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Study of use of Plant Extracts and Copper Nanoparticles in the management of Bacterial Blight disease of Pomegranate

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ABSTRACT
Bacterial blight of pomegranate is a major disease. It is caused by Xanthomonas axonopodis pv. punicae responsible for decrease in production of fruit. Today this disease has become a threat to pomegranate producer in the three major pomegranate growing states viz., Maharashtra, Karnataka and Andhra Pradesh. This disease affects flower, leaves, twigs, stem, buds and fruit. It is dangerous when fruit gets infected. The disease is responsible for 30-50 % losses on an average. However, under favorable environmental conditions losses are 80-100 %. X. axonopodis was isolated from infected pomegranate fruit, stem and leaves and maintained on NAS medium plates. Pure strain proved Gram staining, oxidase, catalase and starch dehydrogenase tests. Plant extracts of Eucalyptus globulus, Azadirachta indica and Lantana camara linn were used. The plant extract of Eucalyptus globulus showed more significant antibacterial activity followed by Lantana camara linn and Azadirachta indica. The synergistic effect of all alcoholic plant extracts with CuNPs found significantly effective over the effect of plant extracts.
Keywords: *Disease management, CuNPs, Xanthomonas axonopodis, Eucalyptus globulus, Azadirachta indica and Lantana camara linn.*

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INTRODUCTION

Pomegranate (*Punica granatum* L.) is belonging to the smallest botanical family *punicacae.* The Pomegranate (*Punica granatum* L.) is an ancient fruit crop of India. Pomegranate has been associated with high nutritional value, many health benefits and its entire plant has great economic and therapeutic value. Pomegranate is also well known for various medicinal properties and nutritive values [1]. It is native to Iran. It is the important fruit crop cultivated throughout the world. India is largest pomegranate producer in the world sharing about 36 per cent of the world's production and above 30 per cent of the international Trade [2]. Plant compounds are used to treat infection since long time in a large part of the world, especially in developing countries, and there is dependence on traditional medicine for a variety of diseases [3]. Plant extracts have played significant role in the inhibition of pathogens and in the improvement of quality and yield of food [4]. There is need to shift towards eco-friendly technologies to avoid the loss. Plants produce hundreds to thousands of diverse chemical compounds with different biological activities. Medicinal plants have been found useful in the cure of a number of diseases including bacterial diseases. Medicinal plants are a rich source of antimicrobial agents [5]. Ayurveda regarded neem (*Azadirachta indica* Family: Meliaceae) as a cure for many ailments, predominantly due to its superb antimicrobial activity. Almost every part of the tree is bitter and finds application in indigenous medicine. Neem extract has been reported to have antidiabetic, antibacterial and antiviral activity [6]. Neem tree is evergreen tree found in most tropical countries. Almost every part of the tree has been in use since ancient times to treat a number of human ailments and also as a household pesticide. The extract from bark, leaves, fruits and root have been used to control leprosy, intestinal helminthiasis and respiratory disorders in children [7]. Flavonoids, flavonoglycosides, dihydrochalocones, tannins and others are also important constituents of bark, leaves, fruits and flowers of neem. The biological activities and medicinal properties of neem have recently been reported [8]. Heavy use of chemicals in agriculture land causes loss of flora and fauna of the soil, plant

pathogen develops resistance against chemicals and residual toxicity remains in plant and animals. To overcome this problem, there is growing interest worldwide in the utilization of sustainable material for pathogen control [9].

MATERIAL AND METHODS

Collection of Plant Material

The infected parts of the pomegranate showing bacterial blight diseases symptoms were collected in sterile plastic bags from different locality pomegranate orchard in Pravara area.

Isolation of *Xanthomonas axonopodis*

The infected parts were first washed in running tap water. Infected portions along with small portions of healthy tissue were cut into 5 mm bits. The bits were further surface sterilized with 0.1 per cent $HgCl_2$ for 1 minute and washed thoroughly with sterile distilled water three times. The surface sterilize bits were then crushed in 2 to 3 ml sterile distilled water and allowed to diffuse for 5 to 10 min at room temperature. A loop full leachate was streaked on Nutrient Agar medium plates aseptically and incubated at 30±1 °C temperature for 48 hours. The colonies grown within 72 hours were picked and again streaked on fresh Nutrient Agar plates. The discrete colonies were sub cultured on nutrient agar slants for further studies.

The isolated bacterial colonies were further picked up with the sterilized inoculation loop and streaked on the surface of sterilized NA medium petri plates. The inoculated plates were incubated at 30^oC for 72 hours. Observations were noted for the developed well-separated typical, bright yellow, mucoid colonies. These pure colonies were further streaked onto the NA medium slants and incubated at 30^oC for 72 hours. The pure cultures slants were stored in the refrigerator at 5^oC, as a stock culture for further studies [0,11].

Collection of plant materials

Fresh plant materials of *Eucalyptus, Azadirachta indica, Lantana camara* linn and *Annona squamosa* were collected randomly from the region near to Pravaranagar, India. Fresh plant materials were washed; shade dried and then powdered using the mixture blender. Methanol extraction 10 g of plant powder was added to 100 ml of methanol in a conical flask and plugged with cotton wool. After 42 hours the supernatant was collected and the solvent was evaporated to make the crude extract and stored at 40^0C [12] . Similarly, 10 % , 5 % , 2.5 % plant extracts were prepared.

Antibacterial activity and Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the crude methanol extracts of leaves were determined by using serial dilution technique [13] . 1 mg/ml of the sample solutions of all the extracts were prepared using Dimethyl Sulfoxide (DMSO). Each test tubes was filled with 1 ml of sterile nutrient broth media and doses of sample solution were added. Then these test tubes were inoculated with the selected organisms (1×10^6 cells/ml) followed by incubation at 37^0C for 24 hours. The test tubes which showed minimum concentration as well as clear content were selected. This lowest or minimum concentration was considered as Minimum Inhibitory Concentration (MIC). Another three test tubes containing medium, medium and sample, medium and inoculum were used as control. Bacterial growth observed was only in test tubes containing medium and inoculum and the other two were clear showing no growth [13]. Experiments were done in triplicate and repeated twice. The sample from these tubes was spread on nutrient agar plated by spread plate technique and bacterial growth was observed after 48 hours.

Percent Reduction in Bacterial Growth

Percent reduction in bacterial growth was estimated with spread plate technique. 1 ml sample was added in separate sterile plates and 20 ml sterile nutrient agar medium at 45^0C was poured in each sterile plate. Plates were incubated at 27 ^0C for 24 h. Percentage reduction in bacterial growth was estimated as per following formula.

Percentage reduction in bacterial count = Initial CFU- Final CFU/ Initial CFU x 100

RESULT AND DISCUSSION

Identification and characterization

The morphological and biochemical characteristics tests were used to identity pathogen. Morphologically all the isolated strains showed light to pale yellow colour, profuse slimy smooth growth. Further characterization through a biochemical test proved Gram negative reaction, positive to citrate utilization, starch hydrolysis, KOH solubility and lysine utilization etc., inconformity with past findings [14,15,16,17].

Antibacterial Activity of Plant Extract

After observation of colonies count was taken. Percentage reduction was calculated using formula.

Table 1: Effect of Alcoholic Plant Extract of *Eucalyptus globulus* on *X. axonopodis*

Sr. No	Alcoholic extract of *Eucalyptus*	O.D. at 0 min	O.D. after 24 hours	Colony count	Percentage reduction
1	10	1.40	0.90	14	93.832
2	5	0.86	0.71	29	87.22
3	2.5	0.87	0.93	31	86.34

Table 2: Effect of Alcoholic Plant Extract of *Lantana camara linn* on *X. axonopodis*

Sr. No	Alcoholic extract of *Lantana camara linn* (%)	O.D. at 0 min	O.D. after 24 hours	Colony count	Percentage reduction
1	10	0.448	1.25	18	92.07
2	5	0.301	0.640	21	90.748
3	2.5	0.134	0.367	36	84.140

Table 3: Effect of Alcoholic Plant Extract of *Azadirachta indica* on *X. axonopodis*

Sr. No	Alcoholic extract of *Azadirachta indica(%)*	O.D. at 0 min	O.D. after 24hours	Colony count	Percentage reduction
1	10	0. 367	0.389	59	74.008
2	5	0. 291	0.241	121	46.69
3	2.5	0. 148	0.236	119	47.57

Table 4: Effect of Combination of Alcoholic extracts of *Eucalyptus globulus*, *Azadirachta indica and Lantana camara* on *X. axonopodis*

Sr. No	Combination of Alcoholic extracts of *Eucalyptus* , *Azadirachta indica and Lantana camara* (%)	O.D. at 0 min	O.D. after 24 hours	Colony count	Percentage reduction
1	10	1.45	0.636	26	88.54
2	5	0.664	0.454	29	87.22
3	2.5	0.349	0.177	30	86.78

Table 5: Synergistic Effect of Alcoholic extracts of *Eucalyptus*, *Azadirachta indica and Lantana camara* (%) and copper nanoparticle

Sr. No	Percentage Combination of Alcoholic extracts of *Eucalyptus, Azadirachta indica and Lantana camara*	CuNP µg/ml	Colony count			Mean	Percentage reduction
			1	2	3		
1	10	2.5	9	7	8	8	98.039
2	5	2.5	31	29	28	29.33	92.89
3	2.5	2.5	41	40	42	41	89.95
4	10	5	8	7	7	7.33	98.28
5	5	5	20	19	22	20.66	95.09
6	2.5	5	35	37	39	37	90.93
7	Control	-	409	407	408	408	00
	SD						±33.0973
	SE						±12.5096

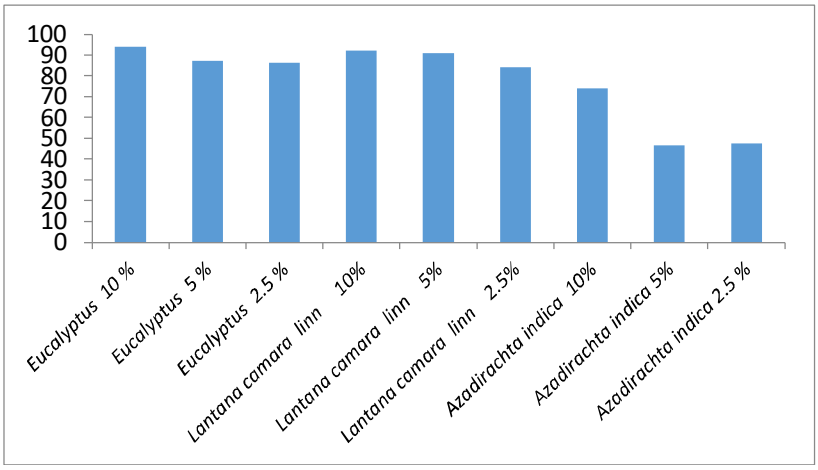

Figure 1: **Alcoholic plant extract Vs percentage reduction**

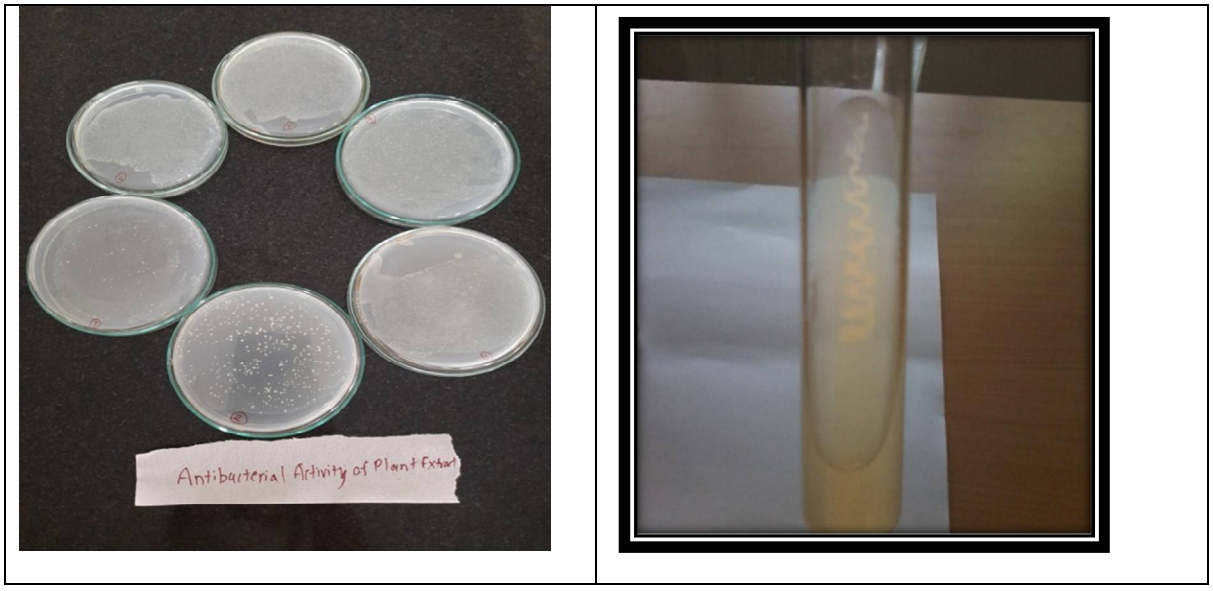

Figure 2: Antibacterial Activity of Plant Extract

CONCLUSION

Results summarized in table no 1, supports that alcoholic extract of *Eucalyptus globulus* proved that about 95% reduction in colony count at 10% alcoholic extract. However, results in table 2 proved that alcoholic extract of *Lantana camara linn* given at 5% concentration proved significantly effective in percentage reduction of colony count up to 90%. Results summarized in table 3 proved that alcoholic extract of *Azadirachta indica* proved only upto 75% reduction in colony count at 10% concentration. In table 4, combination of plant extracts proved insignificant as compared to individual effect. Results presented in table 5 indicates synergistic effect of alcoholic plant extracts and copper nanoparticle. Experimental finding indicates that synergistic effect of all alcoholic plant extracts with CuNPs found significantly effective over synergistic effect of plants. This finding is supported by the researchers [18,19].

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COMPETING INTEREST

The author declares that they do not have any competing interests.

DECLARATION OF CONFLICT OF INTEREST: Nil

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