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Effect of botanicals on *Xanthomonas axonopodis* pv. *vignicola* and bacterial blight severity in Cowpea

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Cowpea is grown throughout the world as vegetable, pulse, fodder and green manure crop. Amongst the diseases of cowpea, bacterial blight caused by *Xanthomonas axonopodis* pv. *vignicola* is most common and wide spread which is known to cause extensive damage throughout the world. The management of the disease is difficult and the chemicals are least effective and are hazardous. Hence, an alternate method of control needs to be developed which is effective, cheap and ecofriendly. The water extract from only two botanicals viz., *Ocimum gratissimum* and *Tylophora asthmatica* were effective in inhibiting the growth of *X. axonopodis pv. vignicola. O. gratissimum* extract showed highest inhibition zone of 28.33 mm at 1:0 dilution and had inhibitory effect up to 1:10 dilution with 21.66 mm inhibition zone. Alcohol extract of *O. gratissimum* was the most effective in inhibiting the growth of *X. axonopodis pv*. *vignicola* followed *by C. gigentea, O. sanctum, T. asthmatica, N. sativa* and *R. graveolens*. Seed treatment with alcohol extract of *Ocimum gratissimum* was most effective in suppressing the blight disease.

Key words: Medicinal plants: *Xanthomonas axonopodis*: cowpea: inhibition zone:

INTRODUCTION

Cowpea (*Vigna anguiculata* L. Walp.) is a legume mainly grown in tropical and subtropical regions in the world for vegetable and grains and to lesser extent as fodder crop. It also serves as cover crop and improves soil fertility by fixing atmospheric nitrogen. Nutritionally, cowpea grains contain 23-25 % protein, 50-67 % starch and several vitamins and minerals thus making it an important economic crop in many developing countries (Sebetha *et al.* 2010).

In India, cowpea is grown as sole crop, inter crop and mixed crop in pulse growing states. It occupies an area of about 3.9 mha with a production of 2.2 mt and productivity of 564 kg ha^{-1}. In Karnataka, it is grown on an area of 0.97 mha with a production of 0.43 mt and productivity of 462 kg ha^{-1} (Anon. 2013).

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The major constraint in cowpea production is by pests and diseases caused by fungi, virus and bacteria. Amongst the diseases bacterial blight caused by Xanthomonas axonopodis pv. vignicola is most common and wide spread which is known to cause extensive damage throughout the world. In the tropical and sub-tropical areas, it can be severe because of high temperatures and alternating wet and dry conditions. It affects the foliage and pods of cowpeas and causes significant yield losses and adversely affects seed quality. Seed-borne inoculum is important in the survival, primary infection and dissemination of the pathogen (Okechuku *et al.* 2010).

The use of chemicals has not been effective in the control of bacterial blight of cowpea because the pathogen is a seed borne and is systemic in its nature. The use of copper based bactericides and antibiotics seldom gave satisfactory control. Botanicals because of their natural origin are biodegradable and they do not leave toxic residues or by-products to accumulate in the environment.

Therefore under this scenario, botanical pesticides seem to be ideal candidate to be exploited in management of bacterial blight of cowpea in view of the safety, renewable nature, cost effective and high target specificity. Hence an investigation has been conducted to study the effect of extracts from few important medicinal plants against *X. axonopodis pv. vignicola* and the cowpea blight disease.

MATERIALS AND METHODS

An experiment was conducted during 2016 at College of Agriculture, V.C. Farm, Mandya to study the inhibitory effect of few plant extracts against *X. axonopodis pv. vignicola* and on the severity of cowpea bacterial blight disease.

Selection of medicinal plants

Medicinal plants mentioned in Table 1 which were reported to contain some antibacterial constituents and being used in Indian system of medicine were selected to screen for their antibacterial properties against *X. axonopodis* pv. *vignicola* causing bacterial blight of cowpea. These plants were collected from College of Agriculture, Mandya.

Isolation of pathogen

Cowpea leaves showing typical symptoms of bacterial blight were collected and cut into small pieces aseptically from the edge of typical lesion along with healthy tissue. The infected leaf bits were surface sterilized in 70 per cent alcohol and was washed in three series of sterile water to remove traces of alcohol. The bits were suspended in a drop of sterile water taken on a sterilized microscope slide and were allowed to stand for five minutes. When a drop of water became turbid due to oozing of bacterial cells from the cut ends of the leaf bits, a loopful of the bacterial suspension was taken on a inoculation needle aseptically and streaked on the surface of nutrient agar contained in sterilized Petri plates. The inoculated plates were incubated at 300C for 48 hours and were observed for the development of well-separated typical *Xanthomonas colonies.*

It was purified by picking the individual colonies and streaked on the surface of yeast extract dextrose calcium carbonate agar (YDCA) medium contained in Petri dishes. Three to four loop ful of well-separated colonies were suspended in sterile distilled water taken in vials. The vials were stored at 5^0C, and served as stock culture for further studies.

The bacterium isolated from diseased plant was identified on the basis of morphological, cultural and biochemical characteristics.

Method of extraction

Two most common methods used in extraction were followed to extract the antimicrobial components contained in eight different plant species in order to screen for their antimicrobial property against cowpea bacterial blight pathogen were (1) Water extract method (2) Alcohol extract method.

Protocol for water extract

The economic parts of the plants noted in Table 1 were used for the purpose of extraction. 50g of leaves or seed as the case may be were taken and cut into small pieces under aseptic condition. The sample was put into waring blender containing 50ml sterilized distilled water at a ratio 1:1 (water: plant material). The sample was spun at low speed for 10-15 minutes in a coffee warring blender till the materials were broken down to fine texture. The blended material was then squeezed through a sterilized muslin cloth so as to get a crude liquid extract. The crude extract was filtered through Whatman no 1 filter paper followed by sterilized Seitz filter. The sterilized filtrate was collected in sterilized glass tubes and the tubes were sealed under aseptic condition and labelled as “WE”. The water extract was kept at 5oC in a refrigerator for further use.

Protocol for alcohol extract

Fifty gram of the economic parts of the respective plant was mixed with a small quantity of 70 per cent ethyl alcohol and macerated in a pestle and morter under aseptic condition. The material was blend to fine texture, transferred to a beaker and the final volume was made up to 50ml with 70 per cent ethyl alcohol in the ration of 1:1 (plant material: alcohol). The beaker was kept overnight under refrigerated condition. Alcohol extract was squeezed through muslin cloth, then passed and finally sterilized through Seitz filter apparatus. The

sterilized filtrate was collected in sterilized glass tubes and the tubes were sealed under aseptic condition and labelled as "AE". The alcoholic extract was stored at 5°C in a refrigerator for further use.

In-vitro evaluation of plant extracts

Both water and alcohol extracts of the medicinal plants were screened at different dilutions viz., 1:0 (undiluted), 1:1, 1:10, 1:100, 1:1000. The efficacy of the extracts were tested by the zone of inhibition assay technique against *X. axonopodis pv. vignicola* causing bacterial blight of cowpea. A heavy suspension of the test bacteria ($7x10^8$cfu/ml) was seeded to the sterilized nutrient agar medium by mixing the bacterial cultural with the cooled nutrient agar (45-50°c) in a 500ml Erylenmeyer flask. The seeded medium was poured on sterilized Petri plates and allowed to solidify.

Sterilized filter paper disc (Whatman no.1) measuring 10mm diameter were soaked for 10 minutes in undiluted (1:10) and diluted (1:1, 1:10, 1:100 and 1:1000) plant extracts and placed on the surface of seeded nutrient agar medium contained in the Petri plates in marked position. The inoculated plates were incubated first at 4°C for 4 hours so as to allow the diffusion of the extract into the medium.The plates were then transferred to incubator maintained at 30°C and incubated for 48 hours. Observations were recorded on the zone of inhibition produced around the filter paper disc in each plant extract at different dilutions, by measuring the diameter of the inhibition zone.

Effect of seed treatment with plant extract on the development of bacterial blight of cowpea

The *X. axonopodis pv. vignicola* isolated from the infected cowpea leaves were multiplied on YDC agar plates and the cells were harvested in sterilized water. The concentration of the cell suspension was adjusted to $7x10^8$ cfu/ml turbidometrically.

Apparently healthy cowpea seeds collected from the field were inoculated with the bacterial suspension by keeping the seed in the bacterial suspension contained in 250ml side arm flask. The bacterial cells were ingressed into the seeds by subjecting the seeds to a vacuum pressure of 20lbs/sq. inch for 15 minutes. The inoculated seeds were air dried over night.

Artificially inoculated cowpea seeds of cv. C-152 were soaked separately in diluted (1:1, 1:10) and undiluted (1:0) alcohol extracts of *Ocimum gratissimum*, *O. sanctum, Tylophora asthmatica* and *Calotropis gigantea* for 30 minutes. The seeds were removed after the stipulated duration of soaking, air dried for 24 hours and were sown in 6" pots containing sterilized soil. Untreated seeds were maintained as positive control. Observations were recorded for per cent leaf area infected per plant.

RESULTS AND DISCUSSION

Effect of water extract of medicinal plants against X. axonopodis pv. vignicola

Water extracts of *O. gratissimum* and *T. asthmatica* had inhibitory effect against X. *axonopodis pv. vignicola* and rest of plant extracts tested had no effect at all. *O. gratissimum* extract showed highest inhibition zone of 28.33 mm at 1:0 dilution and had inhibitory effect up to 1:10 dilution (21.66 mm) which was far better than the control (14.00 mm). Whereas, *T. asthmatica* was effective only up to 1:1 dilution and was superior to control at 1:0 and 1:10 dilution with inhibition of 18.33 mm and 14.33 respectively (Table 2). Saha *et al*. (2013) reported inhibitory effect of extracts of five *Ocimum* species against Gram-positive and Gram-negative bacteria and few plant pathogenic fungi.

Effect of alcohol extract of medicinal plants against X. axonopodis pv. vignicola

The alcohol extracts of six medicinal plants viz., *O. gratissimum, O. sanctum, T. asthmatica, R. graveolens, C. gigentea, and N. sativa* exhibited inhibitory effect whereas, *S. persica* and *T. cardifolia* had no such effect (Table 3). Among the botanicals tested, *O. gratissimum* was the most effective in inhibiting the growth of *X. axonopodis pv. vignicola* followed by *C. gigentea, O. sanctum, T. asthmatica, N. sativa* and *R. graveolens*. Extract of *O. gratissimum* was effective up to 1:100 dilution and produced inhibition zones of 30.33, 28.66, 25.66 and 20.33 mm at 1:0, 1:1, 1:10 and 1:100 dilution respectively which was superior when compared with streptocycline (14.00 mm)

Table 1: List of medicinal plants used to test their efficacy against *Xanthomonas axanopodis* pv. *vignicola*

	Common Name	Scientific Name	Family	Part used for extraction
	Antamul	*Tylophora asthmatica W. & A.*	Asclepiadaceae	Leaves
	Mudar	*Calotropis gigentea L*	Asclepiadaceae	Shoot
	Holy basil	*Ocimum sanctum L*	Lamiaceae	Leaves
	Clocimum	*Ocimum gratissimum L*	Lamiaceae	Leaves
	Tinospora	*Tinospora cardifolia Willd.*	Menispermaceae	Leaves
	Black Cumin	*Nigella sativa L.*	Ranunculaceae	Seeds
	Garden Rue	*Ruta graveolens L.*	Rutaceae	Shoot
	Meswak	*Salvadora persica L.*	Salvadoraceae	Stem

Table 2: Effect of water extracts of medicinal plants against *Xanthomonas axanopodis* pv. *vignicola*

	Zone of inhibition (mm)							
Dilution	*O. gratissimum*	*O. sanctum*	*T. asthmatica*	*R. graveolens*	*C. gigentea*	*N. sativa*	*S. persica*	*T. cardifolia*
1:0	28.33	0.00	18.33	0.00	0.00	0.00	0.00	0.00
	(5.42)	(1.00)	(4.40)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
1:1	25.00	0.00	14.33	0.00	0.00	0.00	0.00	0.00
	(5.10)	(1.00)	(3.91)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
1:10	21.66	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(4.76)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
1:100	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
1:1000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
Streptocycline 400 ppm. (control)	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00
	(3.87)	(3.87)	(3.87)	(3.87)	(3.87)	(3.87)	(3.87)	(3.87)

(Figures in parenthesis are square root transformed values)

	S.Em+	CD(1%)
Factor A	0.0141	0.0397
Factor B	0.0141	0.0397
A x B	0.0345	0.0972

which served as control. *O. sanctum, T. asthmatica and N. sativa* extracts were inhibitory up to 1:10 dilutions whereas, *R. graveolens* and *C. gigentea* extracts were effective up to 1:1 dilutions. But *S. persica* and *T. cardifolia* extracts showed no inhibitory effect at all the dilutions tested against the pathogen.

This work revealed that ethanol could be among the better solvent for extraction from medicinal

Table 3: Effect of alcohol extracts of medicinal plants against Xanthomonas axanopodis pv. vignicola

Dilution	Zone of inhibition (mm)							
	O.gratissimum	*O. sanctum*	*T. asthmatica*	*R. graveolens*	*C. gigentea*	*N. sativa*	*S. persica*	*T. cardifolia*
1:0	30.33	20.33	19.66	14.33	20.66	17.66	0.00	0.00
	(5.60)	(4.62)	(4.55)	(3.91)	(4.65)	(4.32)	(1.00)	(1.00)
1:1	28.66	18.33	16.66	12.33	17.00	12.66	0.00	0.00
	(5.45)	(4.40)	(4.20)	(3.65)	(4.24)	(3.70)	(1.00)	(1.00)
1:10	25.66	15.66	13.33	0.00	0.00	11.66	0.00	0.00
	(5.16)	(4.08)	(3.78)	(1.00)	(1.00)	(3.56)	(1.00)	(1.00)
1:100	20.33	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(4.62)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
1:1000	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)
Streptocycline 400	14.00	14.00	14.00	14.00	14.00	14.00	14.00	14.00
ppm. (control)	(3.87)	(3.87)	(3.87)	(3.87)	(3.87)	(3.87)	(3.87)	(3.87)

(Figures in parenthesis are square root transformed values)

	S. Em+	C. D. (1%)
Factor A	0.0091	0.0257
Factor B	0.0091	0.0257
A x B	0.0224	0.0630

plants. This could be because ethanol diffuses easier into the medium than water. Gena *et al*. (2008)) observed solvent extracts of *Ocimum sanctum* was better in inhibiting the growth of *X. axonopodis pv. vignicola* followed by *Solanum indicum* and *Glycyrhiza glabra*. Similarly, *Ponnanikajamideen et al.* (2013) found that *T. asthmatica* extracts obtained from different extracts showed inhibitory effect against different strains of bacteria. The alcohol and water extracts of R. graveolens exhibited inhibitory activity against many Gram negative bacterial and plant pathogenic fungi tested (Pandey *et al*. 2011). The strong antibacterial activity of essential oil of *N. sativa* seeds was demonstrated against both Gram-positive and Gram-negative bacteria and maximum inhibitory activity was recorded against Bacillus subtilis It also showed inhibitory activity against three *Erwinia* spp. causing soft not of potato by the extract of *Calotropis procera*, thus confirmed the result obtained of present investigation.

Effect of seed treatment with the plant extracts on the development of bacterial blight of cowpea

Undiluted (1:0) extracts of *O. gratissimum, O. sanctum, T. asthmatica* and *Calotropis gigantea* were effective in suppressing the bacterial blight of cowpea as none of the plants developed symptoms of bacterial blight. Also *O. gratissimum*

Table 4: Effect of plant extracts on the development of bacterial blight of cowpea

Alcohol extract	Dilution	Percent leaves infected/plant
Ocimum gratissimum	1:0	0.00
	1:1	0.00
	1:10	12.82
Ocimum sanctum	1:0	0.00
	1:1	7.14
	1:10	10.71
Tylophora asthmatica	1:0	0.00
	1:1	11.76
	1:10	12.50
Calotropis gigantea	1:0	0.00
	1:1	8.33
	1:10	8.62
Control (untreated)		13.41
	S. Em+	0.0915
	C. D. (1%)	0.2538

at 1:1 dilution completely suppressed the disease, whereas at 1:10 dilution 2.82 per cent leaves/plant was infected. In case of *O. sanctum* at 1:1 dilution 7.14 per cent of the leaves were infected/plant, whereas at 1:10 dilution 10.71 per cent of leaves/ plants were infected.

In case of *T. asthmatica* at 1:1 dilution 11.76 per cent of the leaves/plant was infected, whereas at 1:10 dilution 12.50 per cent of the leaves were infected/plant. In case of *C. gigantea* at 1:1 dilution 8.33 per cent of leaves/plant was affected, whereas at 1:10 dilution 8.62 per cent of leaves/plant was affected.

In untreated seeds of cowpea (control) 13.41 per cent of the leaves/plant was affected by bacterial blight (Table 4).

Ganiyu *et al.* (2017) reported that extracts of *Azadirachta indica, Achalypha wilkisiana* and *Carica papaya* effectively controlled the development and spread of common blight of cowpea due to the presence of antibacterial compounds in plant extracts.

The present investigation revealed that extracts of many medicinal plants had inhibitory effect against *X. axonopodis pv. vignicola*. Further, alcohol extracts were more effective than water extracts as in alcohol extract, more number of phytochemicals liberated and their efficacy were enhanced against the pathogen. Hence these could be exploited as an alternate management strategy for chemical pesticides in the management of bacterial blight of cowpea. The future studies should focus on identification and elucidation of the precise active principles present in medicinal plants having potential antimicrobial properties.

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