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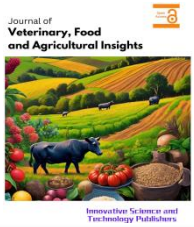

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Research Article

Effectiveness of nano pesticides against *Aschochyta rabiei* causing blight disease in chickpea

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ABSTRACT

Non formulated fungicides and botanical extracts and zinc nitrate formulated plant extracts and fungicides were tested at different concentrations under laboratory conditions. Fungicides gave significantly more control over rest of the treatments during *in vitro* studies. Two fungicides i.e. Alliete and Thiovet$_{Jet,}$ two plant extracts i.e. neem and datura, and zinc nitrate emained significant in their efficacy under laboratory conditions. Alliete and Thiovet$_{Jet}$ @ 150pmm reduced 3.716% and 4.0250% colony growth of *A. rabiei*, respectively while botanical extracts; neem and datura at 50, 100 and 150 ppm concentrations inhibited 4.5667%, 4.3000% and 3.9917% and 4.2167%, 4.1500% and 3.5917% colony growth, respectively. These effective treatments were then tested by formulating with the zinc nitrate nano particles under *in vitro* conditions and proved effective. Under lab conditions, nano-Alliete controlled significantly more disease severity than nano Thiovet$_{Jet,}$ while among formulated plant extracts, nano neem remained significantly more effective than datura which showed significant less effect over ascochyta blight. All nano formulated nano Alliete, nano Thiovet$_{Jet,}$ nano neem and nano datura and zinc nitrate particles alone restricted disease severity to; 5.5583%, 3.8167%, 4.0000%, 3.8667% and 3.7583%, at 150 ppm concentrations, respectively compared to control (6.1750%) under lab conditions.

Keywords: Botanical extracts; Datura; Neem; Nanoparticles

INTRODUCTION

In Pakistan, chickpea is often cultivated as a post moon-soon rotation crop with wheat. This assists in maintaining the soil fertility by enhancing nitrogen availability, and increases water use efficiency (Varshney *et al.*, 2002). Chickpea is the third most vital crop (legume) in the world after peas (*Pisum sativum* L.) and dry beans (*Phaseolus vulgaris* L.) (Pande *et al.*, 2005). Chickpea is cultivated worldwide on 13.5 million hectares with production of 13.1 million tonnes. Pakistan ranks third in the world in chickpea production (FAO, 2013). Chickpea is the largest Rabi crop in Pakistan, mostly cultivated in Barani areas on an area of 977 thousand hectares with yield of 475 thousand tonnes (GOP, 2021).

Chickpea blight or Ascochyta blight (AB) caused by *Ascochyta rabiei* (Pass.) Lab. (teleomorph: *Didymella rabiei*) (Kovachevski) v. Arx, is the most important limiting factor in gram production around the globe (Mahmood *et al.*, 2019). Ascochyta blight disease in severe form may result 100% yield losses (Zikara-Zine and Bouznad, 2007). In Pakistan, this disease is a major threat to chickpea production (Syed *et al.*, 2009). Fungus overwinters in the form of pycnidia in crop disease debris left in field, on seeds and in soil. Survival of *A. rabiei* depends highly on rainfall and low temperatures (Khaliq, 2021). In Pakistan, hot weather and heavy rainfall in summer decreases the survival duration of chickpea blight fungus (Khaliq *et al.*, 2021).

Cultivation of disease resistant varieties of chickpea is an effective way to avoid ascochyta blight (Ahmad *et al.,* 2021). But because of the emergence of new pathotypes/races, available cultivars are becoming susceptible rapidly in Pakistan. Eight to twelve isolates (I-68, I-70, I-71, I-72, I-74, KN-1, KN-30, C-50, C-51, A-32, P-16, and M-12) having different races have been reported in Pakistan (Jamil *el al.,* 2010). Resistance has been developed in advanced lines against these pathotypes, but cultivars become susceptible after few years because of appearance of new pathotype.

Cultivation of resistant varieties is the cheapest source to manage chickpea blight, but lack of resistant source, and for rationale use of control measures, study of Zn based nanoparticle for controlling chickpea blight is vital. Thus, the hypothesis of this study was developed that "preparation of Zn based nano-formulated plant extracts, Zn based nano-formulated commercial fungicides and Zn metallic nano-particles could be predicted for timely management of chickpea blight".

MATERIALS AND METHODS

Collection of diseased samples

Chickpea blight infected pods were collected from Ayub Agricultural Research Institute (AARI) located near to University of Agriculture, Faisalabad. The pods were placed in refrigerator at 5-8°C. These samples were then used for isolation and purification of *A. rabiei.*

Preparation of culture medium

PDA was prepared in the laboratory. The ingredients for the preparation of one liter PDA media was potato starch (200g), agar (20g), dextrose (20g) and water (1,000 ml). Potato starch was taken by boiling of potato 200g in 500 ml of distilled water.

Isolation, purification and mass culturing of *Aschochyta rabiei*

The pathogen *A. rabiei* was isolated by the procedure followed by Ahmad *el al.,* (2021). Pods were placed in forceps grip and heated on sprit lamp flame in a way that outer surface of pods could be sterilized, while inner pod layer remained undamaged. Surface sterilized pods were then opened and infected seed were brought out from the pods with sterilized forceps. Infected seeds were placed on the autoclaved potato dextrose agar medium and placed in incubator at 20 ± 2 °C for fortnight. When colonies of *A. rabiei* formed around the plated infected material on PDA medium, they were isolated, and purified by single spore culture method (Choi *et al.,* 1999). Purified culture of *A. rabiei* was prepared and maintained at 5°C.

Preparation of mass culture

The chickpea seeds were soaked in tap water for overnight (Ahmad *et al.,* 2021). The soaked seeds were spread on paper towels so that excessive moisture could be absorbed. The soaked chickpea seeds were then placed into conical flasks at the rate of 250 g/1liter flask. A cotton plug was tightly fixed into the opening of conical flask. The flasks containing chickpea seeds were placed in an autoclave at 121 ^{o}C and 15 lbs psi for half an hour and same process was repeated after an interval of 24 hours to eliminate the traces of bacterial endospores, if any. On the autoclaved seeds, 6 mm agar plugs (3-4 in numbers) taken from a fortnightly old culture of *A. rabiei* with sterile cork borer were inserted into seeds. Streptomycin (25 mg) was also mixed in autoclaved seeds i.e. 250 g in conical flasks to avoid bacterial contamination. After closing the mouth of flasks with cotton plug, flasks were incubated at 20 ± 2°C for ten days for further growth of pycnidial culture of *A. rabiei*.

Management of chickpea blight

In vitro evaluation of Zn based nano-formulated plant extracts for controlling chickpea blight

Two plant extracts *viz.,* neem and datura with Zn nitrate $Zn(NO_3)_2$ were tested for the management of chickpea blight pathogen by using the poisoned food technique (Nene and Thapliyal, 2002). For this purpose, the neem and datura leaf samples were placed in the shed and allowed to air dry for 5 to 7 days. The air dried samples was ground to powder form with the use of a grinder, separately 10g neem leaves powder and 10g datura leaves powder was added to 50 ml hot distilled water. The water and plant extract powder were mixed together and placed in an orbital shaker for 3 hours at 120 rpm. Zn nitrate $Zn(NO_3)_2$ was added slowly into the 10 ml of the each plant extract solution and allowed to stand in a beaker and then thoroughly mixed with the help of a hand shaker to make the concentrated

solution. In separate beaker, sodium sulphate Na_2SO_4 powder was mixed with the distilled water with help of hand shaking to make the saturated solution. Using the pipette, sodium sulphate solution was added separately into each of the Zn nitrate Zn $(NO_3)_2$ solutions, drop by drop. To allow the precipitation, the solutions were kept at room temperature for 3 to 4 hours. The supernatant was drained out. Then the precipitates was transferred to a new petri plate and kept in an oven for 4 to 5 hours to make the precipitates hard. The precipitates was pressed and crushed to convert into powder form using the spatula.

***In vitro* evaluation of Zn based nano-formulated commercial fungicides against chickpea blight**

The Zn based nano-formulated two commercial available fungicides such as Aliette^R and Thiovetjet^R were generally used for control of pathogen of chickpea blight disease for. The preparation process of Zn based nano-formulated commercial fungicides was the same as used for the production of nano-formulated plant extracts. Separately, 10 g Aliette^R fungicide and 10 g Thiovetjet^R fungicides was added into the 50 ml hot distilled water to make two separate solution of each fungicide. The solution of each fungicide was placed in the orbital shaker for 3 hours at 120 rpm. The fungicide (10 ml of each solution) was passed through a sieve into beakers separately. The Zn nitrate $Zn(NO_3)_2$ was added slowly into each fungicide solution and mixed with 10 ml of each fungicide solution thoroughly with the help of hand shaking to make concentrated solution. In separate beaker, sodium sulphate Na_2SO_4 was added into the distilled water and mixed with the help of hand shaking to make a saturated solution. Using pipette, the sodium sulphate Na_2SO_4 solution was added into the Zn nitrate $Zn(NO_3)_2$ solution drop by drop. The solution was placed 3 to 4 hours at room temperature to allow for the precipitations. The supernatant was drained to keep the precipitates. The precipitates of each fungicide was kept in an oven at 65 ^oC for 3 to 4 hours to allow the precipitates become hard. The precipitates of each fungicide was pressed and crushed using a spatula to make powder form of each nano-formulation fungicide.

***In vitro* evaluation of Zn metallic nano-particles against chickpea blight**

For *in vitro* evaluation of Zn metallic nano-particles against chickpea blight pathogen Zn nitrate $Zn(NO_3)_2$ was added into 10 ml of distilled water and mixed thoroughly to make a concentrated solution. In another beaker, the sodium sulphate Na_2SO_4 was added into the distilled water and mixed with the help of hand shaking to make a saturated solution. Using a pipette, 10 ml of sodium sulphate saturated solution was transferred drop by drop into the Zn nitrate $Zn(NO_3)_2$ solution. The solution was kept for 3 to 4 hours at room temperature to allow for precipitations. The supernatant was drained out and the precipitate was retained. The precipitate was kept in an oven at 65 ^oC for 4 to 5 hours to allow the precipitate to form a hard shape. The precipitates were crushed using a spatula to form a powdered form.

Poisoned food technique

The preparation of Potato Dextrose Agar (PDA) medium was done by the standard protocol and then the medium was allowed to cool to 50°C. Different concentrations (1, 2, 3 and 4% v/v) of flower and shoot extracts in the PDA medium were prepared by adding appropriate proportions of distilled water and stock solution into the PDA medium. Control treatment distilled water in same proportion. Then the complete mixing of extracts and medium was done. Sterilized petri-plates of 9 cm diameter was taken and poured with 20 ml of each medium. After the PDA medium gets solidified, mycelial discs having a diameter of 5 mm with a pre-sterilized cork borer from *Aschochyta rabiei* culture (five to seven days old) was taken and placed in the centre of each Petri plate. Three replications of each treatment were tested. The petri plates were incubated in an incubator for seven days at a temperature of 25±2. The measurement of fungal growth was done. For this purpose, the diameters were determined at right angles for each colony. Inhibition of growth was determined through the formula (Shafique *et al*., 2011) given below:

$$\text{Growth inhibition (\%)} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

***In vitro* evaluation of non-nano fungicides against chickpea blight**

For this purpose, stock solution was prepared. The percentage of active ingredient was divided with 100 and that amount of fungicide was added to 100mL of distilled water. For 50, 100 & 150 ppm 0.5, 1 & 1.5 mL from stock solution was taken respectively and poured into 100mL of PDA.

Results and Discussion

In vitro evaluation of non nano-formulated fungicides and plant extracts against the *Aschochyta rabiei*

Non nano-formulated plant extracts and fungicides significantly reduced the colony growth of fungus. Mean inhibition of fungus colony growth (%) by non nano-formulated plant extracts *viz.*, neem and datura and fungicides i.e. Aliette and Thiovit jet were statistically different at their different concentrations. Among plant extracts neem inhibited maximum colony growth followed by datura compared with zinc alone. Aliette (T_1) at 50ppm, 100ppm and 150 ppm concentrations reduced the colony growth of fungus (*A. rabiei)* by 3.525%, 3.866% and 3.716%, respectively as compared to the control. The fungicide Thiovit jet (T_2) at 50ppm, 100ppm and 150 ppm concentrations remained the 2nd good and reduced the colony growth of *A. rabiei* by 3.866%, 4.5917% and 4.0250%, respectively as compared to the control (Figure 1).

Among plant extracts, Neem (T_3) at 50ppm, 100ppm and 150 ppm concentrations reduced the colony growth *A. rabiei* as; 4.5667%, 4.3000% and 3.9917%. The plant extract datura (T_4) at three concentrations i.e. 50ppm, 100ppm and 150 ppm inhibit the colony growth as; 4.2167%, 4.1500% and 3.5917%. The application of zinc (T_5) alone at three concentrations i.e. 50ppm, 100ppm and 150 ppm remained the least effective in inhibiting the colony growth of *A. rabiei* as; 4.2750%, 3.9083% and 3.7667% respectively as compared to the control (T_6) (Figure 2).

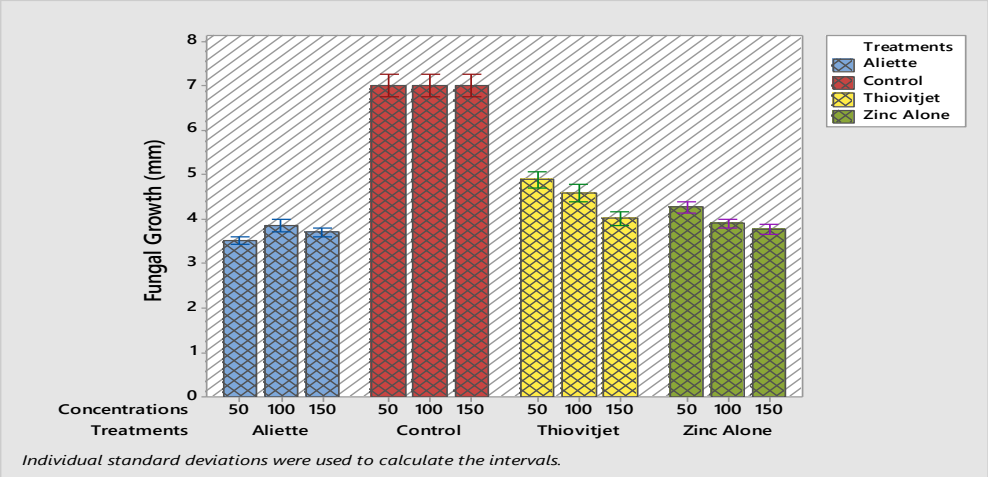

Figure 1. Effect of various non nano-formulated fungicides and zinc source against colony growth of *A. rabiei.*

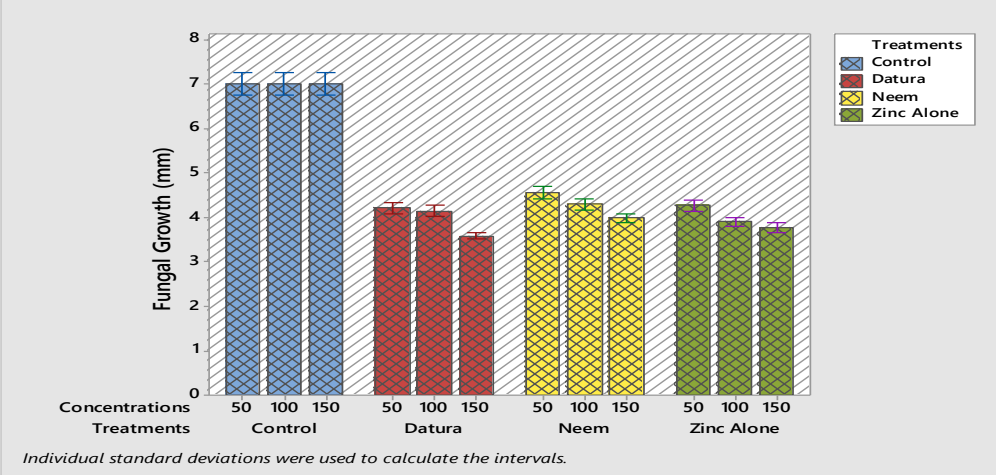

Figure 2. Effect of various non nano-formulated plant extracts and zinc source against colony growth of *A. rabiei.*

Aliette (T_1) in all four days *viz.*, 1, 2, 3, and 4 proved most effective in inhibiting the colony growth of fungus as; 3.1111%, 3.2222%, 3.8667, and 4.6111%, respectively as compared to the control. The

fungicide Thiovit jet (T_2) remained second good in reducing the colony growth of *A. rabiei* in all four days 1, 2, 3 & 4 as; 3.2333%, 3.9333%, 4.8444% & 6.0000%, respectively as compared to the control (Figure 3).

Among plant extracts, Neem (T_3) in all four days i.e. 1, 2, 3 & 4 was best to inhibit the colony growth *A. rabiei* as; 3.4000%, 3.9333%, 4.5333% and 5.2778% respectively. The plant extract datura (T_4) in all four days i.e. 1, 2, 3 and 4 inhibit the colony growth as; 3.2222%, 3.5667%, 4.1778% and 4.9778% and proved the second best plant extract. The application of zinc (T_5) in all four days *viz.*, 1, 2, 3, and 4 remained the least effective in inhibiting the colony growth of *A. rabiei* as; 3.1889%, 3.5444%, 4.1222% and 5.0778% respectively as compared to the control (T_6) (Figure 4).

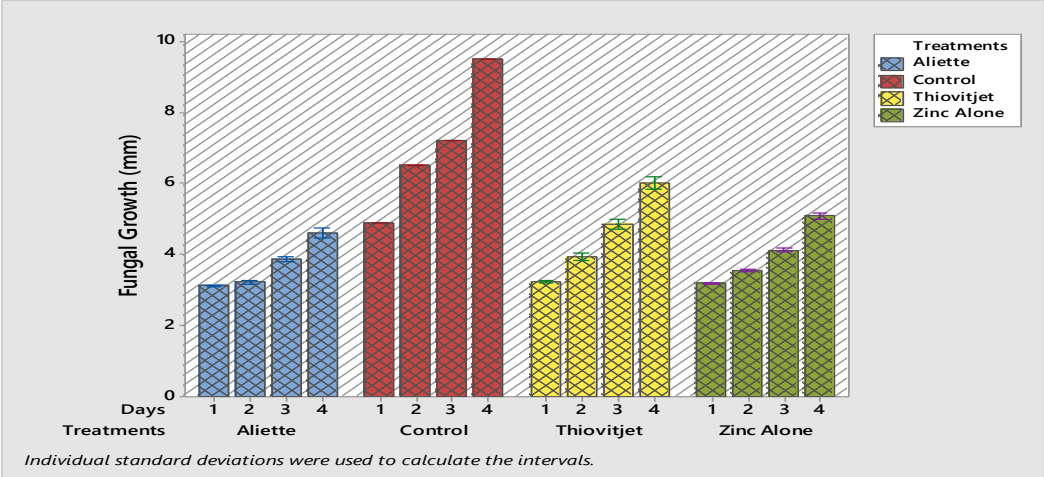

Figure 3. Effect of different concentrations of non nano-formulated fungicides and zinc against colony growth of *A. rabiei* at different days intervals.

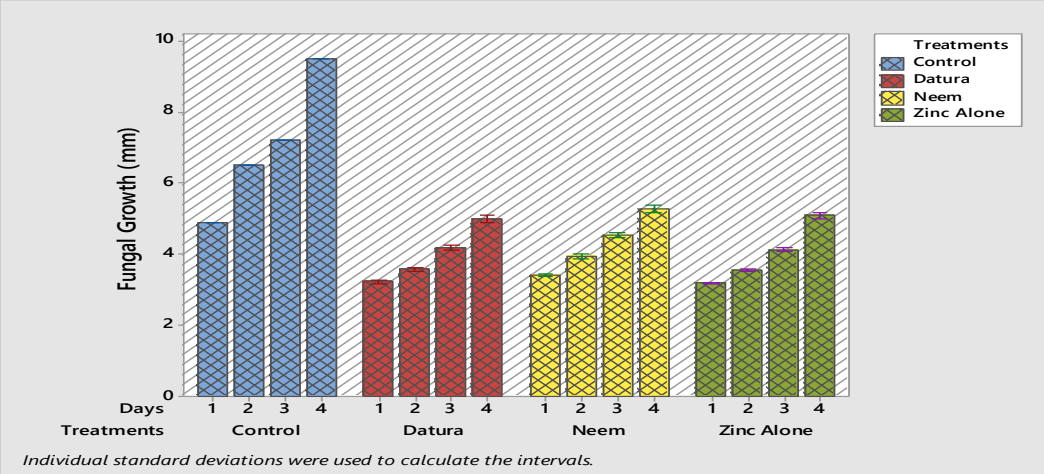

Figure 4. Effect of different concentrations of non nano-formulated plant extracts and zinc against colony growth of *A. rabiei* at different days intervals.

In vitro evaluation of zinc nitrate nano formulated fungicides and plant extracts against the *Aschochyta rabiei*

Analysis of variance (ANOVA) indicated that effects of all applied zinc nitrate nano formulated treatments and their concentrations were significant ($P \leq 0.05$). Two-way interactions between concentrations and treatments also indicated significant ($P \leq 0.05$) association. All zinc nitrate nano-formulated plant extracts and fungicides significantly reduced the colony growth of fungus. Mean inhibition of fungus colony growth (%) by zinc nitrate nano-formulated plant extracts *viz.*, neem and datura and fungicides i.e. Aliette and Thiovit jet were statistically different at their different concentrations as indicated in the Table 4.7. Similarly, mean inhibition of fungus colony growth (%)

by all zinc nitrate nano formulated plant extracts and fungicides was statistically different with the application in different day's intervals. Zinc nitrate formulated nano fungicide Aliette performed best as compared to the Thiovit jet. Among zinc nitrate formulated nano plant extracts neem inhibited maximum colony growth followed by nano formulated datura compared with zinc nitrate alone (Figure 5). Zinc nitrate formulated nano Aliette (T_1) at 50ppm, 100ppm and 150 ppm concentrations reduced the colony growth of fungus (*A. rabiei)* by; 3.8333%, 4.1500% and 5.5583%, respectively as compared to the control. The nano formulated fungicide Thiovit jet (T_2) at 50ppm, 100ppm and 150 ppm concentrations proved the second most effective fungicide and reduced the colony growth of *A. rabiei* by 4.6750%, 4.3083% and 3.8167%, respectively as compared to the control.

Among zinc nitrate nano formulated plant extracts, neem (T_3) at 50ppm, 100ppm and 150 ppm concentrations proved effective in reducing the colony growth *A. rabiei* as; 4.6333%, 4.4500% and 4.0000%. The nano formulated plant extract datura (T_4) at three concentrations i.e. 50ppm, 100ppm and 150 ppm inhibit the colony growth as; 3.5583%, 3.6750% and 3.8667%. The application of nano zinc in the form of zinc nitrate (T_5) alone at three concentrations i.e. 50 ppm, 100 ppm and 150 ppm remained the least effective in inhibiting the colony growth of *A. rabiei* by; 4.1167%, 3.9167% and 3.7583% respectively as compared to the control (T_6) (Figure 6).

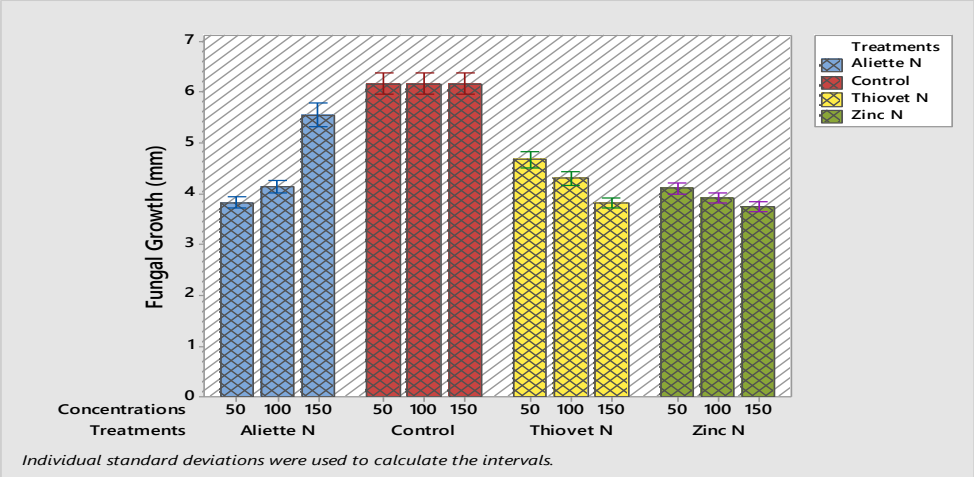

Figure 5. Effect of various zinc nitrate formulated nano-fungicides and zinc nitrate alone against colony growth of *A. rabiei.*

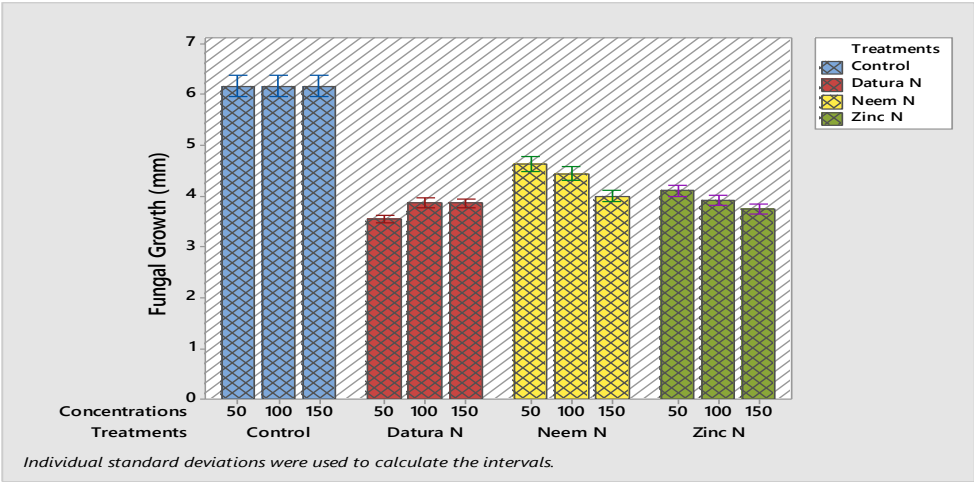

Figure 6. Effect of various zinc nitrate formulated nano-plant extracts and zinc nitrate alone against colony growth of *A. rabiei.*

Zinc nitrate formulated nano Aliette (T_1) in all four days *viz.*, 1, 2, 3, and 4 proved most effective in inhibiting the colony growth of fungus as; 3.1667%, 4.1778%, 5.0111%, and 5.7000%, respectively as compared to the control. The fungicide nano Thiovit jet (T_2) remained second good in reducing the

colony growth of *A. rabiei* in all four days 1, 2, 3 & 4 as; 3.2778%, 3.8344%, 4.5444% & 5.4000%, respectively as compared to the control (Figure 7).

Among plant extracts, nano neem (T_3) in all four days i.e. 1, 2, 3 & 4 was best to inhibit the colony growth *A. rabiei* as; 3.3556%, 3.9556%, 4.6889% and 5.4444% respectively. The zinc nitrate formulated plant extract nano datura (T_4) in all four days i.e. 1, 2, 3 and 4 inhibit the colony growth as; 3.2222%, 3.5000%, 3.8889% and 4.4556% and proved the second best plant extract. The application of zinc nitrate as nano zinc (T_5) in all four days *viz.*, 1, 2, 3, and 4 remained the least effective in inhibiting the colony growth of *A. rabiei* as; 3.1111%, 3.5444%, 4.1222% and 4.8444% respectively as compared to the control (T_6) (Figure 8).

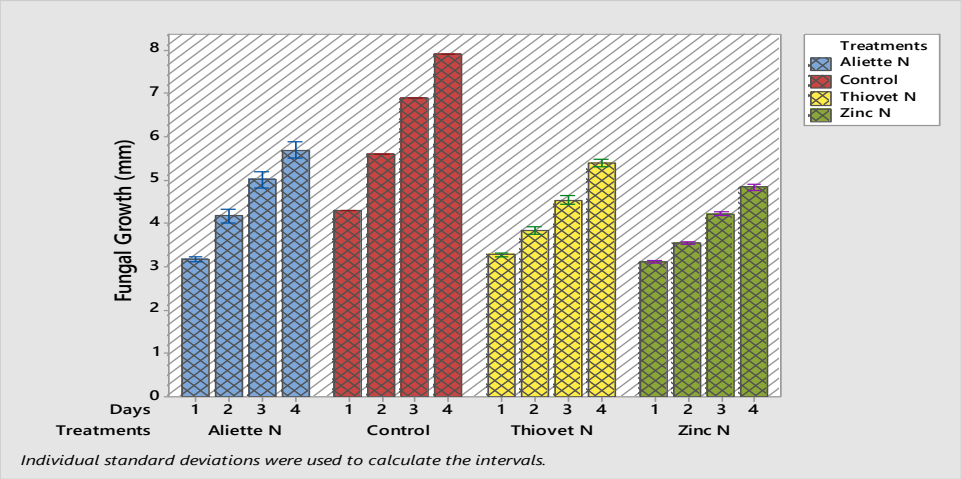

Figure 7. Effect of various concentrations of zinc nitrate nano-formulated fungicides and zinc nitrate alone against colony growth of *A. rabiei* at different days intervals.

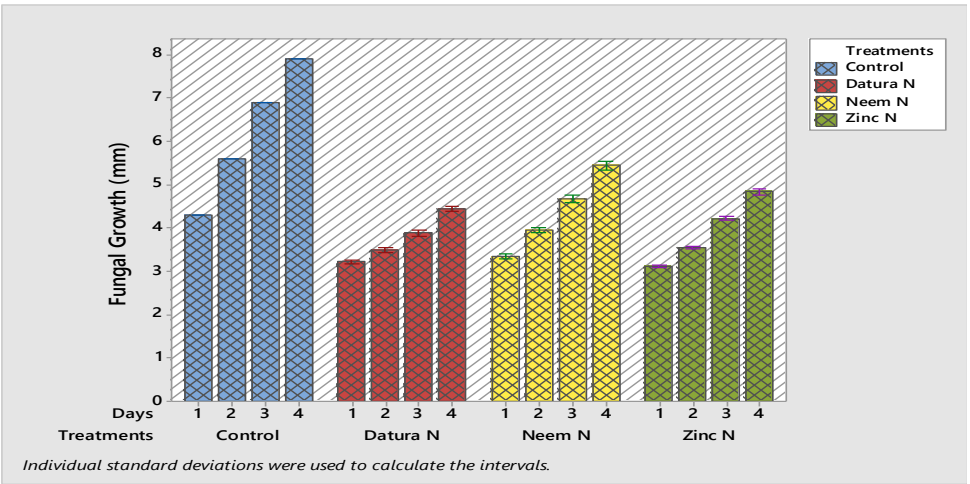

Figure 8. Effect of various concentrations of zinc nitrate nano-formulated plant extracts and zinc nitrate alone against colony growth of *A. rabiei* at different day's intervals.

Fungicides and plant extracts) were evaluated against chickpea blight under *in vitro* and *in vivo* conditions. Among fungicides, Alliete and $Thiovet_{jet}$ inhibited significantly colony growth of *A. rabiei*. Significant inhibition of colony growth by these two fungicides may be attributed to their active ingredients i.e. aluminium ethyle phosphate **(**Alliete) and sulfur ($Thiovet_{jet}$). For example, sulfur containg fungicides inhibit fungus growth by inactivating sulfhydryl groups in amino acids and in enzymes, interfering electron transport, and reducing sulfur to hydrogen sulfide which is highly toxic to fungus. Similarly, aluminium ethyle phosphate may have reduced the mycelial growth by inhibiting cell division and enzyme synthesis, and by denaturation of proteins (Agrios, 2005). When these two fungicides (Alliete and $Thiovet_{jet}$) at 0.15% concentration were applied under field

conditions also significantly controlled disease severity. Significant control of chickpea blight by these fungicides under *in vivo* conditions is due to their systemic ability which allowed these fungicides to kill the fungus in established infection. This is in line with the finding of (Shtienberg *et al*., 2000). Organophosphate systemic fungicides, containg Fosetyl-Al, protect the plants by activating defense mechanism and synthesizing phytoalexins (Agrios, 2005). Maximum control of chickpea blight was obtained using three foliar applications of Alliete and $Thiovet_{jet}$. This shows that curative applications significantly control chickpea blight. This is in agreement with Gan *et al*. (2006) who reported that with fewer applications of curative systemic fungicides, ascochyta blight can be controlled effectively. Successful control of ascochyta blight through these two systemic fungicides, might also have resulted because of their good translocation into tissues of the host. Previous researches have shown that only those systemic fungicides perform well which show movement into newly developed tissues (Gan *et al*., 2006; Davidson and Kimber, 2007). Thus, this study recommends that continuous effort should be made to look for those systemic fungicides which show more translocation in the system of the plant. This will help to fix most appropriate fungicides i.e. systemic, for chickpea blight management.

Plant extracts neem (*A. indica*) remained effective against Ascochyta blight, but less than fungicides during current research. Under *in vitro* conditions, these extracts may have inhibited the ascochyta fungus by different antifungal compounds which they contain (Nunez *et al*., 2006; Lee, 2007). Jabeen *et al*., (2011) found that *M. azedarach* contained; benzoic acid, ursolic acid, maesol, 3,5 dimethoxybenzoic acid, ß- sitosterol and ß-amyrin which were highly toxic to chickpea blight fungus. Similarly, from *M. azedarach*, obacunone, nomilin, limonoids and limonin have also been isolated and proved to be effective against different insects (Koul *et al*., 2004). Botanical extracts also contain secondary metabolites which are antifungal and restrict the mycelial growth of fungi (Sisti *et al*., 2008). Disease control by these plant extracts under field conditions may be ascribed to their ability of inducing systemic acquired resistance (SAR) (Guleria and Kumar, 2006). Foliar applications of *A. indica* produce SAR effectively in chickpea cultivars against ascochyta blight (Sarwar *et al*., 2011).

The zinc nitrate formulated fungicides and plant extracts were aslo evaluated at different concentrations towards *A. rabieie* pathogen Fig. 4.8. The presence of inhibition zone clearly indicates that the mechanism of the biocidal action of ZnO nanoparticles which involves disruption of the membrane with high rate of generation of surface oxygen species and finally lead to the death of pathogens. Interestingly, the size of the inhibition zone was different according to the type of pathogens, synthesis method and the concentrations of ZnO nanoparticles. As it was shown in the study of (Rizwan *et al.,* 2010), it has been found in this study that by increasing the concentration of ZnO nanoparticles in wells and discs, the growth inhibition has also been increased consistently because of proper diffusion of nanoparticles in the agar medium. Both nano and bulk ZnO nanoparticles showed antimicrobial activity against selected pathogens but maximum activity was observed in *S. aureus* followed by *P. mirabilis*, *S. marcescens* and *C. freundii*. The nano formulated fungicides and plant extracts reduced the maximum activity of *R. stolonifer* and *T. harzianum*. Dome¯nech (1886), have described that the release of Zn2þ ions is responsible for the antibacterial activity. In our study, zinc nitrate nanoparticles showed a greater significant zone inhibition when compared to control. However, low enhancement of the antimicrobial activity was recorded in the cases plant extracts with their lower concentrations but medium inhibition was noticed at higher concentrations (Fig. 3.5). All the treatments nano fungicides, plant extracts and zinc nitrate particles showed significant difference on the colony growth of *A. rabiei*. While considering the methods (well and disc), the pathogens were more sensitive to well method when compared to disc method of zone inhibition. From the results of present investigation, we confirm that the zinc nitrate nano particles with smaller particle size showed enhanced activity due to the large surface area to volume ratio and surface reactivity when compared to the non- formulated plant extracts and fungicides. We also noticed in all the cases, zinc nitrate concentrations prepared were more effective than the suspension without concentrations. This can be explained on the basis of the oxygen species released on the surface of Zn nitrate, which cause fatal damage to microorganisms (Sunanda *et al.,* 1998). They react with hydrogen ions to produce molecules of H2O2. The generated H2O2 can penetrate the cell membrane and kill the

pathogen (Fang *et al.,* 2006). The generation of H2O2 depends strongly on the surface area of Zn nitrate, which results in more oxygen species on the surface and the higher antibacterial activity of the smaller nanoparticles (Yamamoto *et al.,* 2008). The results of this study may be applicable to medical devices that are coated with nanoparticles against microbes.

As seen from the growth inhibition rates in Fig. 3.6, Zn nitrate has a stronger inhibitory effect than chemically synthesized NPs. It is clear that ZnO nanoparticle at a concentration 150 ppm inhibited growth of fungal pathogens under *in vitro* conditions, whereas the effect was much less at lower concentration. Increasing concentration of Zn nitrate nano-particle decreases the growth of microbes, and the concentration at which growth stopped altogether was higher in Zn nitrate than non formulated plant extracts and fungicides. According to (Adams et al., 2006), Zn nitrate nanoparticles inhibited growth of gram-positive by 90% but some microbial pathogens *viz.*, gram-negative bacteria are much more resistant.

CONCLUSION

The study demonstrates the effectiveness of various chemical-based fungicides, including Azoxystrobin, Tebuconazole, Flutriafol, and Boscalid, in managing potato diseases. All tested fungicides effectively suppressed the disease, with Azoxystrobin and Flutriafol being the most effective combination for early light management. This highlights the positive impact of these fungicides on disease severity in field conditions.

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