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In-vitro Efficacy of Colloidal Silver Compound against *Phytophthora infestans* and *Xanthomonas axonopodis* pv. *punicae*

SHANTAMMA AND K. T. RANGASWAMY

Department of Plant Pathology, College of Agriculture,UAS, GKVK, Bengaluru-560065

ABSTRACT

In-vitro studies were conducted to know the effect of colloidal silver compound at different concentrations ranging from 2.5 to 500 ppm against *Xanthomonas axonopodis* pv. *punicae* and *Phytophthora infestans.* The fungal and bacterial plant pathogens were grown on potato dextrose agar (PDA) and nutrient agar (NA) , respectively and supplemented with different concentration of colloidal silver compound to find out the suppression effect and growth inhibition. Maximum inhibition of *Phytophthora infestans* mycelial growth (86.13%) was recorded at 500 ppm concentration and lowest at 10 ppm concentration (3.31%). However, the colloidal silver compound showed complete inhibition of *Xanthomonas axonopodis* pv. *punicae* at concentrations of 25, 50 and 100 ppm.

AGRICULTURAL production is reducing worldwide every year due to plant diseases as one among other factors. Hence, millions of dollars are being invested on pesticides to control plant diseases. Various organic and non-organic methods are being used to protect plants from diseases. Among these, use of pesticides is the most prevalent one. In recent years, environmental hazards and ill effects caused by excessive use of pesticides have been widely discussed therefore, agriculture scientists are finding alternative measures by conserving nature and ecosystem. As an alternative to chemical pesticides, use of silver nanoparticles as antimicrobial agents has become more common and various forms of silver ions and nano particles were tested to examine the antifungal activity on plant-pathogenic fungi (Kim *et al.,* 2012).

Silver nanoparticles are basically silver particles suspended in water which is in the range of 1to100 nm in size. Colloidal silver particles have unique properties which help in molecular diagnostics, in therapies, as well as in devices that are used in several medical procedures. Colloidal silver particles have high activity against a wide range of microbial pathogens and are highly antimicrobial to several species of bacteria and fungi. According to the mechanism reported, AgNP is highly antimicrobial to several species and interact with the outer membrane of bacteria and arrest the respiration, which in turn leads to the death of the bacteria (Clement *et al*., 1994). In this study colloidal silver compound was tested to know their efficacy against fungal and bacterial plant pathogens.

To determine the antimicrobial activities, colloidal silver compound was tested at different concentrations such as 10 ppm, 25 ppm, 50 ppm, 100 ppm, 250 ppm and 500 ppm against *Phytophthora infestans* and 2.5 ppm, 5 ppm, 10 ppm, 25 ppm, 50 ppm and 100 ppm against *Xanthomonas axonopodis* pv. *Punicae*.

The fungus was grown on PDA media for 10 days prior to setting up the experiment. The PDA media was prepared and melted; the colloidal silver compound was added to the melted medium to obtain the derived concentration on the basis of active ingredient present in the chemical. 20 ml of poisoned medium was poured in each sterilized petri plate. Suitable check was maintained without addition of colloidal silver compound. The plates were then inoculated with circular discs (with diameter of 5mm) from solid culture of fungal pathogen and incubated at 27 to 28 ^{0}C. Three replications were maintained for each treatment. The radial growth of colony was recorded when maximum growth was observed in control plate and per cent inhibition was calculated by using the formula given by Vincent (1927).

The mycelial growth inhibition percentage was determined by using the following formula,

$$\text{Inhibition (\%)} = \frac{C - T}{C} \times 100$$

Where;

Where,

I = Per cent inhibition

C = Radial growth of fungus in control

A loop full of one week old culture of *Xanthomonas axonopodis* pv. *punicae* was diluted in 10 ml of water and used as stock. From the stock, further dilutions were made up to 10^{-6}. 100µl of each dilution of bacterial suspension was poured into petriplates and sterilized nutrient agar supplemented with different concentration of colloidal silver was added. Inhibition of bacterial growth was observed after 48 hours at different concentrations of colloidal silver by counting the number of colonies.

Among the different concentrations of colloidal sliver compound tested under *in vitro*, maximum inhibition of fungal growth was recorded at 500 ppm concentration (86.13%) followed by 250 ppm (74.81%) and lowest inhibition at 10 ppm (3.31%) (Table I and Plate1). These results indicated that the efficacy of each treatment was significant from lower to higher concentration. Similar studies have been carried out by Ouda (2014) where in, AgNPs treated @15 mg L^{-1} concentration resulted in 59.30 and 52.90 per cent inhibition of *Alternaria alternata* and *Botrytis cinerea* respectively. Silver nanoparticle supplemented medium showed 12, 36 and 41 per cent hyphal growth of soil borne pathogens *viz*., *Rhizoctonia solani, Sclerotinia sclerotiorum* and *Sclerotinia minor,* respectively (Min *et al*., 2009).

TABLE I

Efficacy of colloidal silver compound on the growth of Phytophthora infestans

Colloidal silver conc.	Mycelial growth inhibition (%)
Control	0.00
10 ppm	3.31
25 ppm	19.84
50 ppm	24.14
100 ppm	44.57
250 ppm	74.81
500 ppm	86.13
SE.m	1.08
CD (at 1 %)	4.56

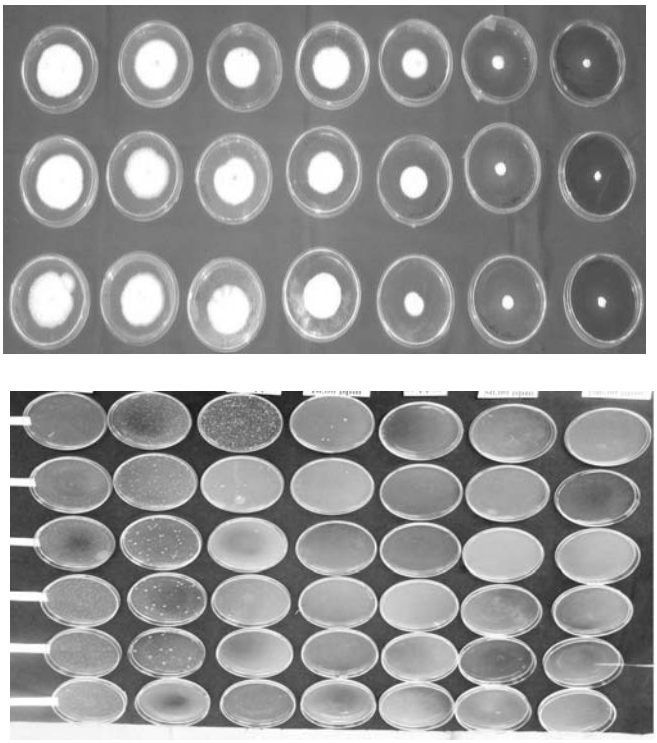

Plate 1. *In vitro* efficacy of colloidal silver compound by poison food technique, colony count method and sap inoculation against *Phytophthora infestans* (A), *Xanthomonas axonopodis* pv. *punicae* (B).

Complete inhibition of *Xanthomonas axonopodis* pv. *punicae* was observed at 25, 50 and 100 ppm of colloidal silver. However, no inhibition was recorded at lower concentrations *viz.*, 2.5, 5 and 10 ppm (Table II & Plate 1). The present results are in line with the studies of Geoprincy (2011) who recorded the combined inhibitory effects of silver nanoparticles @10 mg L^{-1} impregnated with four broad spectrum antibiotics *viz.*, amoxicillin, chloremphenicol, erythromycin and rifamycin against four major bacterial pathogens *viz., Bacillus subtillus, Bacillus cereus, Klebsiella pneumoniiea and Vibrio cholera*.

TABLE II

Efficacy of colloidal silver compound on Xanthomonas axonopodis pv. *Punicae*

Colloidal silver concentrations	Number of bacterial colonies in different dilutions					
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
Control	1088.00	832.33	248.40	182.25	109.62	85.36
2.5 ppm	161.50	85.33	44.23	27.66	2.36	1.00
5.0 ppm	155.00	2.50	2.50	1.00	0.66	0.00
10 ppm	1.66	0.66	0.00	0.00	0.00	0.00
25 to 100 ppm	0.00	0.00	0.00	0.00	0.00	0.00
	Conc.	Dilutions	C x D			
S.Em	1.86	1.72	4.56			
CD (at 1%)	6.95	6.43	17.02			

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