3000	
1.	Copper oxy chloride	6.00 (2.64)*	7.00 (2.82)	8.70 (3.10)	7.20
2.	Copper hydroxide	10.00 (3.31)	11.00 (3.46)	10.00 (3.31)	10.30
3.	Kasugamycin	20.30 (4.61)	20.30 (4.61)	22.00 (4.79)	20.90
4.	Streptocycline	10.00 (3.31)	10.00 (3.31)	11.30 (3.51)	10.40
5.	Streptocycline + Copper oxy chloride	5.70 (2.58)	6.70 (2.76)	7.00 (2.82)	6.40
6.	Streptocycline+ Copper hydroxide	9.70 (3.26)	9.30 (3.21)	9.70 (3.26)	9.60
7.	Control	0.00 (1.00)	0.00 (1.00)	0.00(1.00)	0.00
	Mean	8.80	9.19	9.80	
	Factor	Bactericides	Concentration	B x C	
	SE m ±	0.041	0.029	0.071	
	CD at 1%	0.167	0.102	0.286	

*fig in the parenthesis are sqrare root transformed values

Table 2: *In Vitro* evaluation of botanicsls against *xanthomonas campestris pv. Arecae*

Sl. No.	Botanicals	Mean inhibition zone (mm)			Mean
		Concentration (%)			
		5	10	15	
1.	Glyricidia	6.00 (2.65)*	8.00 (3.00)	10.00 (3.32)	8.00
2.	Neem	7.00 (2.83)	14.00 (3.87)	18.00 (4.36)	13.00
3.	Nilgiri	7.00 (2.83)	8.00 (3.00)	9.00 (3.16)	8.00
4.	Garlic	7.60 (2.94)	10.00 (3.32)	9.60 (3.27)	9.10
5.	Lantana	10.00 (3.27)	10.00 (3.32)	11.00 (3.46)	10.20
6.	Onion	8.70 (3.11)	9.70 (3.27)	10.00 (3.32)	9.40
7.	Pomgamia	8.70 (3.11)	9.70 (3.27)	15.30 (4.04)	11.20
8.	Tridax	6.70 (2.77)	8.00 (3.00)	10.00 (3.32)	8.20
9	Noni	10.00 (3.32)	11.00 (3.42)	12.00 (3.56)	10.80
10	Control	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00
	Mean	7.10	8.80	10.50	
	Factors	Botanicals	Concentration	B x C	
	SE m±	0.06	0.03	0.11	
	CD at 1%	0.22	0.13	0.39	

Fig in the parenthesis are square root transformed values

Table 3: *In Vitro* Evaluation of bio-agents against *Xanthomonas Campestris pv. Arecae.*

Sl. No.	Bio-agents	Mean inhibition zone (mm)
1.	*Trichoderma harzianum*	24.10 (5.01)*
2.	*Bacillus subtilis*	12.40 (3.66)
3.	*Pseudomonas fluorescens*	15.70 (4.08)
	SE m±	0.06
	CD at 1%	0.28

Fig in the parenthesis are square root transformes values.

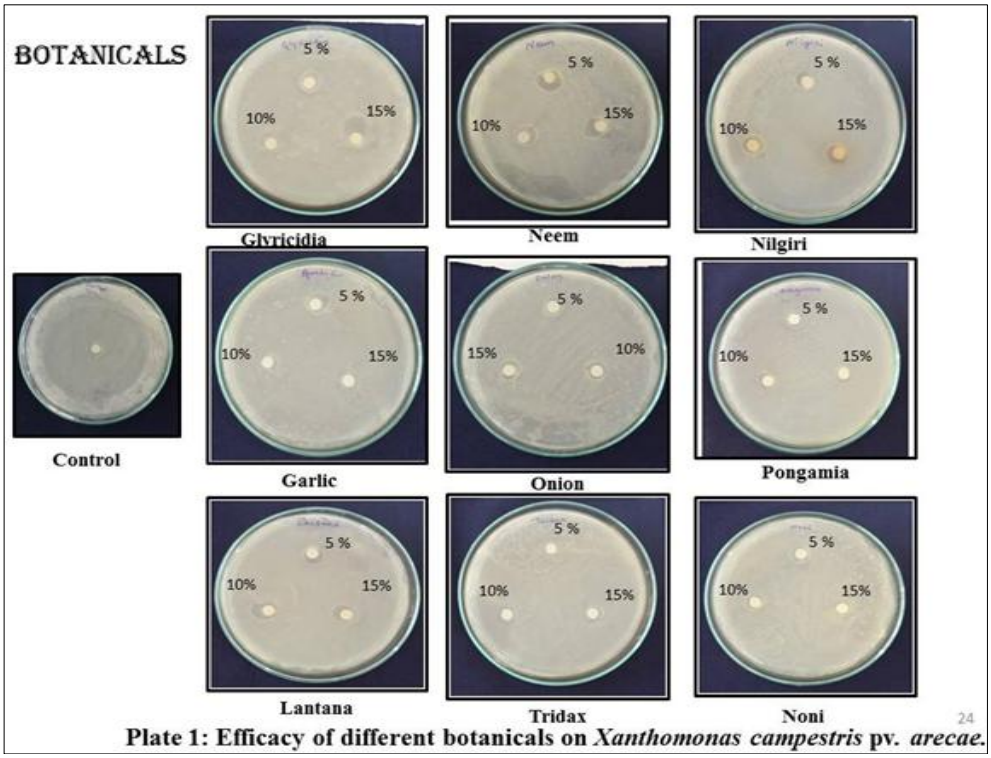

Plate 1: Efficacy of different botanicals on *Xanthomonas campestris* pv. *arecae*.

Plate 2: Efficacy of different bio-agents on *Xanthomonas campestris pv. arecae*

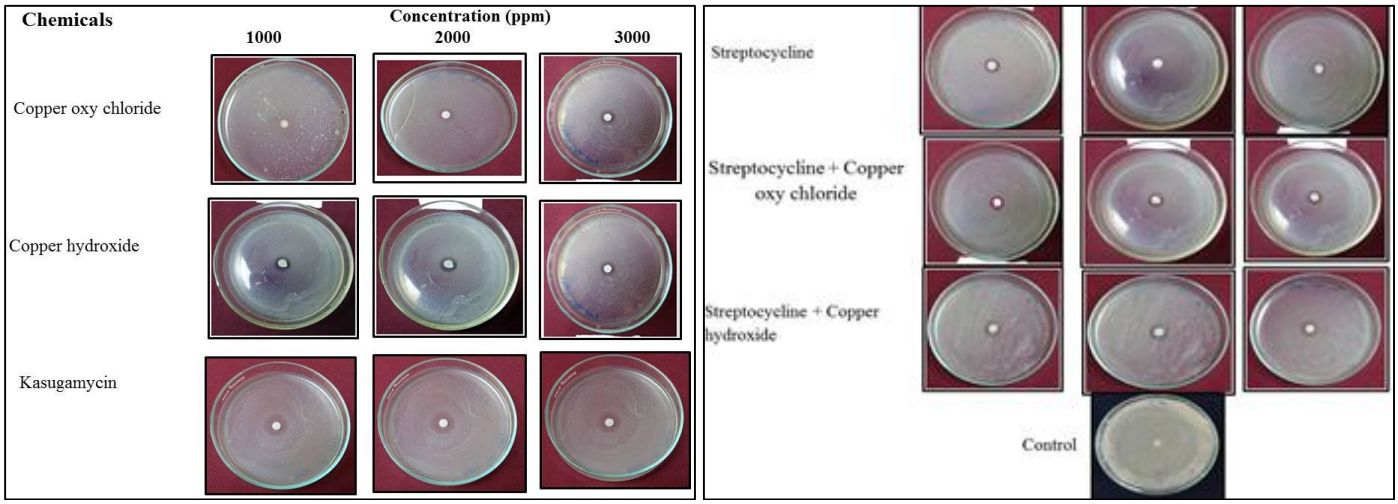

Plate 3: Efficacy of different bactericides on *Xanthomonas camestris pv. arecae*

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