Eco. Env. & Cons. 28 (May Suppl. Issue) : 2022; pp. (S306-S315)
Copyright@ EM International
ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2022.v28i03s.046

Evaluation of efficacy of colloidal silver particles against late blight of potato (*Phytophthora infestans* L.)

Shantamma, K. T. Rangaswamy, N. B. Prakash and Raghavendra Achari

Department of Plant Pathology and Soil Science and Agriculture Chemistry, College of Agriculture, UAS, GKVK, Bengaluru 560 065, Karnataka, India

(Received 1 October, 2021; Accepted 29 October, 2021)

ABSTRACT

Colloidal silver was synthesized by the 'bottom up' approach of wet chemical method and analyzed by laser Dynamic Light Scattering (DLS). The colloidal silver solution synthesized was having a particle size with a mean diameter of 149.4 nm. Tested the efficacy of synthesized colloidal silver particles under *in vitro* conditions (10 ppm, 25 ppm, 50 ppm,100 ppm 250 ppm and 500 ppm) against *Phytophthora infestans.* Among the different concentrations maximum inhibition of mycelial growth was recorded at higher concentrations (250 ppm and 500 ppm). To know the further effectiveness of higher concentrations of colloidal silver particles were tested alone (250 ppm and 500 ppm) and in combination with fungicides (Cymoxanil 8 % + mancozeb 64 % WP@ 0.2 % and Dimethomorph @ 0.2%) under field conditions. The spray solutions of colloidal silver at 500 ppm recorded significantly less late blight disease severity at 90 days after sowing (DAS) (42.22 PDI) when compared to @ 250 ppm (60.74 PDI) and untreated plants (75.56 PDI). The foliar application of colloidal silver @ 500 ppm is found on par with Cymoxanil 8 % + mancozeb 64 % WP@ 0.2 % whereas superior over Dimethomorph 50% WP @ 0.2%. Further, colloidal silver both @ 250 ppm and 500 ppm when used in combination with fungicides could not significantly complemented the efficacy of fungicides *viz*., Cymoxanil 8 % + Mancozeb 64 % WP @ 0.2 % and Dimethomorph 50% WP @ 0.2% in reducing the late blight disease severity at 90 DAS, but recorded significantly less disease severity than untreated control plants. Examined the biochemical response of plant defense mechanism pertaining to defense related enzymesin treated and untreated plants. The increased activity of peroxidase (PO) (1.5 mg/g), polyphenol oxidases (PPO) (0.01 mg/g) and phenylalanine ammonia lyase PAL (1.5 mg/g) was observed in late blight infected potato plants treated with colloidal silver particles @ 500ppm and in combination of 0.2 per cent of Cymoxanil 8 % + Mancozeb 64 % WP fungicide.

Key words *: Colloidal silver, Laser Dynamic Light Scattering (DLS), Phytophthora infestans, In vitro, Field and Biochemical.*

Introduction

Nanotechnology is the application of science and technology to control the matter at molecular level. The term nanotechnology was first defined by Norio Taniguchi of Tokyo Science University in 1974. Nanoparticles are being viewed as fundamental building blocks of nanotechnology. Nanoparticles (NPs) are commonly defined as solid colloidal particles with sizes typically in the range of 10 nm to 1000 nm in one or more dimensions (Taylor, 2011; Kreuter, 1994). The antimicrobial property of silver nanoparticles is exclusively governed by the size of the particles in turn total surface area. Hence, the most effective range of nanoparticles with potential antimicrobial property is 10-100 nm in diameter.

Four main types of silver solutions sold most commonly *viz*., Ionic silver, colloidal silver, protein silver and citrate silver for different uses. The ionic silver cannot exist without water, so when the water is evaporated upon spraying to plant surface, ionic silver reacts either with hydroxides or carbonates and reduced to silver oxide. The silver oxide contributes only about 5 per cent of antimicrobial activity of silver AgNPs and the antimicrobial effectiveness of ionic silver will be lost.

Colloidal silver consists of tiny silver particles in a liquid phase with diameter/size even though ranging from 1-1000 nm, but more concentration of particles will be in the range of >100 to 1000 nm. Colloidal silver containing relatively less concentration of ionic silver like colloidal silver produced by registered and patented "N9 pure silver" technology by Resil Pvt. Ltd. Bengaluru (Registration No. 4273995, Serial No. 85031484) are already in use for imparting antimicrobial property to fabrics without proven harm to the human beings. They are known to have less silver load, less ionic silver concentration and less leaching property makes them much safe to humans.Colloidal silver particles also maintain and enhance the photosynthetic pigments, improve the protein and defense status in plants (Hatami and Ghorbanpour, 2014). The limited research done till now has provided some evidence of the potential applicability of silver for managing plant diseases (Park *et al*., 2006).

To know the antimicrobial efficacy of colloidal silver particles were tested against most potent pathogen of potato causing late blight disease (*Phytophthora infestans*). There are large number of chemicals/pesticides are available in the market for the management of late blight of potato but these are either less effective or can cause major residue related environmental hazards. In order to findan alternative molecules to the chemical fungicides, new molecules such as colloidal silver particles with less concentration of ionic silver and size between 100-1000 nm which have been reported to have inhibitory effect on many fungal plant pathogens besides safe to human beings and environment were synthesized.

Material and Methods

Synthesis of colloidal silver particles

Colloidal silver particles were synthesized at facility established byResil Pvt. Ltd. Bengaluru. Colloidal silver was synthesized by using the 'bottom up' approach of wet chemical synthesis wherein metal salts undergo reduction reaction. The reaction used for the synthesis of colloidal silver was the sodium borohydride reduction of silver nitrate:

$$AgNO_3 + NaBH_4 \rightarrow Ag + 1/2H_2 + 1/2B2H_6 + NaNO_3$$

The initial concentration of the solution synthesized was 1200 ppm. The colloidal silver particles solution was diluted to desired concentrations by using distilled water at room temperature.

Efficacy of colloidal silver particles against late blight disease of potato under *In vitro* condition

In order to test the efficacy of colloidal silver particles against *Phytophthora infestans* under lab condition, an experiment was under taken at UAS, GKVK,Dept. of plant pathology in 2016-17.

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 plates. 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 ^{o}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 (Wheeler, 1969).

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

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

Where;
I = Per cent inhibition
C = Radial growth of fungus in control

Efficacy of colloidal silver particles against late blight disease of potato under field condition

In order to test the efficacy of colloidal silver particles against late blight disease of potato under field condition, an experiment was under taken at ARS

Madenuru (Hassan) of UAS Bengaluru during *rabi*season 2016-17. Colloidal silver particles were applied as foliar sprays at weekly intervals after observing the first visual symptoms. The commercial fungicides namely Cymoxanil 8 % + Mancozeb 64 % WP and Dimethomorph 50 % WP were used both alone and in combination with colloidal silver. The trial was laid out in Randomized Completely Block Design (RCBD) with three replications. Variety grown was KufriJyothi. Chemicals were applied using manually operated high volume (Knapsack) sprayer. Observations were recorded one week after the treatment. Severity of late blight was recorded following 0 – 9 scale (Table 2) and per cent disease index was worked out. The data was analyzed statistically.

Collection and analysis of leaf samples for different biochemical changes

For biochemical studies, the diseased potato plants leaf samples were collected from colloidal silver and fungicides applied plants and used for further studies.

Sampling

For analysis of biochemical parameters, like Peroxidase (PO), Polyphenol Oxidase (PPO) and Phenylalanine Ammonia Lyase(PAL) the sampling was done at 60 and 90 days after sowing at 24 hours after imposing the treatments on potato plants infected with late blight disease.

Assay of peroxidase (PO)

The peroxidase activity was assayed spectrophotometerically (Hartee, 1955). The detail protocol is as below.

Reagents

Hydrogen peroxide solution: 3.3 ml of H_2O_2 was mixed with 97.70 ml of distilled water to get 100 ml of 1 % H_2O_2 solution. The solution was prepared every time freshly.

Pyrogallol, 0.05 M: 6.3005 g of pyrogallol was dissolved in 100 ml of distilled water. The solution was prepared every time freshly.

Phosphate buffer, 01 M, 6.5 pH: Solution A: 27.6 g of sodium phosphate monobasic ($NaH_2PO_4.2H_2O$ Mol. wt. -156.01) was dissolved in small quantity of water and the volume was made up to 1000 ml with distilled water.

Solution B: 28.4 g of sodium phosphate dibasic (Na_2HPO_4 Mol. Wt. 142 g) was dissolved in small quantity of water and volume was made up to 1000 ml with distilled water.265 ml of solution A was mixed with 735 ml of solution B. Finally, pH was adjusted using NaOH.

Preparation of enzyme extract: One gram of leaf sample was homogenized in 3 ml of 0.1 M phosphate buffer, pH 6.5 at 4 °C. This mixture was filtered through 4 layer muslin cloth. The filtrate was centrifuged at 12000 rpm for 20 minutes at 4 °C. The supernatant was collected and used for estimation of peroxidase activity.

Assay: The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of the enzyme extract and 0.5 ml of one per cent H_2O_2. The reaction mixture was incubated at room temperature (28±10 °C). The change in absorbance was recorded at 470 nm at 30 sectime interval for up to 3 min in Hitachi U-2900 spectrophotometer. The boiled enzyme preparation served as blank. The enzyme activity was expressed

Table 2. Disease rating scale for the assessment of late blight severity on potato leaves (Henflig, 1979)

Disease severity rating	Disease severity (%)	Description
0	0.0	No lesions
1	10 %	Lesion area less than 10 %
3	10 % and 20 %	Lesion area between 10 % and 20 % of whole leaflet
5	20 % and 30 %	Lesion area between 20 % and 30 % of whole leaflet
7	30 % and 60 %	Lesion area between 30 % and 60 % of whole leaflet
9	Over 60 %	Lesion area over 60 % of whole leaflet

Per cent disease index-PDI (Wheeler, 1969) was calculated using the formula as given below:

$$\text{Per cent Disease Index} = \frac{\text{Sum of individual disease ratings}}{\text{No. of observations assessed}} \times \frac{100}{\text{Maximum disease rating}}$$

as change in the absorbance at 420 nm $min^{-1}g^{-1}$ on fresh weight basis.

Assay of polyphenol oxidase (PPO)

The polyphenol oxidase activity was determined as per the procedure (Mayer *et al*., 1965).

Reagents:

Phosphate buffer, 0.1 M, 7.0 pH: **Solution A**: 27.6 g of sodium phosphate monobasic (NaH_2PO_4. $2H_2O$ Mol.Wt.-156.01 g) was dissolved in small quantity of water and the volume was made up to 1000 ml with distilled water.

Solution B: 28.4 g of sodium phosphate dibasic (Na_2HPO_4 Mol. Wt. 142 g) was dissolved in small quantity of water and volume was made up to 1000 ml with distilled water.610 ml of solution A was mixed with 390 ml of solution B. Finally the pH was adjusted using NaOH solution. The buffer was stored under refrigerated condition.

Catechol, 0.1M (Mol. Wt. 111.011 g): 11.011 g of catechol was dissolved in small quantity of water and the volume was made up to 1000 ml with distilled water.

Preparation of enzyme extract: One gram of leaf sample was homogenized in 5 ml of 0.1M phosphate buffer, pH 7.0 at 4 °C. This mixture was filtered through a 4 layer muslin cloth. The filtrate was centrifuged at 10000 rpm at for 20 minutes at 4 °C. The supernatant was collected and used for estimation of polyphenol oxidase activity.

Assay: One gram of leaf sample used for phenol oxidase estimation: The reaction mixture consisted of 1.5 ml of 0.1 M sodium phosphate buffer (pH 7.0) and 500 µl of the enzyme extracts. To start the reaction, 500 µl of 0.1 M catechol was added. The change in absorbance was recorded by using spectrophotometer at 495 nm. The polyphenol oxidase activity was expressed as change in absorbance at 495 nm $min^{-1}g^{-1}$ fresh weight of leaf sample.

Assay of Phenylalanine Ammonia Lyase (PAL)

PAL activity was determined as the rate of conversion of L-phenyl alanine to trans-cinnamic acid at 290 nm as per the method (Ross and Senderoff, 1992).

Reagents: **Borate buffer, 0.1M, 8.8 pH**: 6.183 g of boric acid and 1 g of NaOH was dissolved in 800 ml of water and the volume was made up to 900 ml. To this solution 0.1 g of polyvinyl pyrrolidine (PVP) was added.

Substrate solution: L-Phenyl alanine, 12 mM: 1.98 g of L- phenylalanine was dissolved in 1000 ml of distilled water. The solution was prepared every time freshly.

Trans-cinnamic acid: The 29.64 mg of trans-cinnamic acid was dissolved in 10 ml of acetone. Totally 100 µl of this solution was diluted to 10 ml with borate buffer to obtain 2 µl trans-cinnamic acid/ml working standard solution. The buffer was stored under refrigerated condition.

Trichlro acetic acid (TCA, Mol. Wt. 163.39 g), 1 M: 16.339 g of TCA was dissolved in 100 ml of water.

Preparation of enzyme extract: one gram of leaf sample homogenised with 5 ml of 0.1 M ice cold sodium borate buffer (pH 8.8). The homogenate was filtered through a 4 layered muslin cloth. The filtrate was centrifuged at 15000 rpm for 20 min at 4 °C. The supernatant was collected and used for estimation of PAL activity.

Assay: Samples containing 0.4 ml of enzyme extract were incubated with 0.5 ml of 0.1 M borate buffer, pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30 °C. The reaction was arrested by adding 0.5 ml of 1 M TCA and incubated at 37 °C for 5 min. The blank contains 0.4 ml of crude enzyme extract and 2.7 ml of 0.1 M borate buffer (pH 8.8) and absorbance was measured at 290 nm in spectrophotometer. Standard curve was drawn with graded amounts of cinnamic acid dissolved in acetone. The enzyme activity was expressed as µM of trans-cinnamic acid $min^{-1}g^{-1}$ fresh weight of leaf sample.

Results and Discussion

Synthesis of colloidal silver particles

The results of the synthesized colloidal silver revealed that particles size ranged from 11.17 to 5186 nm in diameter, of which 93.60 per cent particles measuring mean size of 171.8 nm, 5.50 per cent particles measuring mean size of 5186 nm and only 0.90 per cent of particles measuring mean size of 11.17 nm in diameter. The Z-average also called as 'cumulants mean' equivalent to intensity mean of 149.4 nm in diameter (Fig. 1). The colloidal solution with 0.492 Polydispersity index (PdI) indicating the suitability of the methodology applied for assessing the particle size (If the value is more than 1 means,

the methodology is not suitable for analyzing the sample tested). The intercept value of 0.924 indicating the good quality of the colloidal solution (The intercept value below 0.85 indicating the poor scattering property, whereas values more than 1.00 indicating the samples with dust). The results of DLS (Distribution of Light Scattering) analysis of synthesized colloidal silver clearly indicates that, critical parameters of quality like PdI and intercept were well within the range and most importantly mean particle size was more than 100 nm (149.4 nm in diameter) as against pure silver nanoparticles size ranges between 10-100 nm, which were reported to be hazardous to organisms and environment (Asharani *et al.*, 2008). Hence, the colloidal silver synthesized was used for field studies against late blight of potato disease.

Invitro efficacy of colloidal silver against *Phytophthora infestans*

The colloidal silver particles at different concentrations *viz*., 10, 25, 500, 100, 250 and 500 ppm were evaluated under *in vitro* for their efficacy against the *P. infestans*. Among the different concentrations of colloidal silver particles tested under *in vitro*, maximum inhibition of fungal mycelia growth was recorded at 500 ppm concentration (86.13%) which was significantly superior over all other concentrations and 74.81 and 44.57 per cent mycelia inhibition was recorded with 250 ppm and 100 ppm concentration, respectively. Colloidal silver particles below 50 ppm had least inhibitory effect on pathogen (Table 3 and Plate 2). The results from *in vitro* experiment showed that inhibition effect increased with increase in concentration of colloidal silver particle. High density of silver particles at which the solution was able to saturate, and cohere to fungal hyphae to deactivate the fungal growth. Similar studies have been reported (Ouda, 2014) that the treated with 15 mg L^{-1} concentration resulted in about 59.30 and 52.90 per cent inhibition with *Alternaria alternate* and *Botrytis cinerea*, respectively. Hyphal growth rate was studied for *Rhizoctoniasolani, Sclerotinia sclerotiorum* and *Sclerotinia minor* was 12 per cent, 36 per cent, and 41per cent at 7 ppm of silver nanoparticles supplemented medium, respectively (Min *et al*., 2009).

Table 3. Efficacy of colloidal silver compound on themycelial growth of *Phytophthorainfestans*

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

In vitro evaluation of colloidal silver compound provided the preliminary information regarding the efficacy of particular concentration of the compound in a shortest period of time and therefore it serve as a basis for further field assay.

Evaluation of colloidal silver particles against *Phytophthora infestans* causinglate blight of potato under field condition

The results of the field trial conducted during *rabi* are presented in Table 4. The colloidal silver at 500 ppm reduced the late blight severity (17.78 PDI) when compared to untreated control (23.70 PDI), whereas in combination with Dimethomorph 50 % WP @ 0.2 % recorded lowest disease severity (7.70 PDI) which was significantly superior over Dimethomorph 50 % WP alone @ 0.2 % at 60 DAS. Colloidal silver both at 250 and 500 ppm along with Cymoxanil 8 % + Mancozeb 64 % WP @ 0.2 % (11.85 PDI) could not influence the efficacy of Cymoxanil 8 % + Mancozeb 64 % WP @ 0.2 % (10.37 PDI) in reducing the late blight disease at 60 DAS.

At 90 DAS colloidal silver a both at 250 and 500 ppm significantly reduced the late blight disease severity and recorded PDI of 60.74 and 42.22, respectively against untreated control (75.56 PDI). Colloidal silver could not influence the efficacy of Cymoxanil 8 % + Mancozeb 64 % WP @ 0.2 % when combined (37.04 PDI) compared to fungicide alone (37.78 PDI) in reducing the disease severity. Colloidal silver could not influence the efficacy of Dimethomorph 50 % WP when combined @ 250 ppm (51.85 PDI), whereas, @ 500 ppm the efficacy of Dimethomorph 50 % WP was significantly enhanced (48.15 PDI) when compared to Dimethomorph 50 % WP alone @ 0.2 % (53.33 PDI). The per cent reduction of mean PDI was differed significantly when colloidal silver @ 500 ppm was combined with Dimethomorph 50 % WP (43.73 %) when compared to Dimethomorph 50 % WP alone @ 0.20 % (32.84 %), whereas colloidal silver could

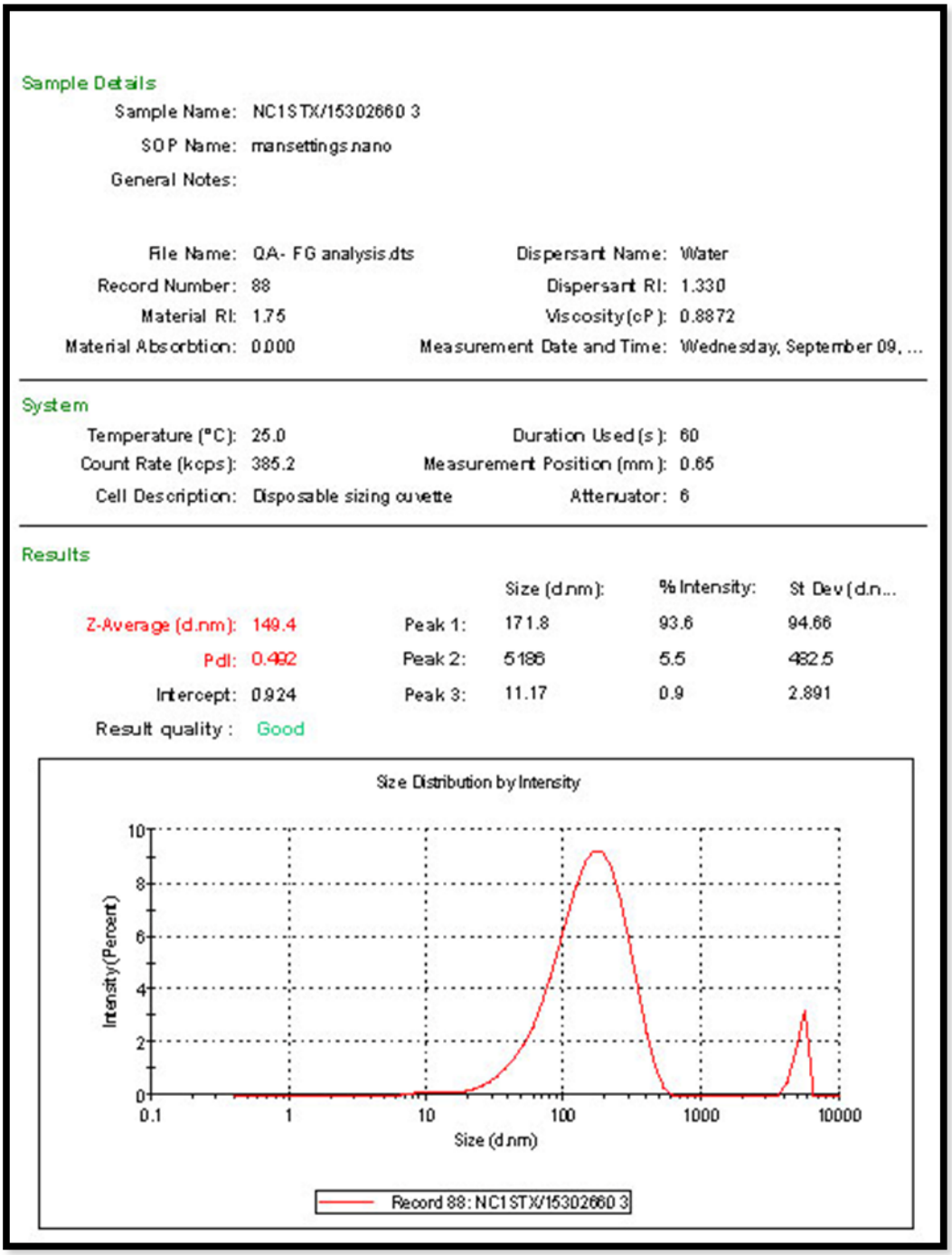

Fig. 1. Distribution of Light Scattering (DLS)- Size distribution report by intensity of colloidal silver

not significantly influence the efficacy of Cymoxanil 8 % + Mancozeb 64 % WP @ 0.2 % (51.49%). Colloidal silver at 500 ppm along with Cymoxanil 8 % + Mancozeb 64 % WP @ 0.2 % recorded highest yield (8.52 tonnes ha^{-1}) when compared to untreated control (4.43 tonnes ha^{-1}).

The results showed that, colloidal silver particles were individually less effective in reducing the late blight of potato than commercial fungicides tested (Cymoxanil 8 % + Mancozeb 64 % WP and Dimethomorph 50 % WP @ 0.2 %). The present findings are contradictory to the results of another work

(Lamsal *et al*., 2012) that is higher inhibitory effect of colloidal silver against *Colletotrichum* as compared to fungicides and an ectophytic powdery mildew pathogen/ disease on cucumber and pumpkin compared to commercial fungicides. This might be due to variation in average size of the silver particles in colloidal solution they used and also due to difference in response of patho system.

The size of the particles reported to be an important criteria and antifungal activity is dependent on particle size. The average size of colloidal silver particles used in the current study were of 149.4 nm as compared to average particle size 7 – 7.5 nm used (Lamsal *et al*., 2011) against *Colletotrichum* spp. and *Gibberellafujikuroi,* a seed borne pathogen (Jo *et al.,* 2015). Probably this could be one of the reasons for low inhibitory effect of colloidal silver particles against late blight of potato under field conditions. Further, the physical incompatibility coupled with interaction between colloidal silver and Cymoxanil 8 % + Mancozeb 64 % WP resulted in inability of colloidal silver to influence the efficacy of Cymoxanil 8 % + Mancozeb 64 % WP in reducing the late blight of potato. Whereas, interaction between colloidal silver with Dimethomorph 50 % WP resulted in increased efficacy of Dimethomorph 50 % WP when combined with colloidal silver @ 500 ppm.

The study was carried out to understand the resistance mechanism induced by application of colloidal silver particles and combination with fungicides. Colloidal silver at two concentrations alone and in combination with two fungicides were evaluated for their efficacy in inducing defense in potato against *Phytophthora infestans*. The activities of peroxidase (PO), polyphenol oxidases (PPO) and phenylalanine ammonia layse (PAL) were analyzed from potato leaf sample infected with *P. infestans* (Fig. 2).

Peroxidase is a key enzyme in the biosynthesis of lignin and suberin. Peroxidases have been associated with a number of physiological functions that may contribute to resistance by depositing the phenolic material in plan cells.

The PO activity reached maximum level at 90 DAS in infected potato plants treated with 500 ppm of colloidal silver particles in combination with Cymoxanil 8 % + Mancozeb 64 % WP at 0.2 per cent (1.5 mg/g) as compared to individual treatments of colloidal silver at 500 ppm (1.2 mg/g) and 0.2 per cent of Cymoxanil 8 % + Mancozeb 64 % WP (1.2 mg/g).

PPO is a copper containing enzyme which oxidizes phenolics to quinines and is concerned to terminal oxidation of diseased plants, which has an accredited role in disease resistance. The observations on PPO activity in late blight infected potato plants treated with colloidal silver in combination with fungicides.

The PPO activity reached maximum levels at 90 DAS in infected potato plants treated with 250 and 500 ppm of colloidal silver in combination with Cymoxanil 8 % + Mancozeb 64 % @ 0.2 per cent (0.01 mg/g) as compared to individual treatments of Cymoxanil 8 % + Mancozeb 64 % at 0.2 per cent (0.007 mg/g) and colloidal silver @ 250 and 500 ppm (0.009 mg/g) concentrations.

PAL is the most important enzyme in phenylpropanoid metabolism for the synthesis of phenolics, phytoalexins and lignin, which in turn impart resistance against infection by pathogens in plants. Effect of colloidal silver application on induction of PAL activity was studied by analyzing the samples of *Phytophthora infestans* affected leaf samples.

The PAL activity reached maximum level at 90 DAS treated with 500 ppm of colloidal silver particles in combination with Cymoxanil 8 % + Mancozeb 64 % WP @ 0.2 per cent (1.5 mg/g) compared to 250 and 500 ppm of colloidal silver particles in combination with 0.2 per cent of Dimethomorph 50 % WP (1.4 mg/g) but the differences were non-significant.

The substantial increase in the PO, PPO and PAL was observed at early stages of disease development in infected potato leaves treated with colloidal silver alone and in combination with fungicides, which indicating that a strong oxidative burst occurred and defense response might involve in lignin production and cell wall fortifications and the combined up regulations of PO, PPO and PAL leading to formation of barriers for pathogen.

The silmilar findings (Sendhil Vel, 2003) were observed that high activity of PO, PPO, PAL, β-1, 3 glucanase, chitinase and total phenols in azoxystrobin treated grapevine plants. The systemic fungicide probenazole treatment induced PO, PPO, PAL, tyrosine ammonia lyase and catechol- o-methyl transferase accumulation in the treated leaves, which indicated that, the disease controlling effect of azoxystrobin and probenazole was attributed to a host mediated reaction (Hewitt, 1988).

PO and β-1, 3 glucanase are related to crosslinking of cell wall components, polymerization of lignin and suberin monomers and subsequent resistance to pathogen in several host pathogen interactions (Ross and Senderoff, 1992). The mustard leaves sprayed with BTH (benzothiadiazole) three days prior to inoculation with *Albugo candida* showed elevated levels and enhanced activity of PO at 11 days after inoculation (Kaur and Kolte, 2001). Earlier, several workers have shown PO enzyme involved in lignin biosynthesis, production of toxic quinones and phytoalexins with the onset of resistance (Glazener, 1982, Hammerschmidt *et al*., 1982 and Daayf, *et al.*, 1997).

Similar studies were reported (Anand *et al.*, 2007) the activity of defense enzymes peroxidase (PO), polyphenol oxidase (PPO),phenylalanine ammonia lyase (PAL), β -1, 3 glucanase, chitinase, catalase and defense-inducing chemicals (total phenols) was found to be increased in azoxystrobin and *Pseudomonas fluorescens* treated tomato plants. The activity of these defense enzymes and chemicals was higher in azoxystrobin (125 g a.i. ha^{-1}) and *P. fluorescens* treated tomato plants challenge inoculated with the pathogens as compared to other treatments. Increased expression of specific isoforms of PO and PPO was also observed due to ISR induction. The effect of non-conventional chemical *viz*., salicylic acid, zinc sulphate, magnesium sulphate, indole acetic acid, indole butyric acid with fungicide (Carbendazim). Results revealed that, increase in total phenol, flavonol, tannin and electrolyte leakage of red fruits of chilli varieties against *Colletotrichumcapsici,* the causal agent of fruit rot of chilli (Geat, 2016).

Conclusion

Management of fungal diseases of food crops is economically important. Recently, a greater effort has been given to development of safe management methods that cause less danger to humans and animals with a focus on overcoming deficiencies of synthetic fungicides. In the present work it was demonstrated that, Colloidal silver particles confirms that in addition to antimicrobial action against pathogen

Table 4. Inhibitory effect of colloidal silver particles and its combinations with fungicides on disease severity of late blight of potato during *rabi* season

Treatments	Per cent disease index (PDI)		Mean PDI	Mean PDI reduction over control	Yield (tonne/ ha)
	60 DAS	90 DAS			
T_1: Colloidal silver @ 250 ppm	20.74 (27.09)	60.74 (51.20)	40.74	17.91	7.01
T_2: Colloidal silver @ 500 ppm	17.78 (24.94)	42.22 (40.52)	30.00	39.55	7.33
T_3: Colloidal silver @ 250 ppm + Cymoxanil 8 % + Mancozeb 64 % WP @ 0.2 %	11.85 (20.12)	37.04 (37.48)	24.45	50.75	7.73
T_4: Colloidal silver @ 500 ppm + Cymoxanil 8 % + Mancozeb 64 % WP @ 0.2 %	11.85 (20.12)	37.04 (37.48)	24.45	50.75	8.52
T_5: Cymoxanil 8 % + Mancozeb 64 % WP @ 0.2%	10.37 (18.76)	37.78 (37.93)	24.08	51.49	7.73
T_6: Colloidal silver @ 250 ppm + Dimethomorph 50 % WP @ 0.2 %	17.04 (24.37)	51.85 (46.06)	34.45	30.60	7.04
T_7: Colloidal silver @ 500 ppm + Dimethomorph 50 % WP @ 0.2 %	7.70 (8.15)	48.15 (43.94)	27.93	43.73	7.46
T_8: Dimethomorph 50 % WP @ 0.2 %	13.33 (21.41)	53.33 (46.91)	33.33	32.84	6.55
T_9: Control	23.70 (29.13)	75.56 (60.39)	49.63		4.43
S E. m±	1.53	1.36			0.13
CD (5 %)	**4.57**	**4.07**			**0.39**

DAS- Days After Sowing

* Figures in the parenthesis are angular transformed values**3.4 Biochemical analysis in colloidal silver treated potato plants infected with *Phytophthorainfestans***

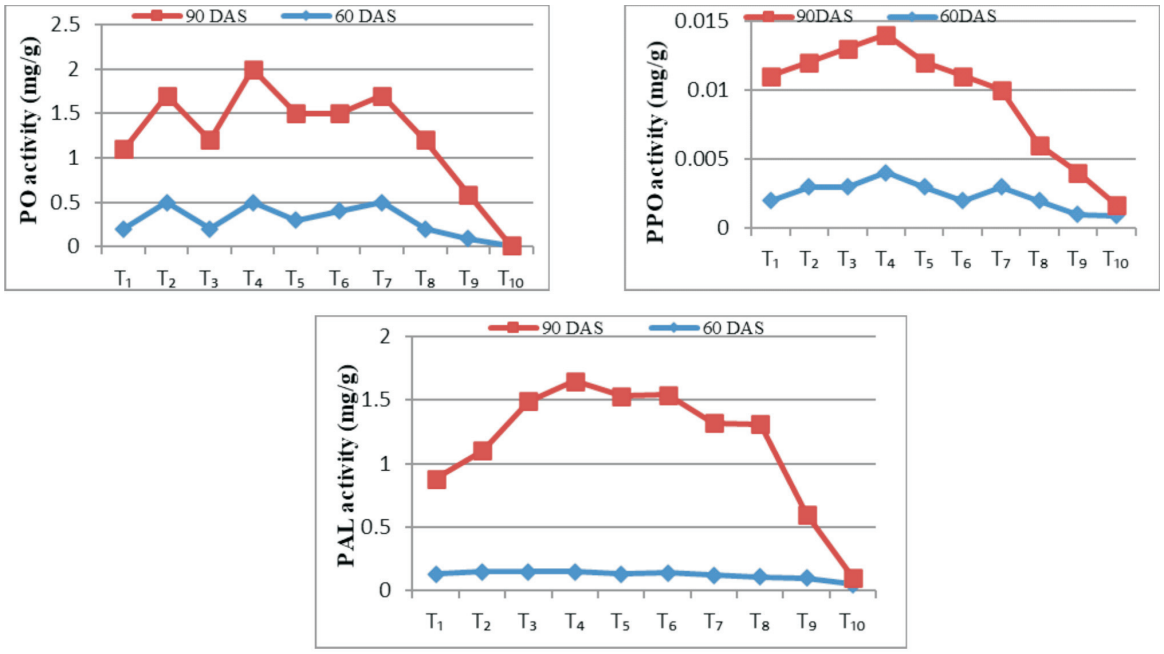

Fig. 2. Biochemical changes in potato leaves infected with *Phytophthora infestans* after application of colloidal silver and in combination with fungicides

T_1: Colloidal silver @ 250 ppm
T_2: Colloidal silver @ 500 ppm
T_3: Colloidal silver @ 250 ppm + Cymoxanil 8 % + Mancozeb 64% @ 0.2%
T_4: Colloidal silver @ 500 ppm + Cymoxanil 8 % + Mancozeb 64% @ 0.2 %
T_5: Colloidal silver @ 250 ppm + Dimethomorph 50 % WP @ 0.2 %
T_6: Colloidal silver @ 500 ppm + Dimethomorph50 % WP @ 0.2%
T_7: Cymoxanil 8 % + Mancozeb 64% @ 0.2 %
T_8: Dimethomorph50 % WP
T_9: Control
T_{10}: Healthy

also involved in the activation of defense responses in plants. This report have generated the data for understanding the biochemical changes in colloidal silver treated plants. The knowledge on plant defense related enzymes can definitely beneficial for the development of new control strategies. Thus, to know the specific effects of colloidal silver particles needs to be tested on different crops against different diseases. Moreover, this report opens up for further research on the areas of understanding the size of the colloidal particles mainly influence their antimicrobial property and other physicochemical properties. Hence, future studies on standardization of size of colloidal silver particles and itsmode of action on the phytopathogenic fungi and bacteria, and impact on plant metabolism could help us to use nanoparticles more wisely in agriculture.

Conflict of interest: Resil Chemicals Pvt. Ltd, Bengaluru provided financial assistance and colloidal silver particles to conduct the research experiments in collaboration with UAS, GKVK, Bengaluru.

References

Anand, T., Chandrasekaran, A., Kuttalam, S., Raguchander, T. and Taniguchi, N. V. 2007. Association of some plant defense enzyme activities with systemic resistance to early leaf blight and leaf spot induced in tomato plants by azoxystrobin and *Pseudomonas fluorescens*. *Proc. Inter. Conf. Prod. Eng. Tolcyo.* 11 : 18-23.

Daayf, F., Schmitt, A. and Belenger, R. R. 1997. Evidence of phytoalexins in cucumber leaves infected with powdery mildew following treatment with leaf extracts of Reynoutriasachalinesis. *Pl. Physiol.* 113 : 719 - 727.

Geat, N., Singh, D. and Khirbat, S. K. 2016. Effect of non-conventional chemicals and synthetic fungicide on biochemical characteristics of chilli against fruit rot pathogen *Colletotrichumcapsici*. *J. Pl. Pathol. Microbiol.* 7 : 1.

Glazener, J. A. 1982. Accumulation of phenolic compounds in cells and formation of lignin-like polymers in cell walls of young tomato fruits after inoculation with *Botrytis cinerea*. *Physiol. Mol. Pl. Pathol.* 20 : 11–25.

Hammerschmidt, R. Nuckles, E. M. and Kuc, J. 1982. Association ofenhanced peroxidase activity with in-

duced systemic resistance of cucumber to *Colletotrichumlagenarium*. *Physiol. Pl. Pathol*. 20 : 73 - 82.

Hartee, E.F. 1955. Haematin Compounds. In: *Modern Methods of Pl. Analysis.* pp 197-245

Hatami, M. and Ghorbanpour, M. 2014. Defense enzyme activities and biochemical variations of *Pelargonium zonale* in response to nanosilver application and dark storage. *Turk J. Biol*. 38 : 130-139.

Henflig, W. 1979. Late blight of potato. *Tech. Information Bulletin*, pp. 13.

Hewitt, H.G. 1988. Systemic acquired resistance. *Fungicides in Crop Protect.* pp. 140 -145.

Jo, Y. K., Cromwell, W., Jeong, H. K., Thorkelson, J., Roh, J. H. and Shin, D. B. 2015. Use of silver nanoparticles for managing *Gibberellafujikuroi* on rice seedlings. *Crop Protect*. 74 : 65-69.

Kreuter, J. 1994. Nanoparticles. *Colloidal Drug Delivery Systems,* pp. 219-277.

Kaur, A. and Kolte, S. J. 2001. Protection of mustard plants against the stag head phase of white rust by foliar treatment with benzothiadiazole, an activator of plant defense system. *J. Mycol. Pl. Pathol*. 31(2) : 133-138.

Lamsal, K., Kim, S. W., Jung, J. H., Kim, Y. S., Kim, K. S. and Lee, Y. S. 2011. Inhibition effects of silver nanoparticles against powdery mildews on cucumber and pumpkin. *Mycobiol.* 39(1) : 26-32.

LAmsal, K., Kim, S. W., Jung, J. H., Kim, Y. S. K. S. and Lee, Y. S. 2012. Application of silver nanoparticles for the control of *Colletotrichum* species *in vitro* and pepper anthracnose disease in field. *Mycobiol.* **39**: 194-199.

Mayer, A. M., Harel, E. and Shaul, R. B. 1965. Assay of catechol oxidase, a critical comparison of methods. *Phytochem.* 5 : 783-789.

Min, J. S., Kim, K. S., Kim, S. W., Jung, J. H., Lamsal, K., Kim, S. B., Jung, M. and Lee, Y. S. 2009. Effects of colloidal silver nanoparticles on *Sclerotium* forming phytopathogenic fungi. *Pl. Pathol. J.* 25 4: 376-380.

Ouda, S. M. 2014. Antifungal activity of silver and copper nanoparticles on two plant pathogens, *Alternaria alternate* and *Botrytis cinerea. Res. J. Microbiol*. 9(1) : 34-42.

Park, H. J., Kim, S. H., Kim, H. J. and Choi, S. H. 2006. A new composition of nanosized silica-silver for control of various plant diseases. *Pl. Pathol. J.* **22:** 295–302.

Reuveni, M., Agapov, V. and Reuveni, R. 1995. Induced systemic protection to powdery mildew in cucumber plants by phosphate and potassium salts. *Environ.Sci. Technol.* 23 : 73–78.

Ross and Senderoff. 1992, *Induction of defense enzymes and phenolic content*. *Ph.D Thesis, Tamil Nadu Agril. Univ., Coimbatore, India*, pp. 192.

Sendhil Vel, V. 2003. Evaluation of azoxystrobin 25 SC against downy mildew and powdery mildew of grapevine. *Ph.D Thesis, Tamil Nadu Agril. Univ., Coimbatore, India*, pp. 190.

Taylor, A. C. 2011. Adhesives with nanoparticles. *Handbook of Adhesion Technol.* 1437-1460.

Vincent, J. M. 1927. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature*. 159 : 850.

Wheeler, B. E. J. 1969. *An Introduction to Plant Diseases,* John Wiley and Sons Limited, London, pp. 301.