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Evaluation of Silver Nanoparticles for the Control of *Phragmidium* Species in Vitro and Taif rose Rust Disease in Field.

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ABSTRACT

Rose rust caused by Phragmediumsp is one of the most important diseases adversely affecting its yield in Saudia Arabia. In this study, Identity of the causal agent fungus was confirmed by LSU rDNA sequencing and identified as Phragmidiummucronatum. Also, the present study is focused on the extracellular synthesis of silver nanoparticles (AgNPs) using culture supernatant of an agriculturally important bacterium, Proteus maribials and demonstrates its effective application for the management of rust disease in rose. The biosynthesis of AgNPs by Proteus maribials was monitored by UV–visible spectrum that showed the surface plasmon resonance (SPR) peak at 406 nm, an important characteristic of AgNPs. Further characterization of synthesized AgNPs carried out using the X-ray diffraction (XRD), transmission electron microscope (TM) and FTIR spectroscopy, respectively. The AgNPs were spherical in shape with size range of ~10 to 20 nm. The XRD analysis confirmed successful biosynthesis and crystalline nature of AgNPs. The AgNPs exhibited strong antifungal activity against P. mucro, the rust disease pathogen of rose. Interestingly, all concentrations of AgNPs (0.25, o.5 and 1 mM) used displayed a strong inhibition of urediospore germination, 3.05, 2.2, and 1.1% respectively, whereas in the absence of AgNPs, urediospore germination was 62.32 %. The results were further tested under field conditions, where application of AgNPs significantly reduced P.mucronatuminfection in rose plants. The averages of disease severity were reduced from 3.6 in non-sprayed plants to 1.06 % in case of spraying with AgNPs (1mM). In summary, our findings represent the efficient application of AgNPs in rust disease management, therefore, it is recommended to consider biological synthesized silver nanoparticles in further studies for possible controlling of rose rust disease.

Keywords: Phragmidiummucronatum, silver nanoparticles, Rose rust disease.

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INTRODUCTION

Crop production is reduced worldwide every year due to phytopathogenic fungi which caused plant disease; therefore, a lot of efforts have been done to control these plant diseases. Various natural and artificial methods have been applied for protection of plants from these diseases. Among these methods, using of synthetic pesticides is the most prevalent way to control the plant diseases. In recent years, environmental hazards caused by excessive use of pesticides have been paid attention; therefore, researchers in the plant protection field are searching for alternative methods rather than using pesticides. As an alternative to chemically manufactured pesticides, use of silver nanoparticles as antimicrobial agents has become more promising technology for plant protection against fungal diseases [1].

The use of nano-sized silver particles as antimicrobial agents has become more common as technological advances make their production more economical. One of the potential applications in which silver can be utilized is in management of plant diseases. Since silver displays multiple modes of inhibitory action to microorganisms [2, 3] it may be used for controlling various plant pathogens in a relatively safer way compared to synthetic fungicides [4]. Until now, limited research provided some evidence of the applicability of silver for controlling plant diseases. [5] demonstrated a successful evaluation of the antifungal activity of three different forms of silver nanoparticles against ambrosia fungus *Raffaelea* sp. Likewise, successful reduction of sclerotium-forming fungi was achieved in a dose-dependent manner when silver nanoparticles (AgNPs) were used [1,6].The antifungal effectiveness against rose powdery mildew using antimicrobial nanosilver colloidal solution was investigated [2,7]. They concluded that the photographic results showed that the effects of nanosilver colloidal solution against rose powdery mildew were very high and durable for a week. In addition, the nanosilver did not have phytoxicity about the plants cell of leaves, stem and bud of roses [2,9] investigated the effectiveness of various forms of silver ions and silver nanoparticles on two plant-pathogenic fungi, *Bipolarissorokiniana* and *Magnaporthegrisea*. In vitro petri dish assays indicated that silver ions and nanoparticles had a significant effect on the colony formation of these two pathogens [8].

Krishnaraj et al. examined the effect of silver nanoparticles on plant pathogenic fungi, *Alternariaalternata, Sclerotiniasclerotiorum, Macrophominaphaseolina, Rhizoctoniasolani, B. cinerea* and *Curvularialunata* and found that 15 mg L^{-1}concentration of NPs greatly inhibited the activity of all the tested pathogens [9]. The zinc nanoparticles (25 mg mL^{-1}) suppressed the colonization of *Aspergillusflavus* . The zinc nanoparticles (25 mg mL^{-1}) suppressed the colonization of *Aspergillusflavus* [10].Although, colloidal silver nanoparticles have been applied for controlling various phytopathogenic diseases [9,11], this is the first report for using silver nanoparticles against rose rust diseases caused by *Phragmidiummucronatum* in the field .Furthermore, all of nanosilver colloid have been used for controlling plant fungal diseases were synthesized chemically that cause environmental concern. The objectives of this study were to determine the inhibitory property of biological synthesized silver nanoparticles on colony formation of plant-pathogenic fungi, and to evaluate the efficacy of the silver compounds for rust disease control on rose plants.

MATERIAL AND METHODS

Biosynthesis of silver nanoparticles

Biosynthesis of silver nanoparticles was conducted according to Bhainsa and D'Souza with some modifications [12]. To obtain stable silver nanoparticles, *Proteus mirabilis* grown in a 500 mL Erlenmeyer flask containing nutrient broth. The flasks were incubated for 24 h in a shaker set at 120 rpm and 28 °C. After the incubation period, the culture was centrifuged at 10,000g and 2 g of biomass (wet weight) was brought into contact with 100mL sterile double-distilled water for 48 h at 28 ◦C in an Erlenmeyer flask and was shaken at 200 rpm. After incubation, the cell filtrate was obtained by centrifugation. The cell filtrate was used for the synthesis of silver nanoparticles. For synthesis of silver nanoparticles (AgNPs); silver nitrate was mixed with 100 mL of cell free filtrate to obtain a final concentration of 1 mM silver ions. The resulting solution was incubated at 37 °C for 24 h in dark. The extracellular synthesis of AgNPs was monitored by visual inspection of the filtrate for a change in the color of culture medium from a clear, light-yellow to brown, and by measurement of the peak exhibited by silver nanoparticles in the UV-Vis spectra

Characterization of silver nanoparticles

Samples for transmission electron microscopy (TEM) analysis were prepared on carbon-coated copper TEM grids. Studies of size, morphology and composition of the nanoparticles were performed by means of transmission electron microscopy (TEM) operated at 120 kV accelerating voltage (JTEM-1230, Japan, JEOL) with selected area electron diffraction (SAED). Finally, the obtained images were processed using the software Image J. Image J developed at the National Institutes of Health (NIH), USA is a Java-based public domain image processing and analysis program [13].

Causal agent pathogen identifications:

About 20 g of fresh urediniospores of *Phragmidium*sp were collected from rose rust uredinia lesions on rose field-infected in May 2015 by a vacuum-trap method [35].

DNA extraction

Total DNA was extracted from urediniospores using the DNeasy Plant Mini Kit (Qiagen,Hilden) according to the manufacturer's protocol. The protocol was modified by shaking the dried samples with the help of a mixer mill (MM 300, Retsch, Haan) for 3 min at 30 Hz in a 1.5 ml tube together with one tungsten carbide ball (3 mm diam).

DNA amplification

The 5k end of the nuclear 28S rRNA gene (nrLSU) was amplified from diluted extracts (10x1 and 10). Primers for the amplification were LR0R (5k-ACC CGC TGA ACT TAA GC) described by [13] and LR6 (5k-CGC CAG TTC TGC TTA CC) described by [15]. PCR conditions consisted of an initial denaturation at 94 xC for 180 s, 12 cycles of 94 x for 35 s, 45 x for 45 s, 72 x for 60 s, 12 cycles of 94 x for 35 s, 45 x for 55 s, 72 x for 90 s, 12 cycles of 94 x for 35 s, 45 x for 60 s, 72 x for 120 s and a final elongation of 72 x for 10 min.

DNA Sequence

The amplified DNA was purified using Qiaquick PCR purification kit (Qiagen) following the manufacturer's instructions, and was then sequenced directly in both directions. Cycle-sequencing was performed using theABI PRISM Big DyeTM Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Warrington) or the Thermo Sequenase labelled primer cycle sequencing Kit (Amersham Pharmacia, Uppsala) with the unlabelled or IRD-labelled primers NLMW1 (5k-TCA ATA AGC GGA GGA AAA GA; [15] and NL4 (5k-GGT CCG TGT TTC AAG ACG G; [16]. The cycle sequencing profile was 25 cycles of 96 x for 10 s, 50 x for 5 s, and 60 x for 4 min. The resulting DNA fragments were separated on an acrylamide gel, using an automatic LI-COR DNA sequencer 4000L or an ABI 373A Stretch (PE Applied Biosystems, Foster City, CA).

Disease assessment:

Rose Plants were left for natural infection. The evaluation of leaf rust severity was assessed as mean of number of uredinia of 7^{th}, 8^{th} and 9^{th} leaves of 50 plants of each plot. Rose plants was performed only once, 7 days after completing a series of treatments, using a conventional 5-degree classification scale: (0) no uredinia; (1), up to 5 uredinia on the whole leaf; (2), on the average 1–5 uredinia on one leaflet; (3) on the average 6–20 uredinia on one leaflet; (4) above 20 uredinia on one leaflet [18].

Field experiments

Field trials and data analysis

To determine the efficacy of silver nanoparticles against rust disease in the field,A field (1/2 fed.) location in El Shafa farm, TaifGovernorate, KSA cultivated by Rose plants (*Rosa damascena* Miller var. *trigintipetala*Dieck) was chosen to carry out this experiment during the agricultural seasons (2014 and 2015). This location was chosen because its long history of Leaf rust epidemic on rose plants. Field was divided into 5 plots, every plot contained at least 5 rows (replicates), and every row contained at least 20 plants. Every plot

was sprayed by treatment agents when the infection rate of the disease ranging from 1-2%. Silver nanoparticles were used at 0. 25, 0.5, and 1 mM simultaneously. The silver nanoparticles were applied on the leaves of infected plant. The commercial fungicide 'Eminent,' which contains Tetraconazole fungicide 16%, 1 mlL^{-1} Eminent, Sipcam, and $AgNO_3$were used as positive controls. Distilled water was used as a negative control. Every plot was sprayed with 10 liters water solution of tested agents and then repeated after ten days. Disease incidence (%) was calculated by counting the numbers of infected leaves out of 150 leaves among the treated plants in field trials. Each experiment was repeated three times.

Uredospore germination

The mature uredospores of *Phragmidiummucronatum*were collected by placing a drop of water on a pustule and then lifting the floating ones with a needle. Silver nanoparticles were prepared under aseptic conditions. The urediniospores suspension was prepared separately in sterile water to obtain 4×10^8 urediniospores per ml. Then a drop of a spore suspension was mixed with one drop of each concentration of AgNPs, and fungicidal solution in a cavity slide to achieve the required concentration. In each treatment three replications were maintained. The slides were then incubated at various temperatures for different intervals of time. The observation on spore germination was recorded 12, 24 and 36 h after incubation under microscope at 40 X magnification. An untreated control treatment was maintained with sterile water. Percent urediniospores germination was calculated by following formula [28].

Per cent spore germination (PG)= A\B*100

Where, A = No. of uredospores germinated; B = No. of uredospores observed.

Statistical analysis

Data were statistically analyzed by analysis of variance (ANOVA), by using statistical analysis cycle (SAS) followed by Duncan's multiple range test [19] at (P≤0.05).

RESULTS AND DISCUSSION

Characterization of Biosynthesis of silver nanoparticles

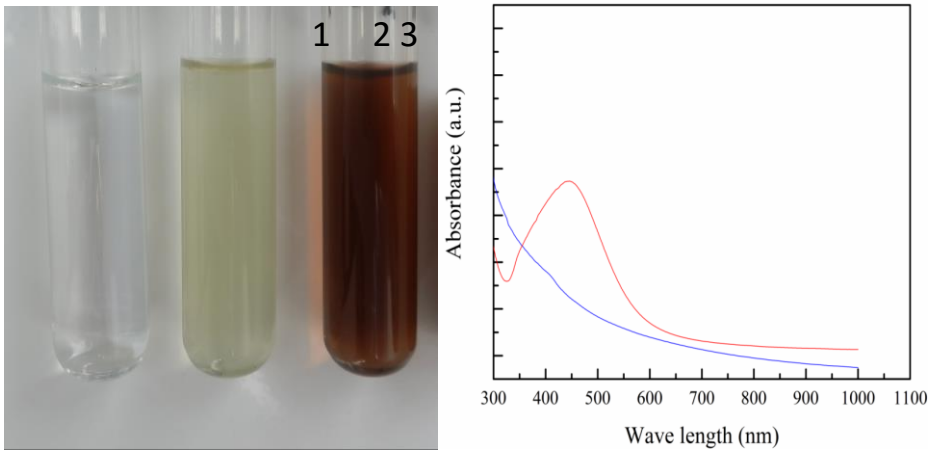

Fig 1: Inset (a) digital photograph of test tubes containing the bacteria *Proteusmariabils* supernatant reacted with aqueous solution of 1 mMAgNPs. It is observed that the color of the solution turned from colorless to brown after 12 hr (tube 3) of the reaction at 30 *o*C, indicating the formation of AgNPs. Blank control, (tube 2) containing supernatant only, and (tube 1) containing $AgNO_3$ only.

(b) The UV-Vis spectra illustrated in Figure 1b, show a well-defined absorption peak corresponds to the wavelength of the surface plasmon resonance (SPR) of AgNPs at 440 nm.

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Visual observation of the culture supernatant incubation with silver nitrate at room temperature in dark showed a color change from light yellow to yellowish brown whereas no color change could be demonstrated in culture supernatant without silver nitrate or media with $AgNO_3$ alone (Fig 1a). The characteristic yellowish brown color of colloidal silver solutions is due to excitation of surface plasmon vibrations in the nanoparticles and provides a convenient spectroscopic signature of their formation. Nanoparticle formation was initially visibly monitored by color change colourless to yellowish brown in the case of Ag [20].

Various reports have established that the resonance peak of silver nanoparticles appears around this region, but the exact position depends on a number of factors such as particles size, and the surface-adsorbed species [21, 22]

TEM analysis

TEM image of Protein-capped AgNPs are shown in Figure 2a. The sample showed mainly spherical, monodispersed nanoparticles without any agglomeration. The particle size histogram is shown in Figure 2 b. The average particle size is 13 nm, while. The X-ray diffraction pattern of the silver nanoparticles is shown in Figure 4. Indexing process of powder diffraction pattern is done and Miller Indices (h k l) to each peak is assigned as shown in the Figure 3. A number of strong Bragg reflections can be seen which correspond to the (111), (200) and (220) reflections of crystalline

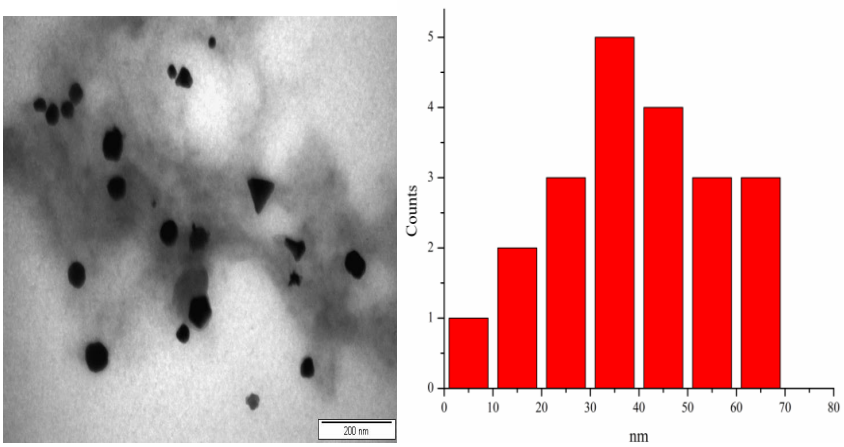

Fig 2: (a) TEM image and (b) particle size distribution of Silver Nanoparticles (AgNPs) Synthesized using *Proteus mariabils*

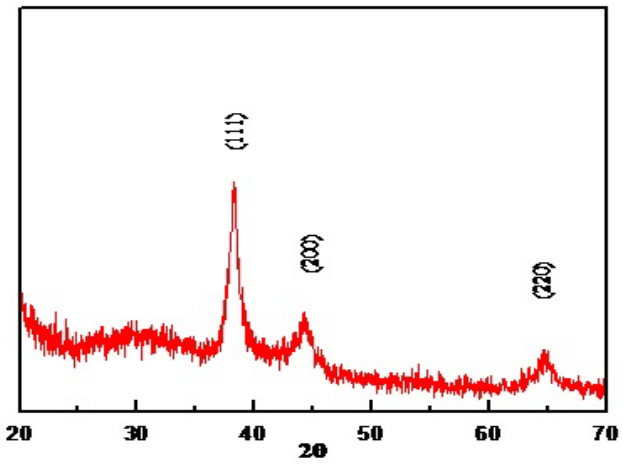

Figure 3: Representative XRD pattern of AgNPs synthesized by the reaction of 1 mM $AgNO_3$ solution with *P. mirabilis* supernatant s at pH 7.

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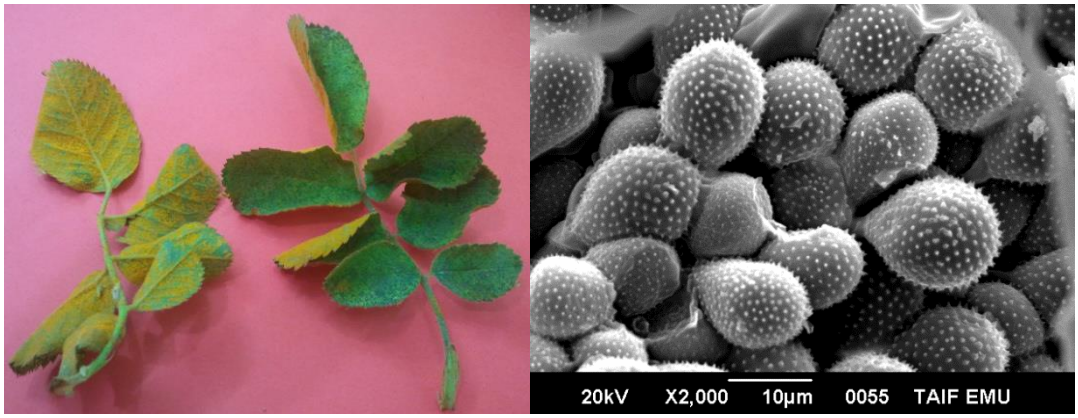
Face center cubic structure (fcc) (JCPDS, File No. 00-001-1164). No spurious diffractions due to crystallographic impurities are found. All the reflections correspond to pure silver metal with face centered cubic symmetry. The intensity of peaks reflected the high degree of crystallinity of the silver nanoparticles. However, the diffraction peaks are broad which indicating that the crystallite size is very small [22].

Characterization of rose rust disease and Causal agent pathogen identifications

*Rosa damascena*is considered one of the most important commercial crops in Taif region in Saudi Arabia. Since three centuries, about 2000 farms have been cultivated at high altitude (Lat. 99° 44/and 79°45/ E) with rose that has been processed into rose oil. During the course of a disease survey, a leaf rust was observed on different farms roses in early March 2014 (Fig.4a). The disease occurred on all green parts of the plant, but mainly abundant on the lower surface of the leaves. Symptoms were orange pustules of urediospores (Fig.4a). Uredinahypophyllous(Fig 4b) were scattered or gregarious, sometimes scattered over the whole leaf surface, minute, pulverulent, pale yellow; paraphysesclavate or cylindrical 52.5-75.5 X 15.4-21.8 µm, wall 1.6-2.1 µm, pale yellow, closely echinulate, germ pores numerous, scattered [24]. The disease severity ranging from 3.5-3.7 using scale as described by [18] on 7th, 8th and 9th leaves. The disease incidence ranged between 90-95 %. Identity of the causal agent fungus was confirmed by LSU rDNA sequencing. The strain was identified as *Phragmidiummucronatum* (accession No. KJ867552) with identity percentage of 99% with the other *P.mucronatum* from Oman [25] To the best of our knowledge, this is the first report on the rust fungus *P. mucronatum* on *Rosa damascena* at Taif, Saudia Arabia.



(a) (b)

Fig (4): Showing that (a) leaf rust smptoms (b) SEM of Urediniospores with pustule on infected rose plants.

Effect of silver nanoparticles against rust disease in rose under field condition

The results in Table (1) Indicated that three spraying of infected plant with different concentrations of AgNPs(0.25,0.50 and 1mM), fungicide Eminent16 % and$AgNO_3$ as a positive control Score reduced leaf rust disease in compared with water control, during growing seasons of 2014 and 2015 . Final determination of the disease severity showed that three sprays gave the best results in management the disease (Table 1). The averages of disease severity were reduced from 3.6 in non-sprayed plants to 1.9, 1.6, and 1.01 in case of spraying with different concentration of AgNPS; 0.25, 0.5, and 1 mm, respectively. However, The averages of disease severity were reduced in case of spraying with $AgNO_3$ as positive control to 2.3 in compared with water control. Furthermore, in case of spraying with Eminent16%the averages ofdisease severity was reduced to 0.81 (Table 1). These results concluded that Score fungicide and AgNPswas the best treatment followed by $AgNO_3$ in descending order, and all treatments led to great reduction to the disease comparing to non-treated plants. [38].

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Table 1: Effect of different treatments by Ag+*Nanoparticle* on leaf rust disease of rose plants under field condition during two successive growing seasons,.2014 and 2015.

Treatments	Leaf rust severity								
	2014			2015			Average of the two years		
	1st spray	2nd spray	3rd spray	1st spray	2nd spray	3rd spray	1st spray	2nd spray	3rd spray
$AgNO_3$ 1mM	2.9^{bA}	2.5^{bA}	$2.1^{\mathbf{bB}}$	3^{bA}	2^{cB}	2.5^{bA}	2.95	2.25	2.3
AgNPs 0.25mM	2.5^{cA}	2.3^{bA}	$2^{\mathbf{bB}}$	2.5^{cA}	2.3^{bA}	1.8^{cB}	2.5	2.3	1.9
AgNPs 0.5 mM	2^{dA}	1.8^{cB}	$1.7^{\mathbf{bB}}$	2.1^{dA}	2^{cA}	1.5^{cB}	2.05	1.9	1.6
AgNPs 1 mM	1.8^{dA}	1.6^{cA}	$1.1^{\mathbf{cB}}$	2^{dA}	1.5^{dB}	0.96^{dC}	1.9	1.55	1.03
Eminent16%	1.5^{eA}	1.4^{dA}	$0.97^{\mathbf{dB}}$	2^{eA}	1.2^{eB}	0.66^{eB}	1.75	1.3	0.815
Control	3.7^{a}	3.7^{a}	3.7^{a}	3.5^{a}	3.5^{a}	3.5^{a}	3.6	3.6	3.6
MSD	0.28	0.283	0.358	0.358	0.253	0.234	-	-	-
F value	204.8	257.2	216.6	89.8	282.2	246	-	-	-

Means followed by the different small letter in each column and capital letters in each rows are significantly different at 0.05 level of probability

The comparative analysis of silver nanoparticles efficiency to reduced rose rust disease severity was also assessed. By calculating the efficiency of tested AgNPs to reduced rose rust disease severity indicated that AgNPs(1mM) gave 71.36 %, and AgNPs(0.25 and 0.5mM) gave 47.22 and 55.55%, respectively. However, $AgNO_3$ (1mM) gave 36.11 (Table2). These results concluded that silver in nano-size was more effectiveness than silver ions against fungal growth. This can be attributed to the fact that silver nanoparticles have the high specific surface area and high fraction of surface atoms, will have high antimicrobial activity compared to bulk silver metal[26]. Therefore, silver nanoparticles are highly reactive as they generate Ag^+ ions while metallic silver is relatively unreactive [27]. Also, the nanoparticles efficiently penetrate into microbial cell, which implies lower concentrations of silver in nano-sized sufficient for microbial control. A previous study indicates that silver nanoparticles disrupt transport systems including ion efflux [27].In addition, silver ions are known to produce active oxygen species (ROS) via their reaction with oxygen, causing damage to cells proteins, lipids, and nucleic acids [29,30].

Table 2: Efficiency of different treatments leaf rust disease severity on rose plants under field condition during growing season 2014 and 2015.

Treatments	% Efficiency of the tested compounds		
	1st spray	2nd spray	3rd spray
$AgNO_3$ 1mM	18.05	37.5	36.11
AgNPs 0.25 mM	30.55	36.11	47.22
AgNPs 0.5 mM	43.05	47.22	55.55
AgNPs 1 mM	47.22	56.94	71.38
Eminent16%	51.39	63.88	77.36
Control	0	0	0

Effect of AgNPs treatment on Urediniospores germination

The process of infection starts with urediniospore germination on the leaf surface followed by appresorium formation after which hyphae colonizes inter and intracellular leaf tissues[1] Information about environmental effects on urediniospore germination can be used to help interpret the life cycle of specific rust fungi. Thus, the effect of temperature on urediniospore germination, a necessary step in the infection process, can be studied in vitro. In this study, each data point is the average of four samples per two replications (Table 3), the effect of temperature on germination of urediniospore of *P. macronatum* on leaves surface of rose plant indicated that germination varies both in percentage and rate depending on temperature

and time interval. Urediospore germination was maximum within 24 h of incubation, in the temperature range of 15-25°C. The mean maximum spore germination was recorded at 20°C (65.32 % germination). However, slight increase in germination percentage recorded at all temperature levels as the incubation period extended.Similar values were reportedby Mueller and Buck whom reported an optimal temperature of 22–24 ^{o}C for germination of urediniospores of *Pucciniahemerocallidis* (causing daylily rust) [41]. Similar results have been reported for other rusts, *Pucciniasubstriata* var. *indica* (causing pearl millet rust), maximum germination rates were at 20–25 ^{o}C[32]. Moreover, urediniospore germination rates in *Thekopsora minima* (a pathogen of hemlock and ericacious species) were consistent at 20 ^{o}C and 25 ^{o}C and reduced at 30 ^{o}C (Pfister et al 2004). However leek rust (caused by *Pucciniaallii*) was limited by lower temperatures, with the highest percent germination of urediniospores at 12-21 ^{o}C[34].

Efficacy of fungicide Eminent® 16% and AgNPs treatment on urediniospore germination of *P. macronatum* are also presented in Table 3. Eminent® 16% was significantly reduced the urediniospore germination (0.99 %) within 24 h at 25 ^{o}Cas compared to water control (62.32%). Similar results have been reported for Reduction of urediniospore germination of *Pucciniasorghi* in vitro using different fungicides [35].

Table (3): Effect of different treatments by Ag^{+}*Nanoparticle* on urediniospores *germination*

Treatments	% Uredinia Germination			
	Temp.	12h	24h	36h
$AgNO_3$ 1mM	15°C	2.41	6.10	6.9
	20°C	5.23	7.02	7.51
	25°C	3.5	5.32	5.62
AgNPs 0.25 mM	15°C	1.5	3.21	3.5
	20°C	3.15	4.25	5.0
	25°C	1.26	2.91	3.05
AgNPs 0. 5 mM	15°C	1.1	1.76	2.18
	20°C	1.21	2.01	2.5
	25°C	0.90	1.85	2.22
AgNPs 1 mM	15°C	1.0	1.2	1.3
	20°C	1.2	1.6	1.8
	25°C	0.8	1.4	1.1
Eminent® 16%	15°C	0.8	0.95	1
	20°C	0.91	1.2	1.5
	25°C	0.9	0.97	0.99
Distilled water	15°C	25.6	34.26	57.6
	20°C	35.3	55.5 %	65.32
	25°C	25.8	40.5	50.6

On the other hand, the inhibitory effect of AgNPs and $AgNO_3$ as a positive control on germination of *P. macronatum*urediniospores was also tested under in vitro condition. Our results revealed that Silver in ionic or nanoparticle forms significantly reducted of urediniospore germination (Table 3). Interestingly, silver nanoparticles was highly effective against urediniospore germination at all the concentrations tested. Significantly lowest urediniospores germination was recorded in 1 mM (1.01 %) followed by 0.5 mM (2.5%), and 0.25 mM (3.05 %) within 24 h at 25 ^{o}C, as compared to water control (62.32%). However, treatment of urediniospores suspension with $AgNo_3$ (1mM)reduced urediniospores germination (7.5%) compared to water control. We concluded that silver in nano-scale was more effectiveness in urediniospores germination reduction than silver ions(Table 3). Silver nanoparticles may directly attach to and penetrate the cell membrane to kill spores, although penetration of silver nanoparticles into microbial cell membranes is not completely understood [1]

This result was further confirmed after observing microscopic images which showed prominent uredinispore germination on rose leaves inoculated with *P. macronatum*uredinispore suspension alone, while it was inhibited on treatment with AgNPs (Figure 5)

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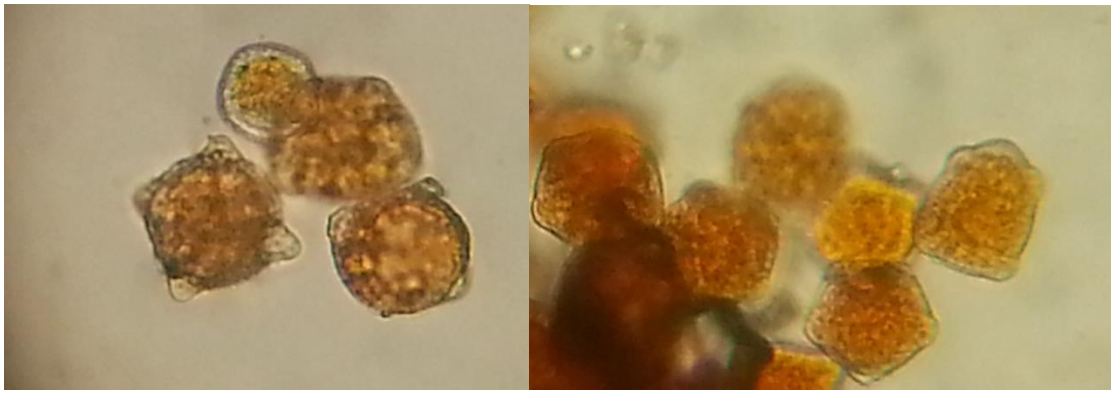

(a) (b)

Fig (5) illustrated that (a) uredinispore germination (water control) (b) uredinispore germination with AgNPs 1 mM after 12 h.

These findings confirm the biocontrol potential of AgNPs against *P. macronatum* under in vitro conditions. Furthermore, field experiment was also confirmedthat application ofAgNPs strongly controlled *P. macronatum* infection in rose (Figure 6).

(a) (b)

Fig (6): (a) Photograph of infected plant before treatment with AgNPs and (b) plant after treatment with 1mMAgNPs (3rd sprays) in field.

CONCLUSION

This study contributes to comparative AgNPs efficacy of biological synthesis of silver nanoparticles against rust disease in rose. It confirms that nano silver is a product effectively active against *P. macronatum*. Silver displays multiple modes of inhibitory action against microorganisms, but it was revealed that silver in nano scale shows a better inhibitory effect on rust disease. However, much works needed for the potential use of silver in control of plant disease include the need for more information on antifungal activity of various silver compounds to plant pathogens and development of better application strategies to increase the efficacy of disease suppression.

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