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Evaluation of Fungicides and Biosynthesized Silver Nanoparticles Against Leaf Spot Pathogen in Alfalfa (*Medicago sativa*)

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Alfalfa (*Medicago sativa*), a member of the Fabaceae family, is recognized as the world's oldest and most significant forage crop, often called the 'queen of forage'. There are various distinct fungal, bacterial and viral diseases commonly found in alfalfa. There are many challenges to alfalfa production but leaf spot has a major threat to alfalfa crop in Pakistan. It is caused by *Curvularia buchloes*. It causes significant economic losses worldwide. Keeping in view high yield losses due to this disease, this research was mainly dealt with disease management. Alfalfa crop was growing in green house and field. *Curvularia spp*. was acquired from Fungal Molecular Biology Lab (FMB-CC), University of Agriculture Faisalabad. Culture was revived for pathogenicity confirmation. Different disease management strategies included fungicides and silver nanoparticles were used. Silver nanoparticles were biogenically synthesized using a fungal/bacterial strain, and their antifungal potential was evaluated. Selected fungicides at different concentrations were also used to evaluate and in combination with Silver nanoparticles to find out the optimum and most effective combination and concentration reduce the growth of fungal pathogens. Data was statistically analyzed. The research was conducted in experimental area of the Department of Plant Pathology, University of Agriculture, Faisalabad under randomized complete block design (RCBD).
Keywords: Alfalfa, queen of forage, leaf spot disease, silver nanoparticles, *Curvularia buchloes*, fungicides.

INTRODUCTION

Alfalfa *(Medicago sativa)* belonging to family Fabaceae known as one of the most important cultivation of forage. Commonly known as Lucerne, alfalfa ranks third in economic value among crops, behind only corn and soybeans. In the USA, it is the fourth most cultivated crop. It has a high forage value, a large area of cultivation and high digestibility and is also known as the "Queen of Fodder Herbs" among farmers and agricultural scientist worldwide. Alfalfa has been reported by archaeological ancient philosopher reports. As early as 4000 B.C. crop was cultivated in southwest Asia (Dale et al., 2012).In most continents alfalfa is grown with more than 35 million hectares in over 80 countries.

Alfalfa is a legume that has been cultivated for over 2000 years ago and it is mostly used in animal feed due to large amount of protein and fiber content of 170-220g of protein and around 40-50% cellulose and lignin per pound of shooting dry mass (Gawel & Grzelak, 2014). This legume is used mainly as feed for animals but is now readily used in human diets because of its vital nutrient value of amino acid, calcium, vitamins and minerals (Apostol et al., 2017*)*.

In the USA, millions of acres are under cultivation of fodder crops. Perennial varieties of alfalfa are most common around the world, and the most prevalent verities depending on climate are *Medicago falcata.* (yellow alfalfa), *Medicago sativa* (common purple alfalfa) and *Medicago media Pers.* (hybrid alfalfa) (Pajić & Marković, 2016). Alfalfa economic importance can be measured by keeping in view its high production of green and dry matter which is 80 t ha^{-1} of green and 20 t ha^{-1} for biomass (Nešić et al., 2005*)*.

Alfalfa *(Medicago sativa L.)* is known to be the most significant forage crop in more than 80 countries with an area of more than 35 million hectares worldwide (Radovic et al., 2009). By taking important steps in alfalfa history, alfalfa is considered to be the 4th largest crop as the current status of western states (Fernandez et al., 2019).

Curvularia had significant implication for the development of alfalfa in Pakistan and many other countries. *Curvularia* leaf spot affects many grasses species all over the world (Smith et al., 1989). *Curvularia* leaf spot disease first recorded in

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Louisiana in the USA, 2017 and in 2018 it was confirmed in Kentucky. *Curvularia* is the fungal genus consist of variety of plant diseases, which typically cause foliar spots in forage grasses. Thirty types of fungi are known to restrict the growth and productivity of alfalfa out of 70 types (Thal & Campbell,1987).The genus *Curvularia*caused number of diseases symptoms such as leaf spots and leaf blight on crop (Benoit & Mathur, 1970). By developing persistent, competitive and resistant varieties of alfalfa disease chances can be minimized and its production can be improved (Bouton, 2012).

Application of nanoparticles in crop preservation has great potential in insect and disease management, in the controlled and targeted distribution of agrochemicals as well as in provision of early detection diagnostic tools (Yadav & Yadav, 2018).

Nano-particles can be used for the detection of pathogens as well as for the detection of analytical compound used for disease inhibition (Younas et al., 2020). Diagnosis of disease, pathogen detection and residual analysis by using nanosensor testing can be much more precise and quicker (Kashyap et al., 2019). Silver nanoparticles can cause and suppress protein expression in different biological microorganisms (Mikhailova, 2020). Plant diseases can be managed with use of silver particles. The mode of microorganism can be inhibited with the application of silver particles, biocontrol agents has the ability to overcome numbers of pathogen of plants in the safer manners rather than the application of scientific chemicals (Rozhin et al., 2021).

Nanoparticles and silver ions effectively used for the reduction of leaf spot disease of crop. Silver is used as a strong disinfectant by inactivating metabolic enzymes to kill single cell microorganisms (Hwang et al., 2008). There are several benefits of nanoparticles such as (1) increased shelf-life (2) increased the solubility of poorly water-soluble pesticides (3) decreased toxicity (4) increased site-specific penetration into the target pest (Hayles et al., 2017).

MATERIALS AND METHODS

Preliminary research work has been done in the Fungal Molecular Biology Lab. Department of Plant Pathology on the management of alfalfa crop by using biocontrol agents, chemicals and nanoparticles. The present research was planned to determine the interaction of pathogenic fungal species i.e., *Curvularia buchloes* in the development of alfalfa leaf spot at a high level of incidence and severity. For this purpose, food poisoned technique were done in Fungal Molecular Biology Lab. Different methodologies were used for the assessing the effect of integrated management on *Curvularia buchloes* in alfalfa and the most effective control was find out.

***Sowing of seed*:** Sowing of alfalfa seed was done in September 2019. Seed rate was 20kg/ha. The seeds were broadcasted on the pots. After sowing the seeds in the pots, first irrigation was applied immediately and the second irrigation was done after five days of sowing. Subsequent irrigations were carried out at an interval of 7-10 days. Number of irrigations depended on atmospheric conditions. Harvesting of crop was done after 70 days of sowing, when 50% plants were in bloom. Plant cutting was done just above the soil surface. The plants were left in the field to produce seeds which were harvested in appropriate time

Acquisition of pathogen

***Revival of culture*:** Culture number FMB0177 of *Curvularia spp.* was acquired from Fungal Molecular Biology Culture Collection (FMB-CC UAF), University of Agriculture Faisalabad. The whole procedure for revival of *Curvulria* culture was done in Laminar flow chamber using PDA media. Cultures were incubated and the fungal identity was confirmed based on morphological and microscopic characteristics.

***Mass culturing and preservation of fungal culture*:** Isolate of the fungal pathogen were mass cultured on PDA media. Test tubes containing pure of each isolated fungal pathogen were preserved on Fungal Molecular Biology Lab, Department of Plant Pathology, University of Agriculture Faisalabad. The isolated cultures were used for further experimentation.

***Identification*:** The identification of isolated and purified fungi was examined microscopically on the basis of morphological characteristics of spore including color, shape, and size.

***Pathogenicity test*:** For the conformation of the pathogen on host plant, pathogenicity test was performed by following the Koch's postulate. For the pathogenicity test, juvenile of alfalfa crop were collected from growing area of alfalfa crop and maintained into Green house of Fungal Molecular Biology Lab. Department of Plant Pathology, University of Agriculture Faisalabad. Pathogen was multiplied or mass culture in the potato dextrose agar (PDA) media and spore suspension was prepared. Spore concentration was adjusted with the help of hemocytometer in the lab concentration. Pathogen was inoculated in the foliar portion of the host plant by rubbing to cause injury with the help of carborendum powder. First of all, host plant foliar part were washed with distilled water to remove dust and other contamination from the surface and then surface sterilize with the help of 70% Ethanol for 1-2 mint and wash with the successive changes of sterile distilled water for 3 times to remove toxic residue of chemical from the sample surface. After that, clean the surface of host plant with cotton swab and rub the foliar part with the help of carborendum powder for inoculation of pathogen. Twenty (ml) pathogen suspension was taken in the beaker and mix it 80 ml distal water to dilute it and sprayed on the foliar part of the host plant with the help of hand sprayer and covered with polythene sheet. Data regarding with one week interval on visual observation, based on

following disease ratting scale given by (Bhunjun et al., 2021).

I 1= No lesions
H.R 2=0-25% lesions infected leaf surface
M.R 3=25.1-50% lesions infected leaf surface
S 4=50.1-75% lesions infected to leaf surface
H.S 5=75.1-100% lesions infected leaf surface

(I=Immune, H.R=Highly Resistant, M. R= Moderately Resistance, S=Susceptible and H.S=Highly Susceptible).

***Applications of nanoparticles on crop*:** Antifungal potential of silver nanoparticles were evaluated against *curvularia spp.*

***In vitro evaluation of antifungal activity of Silver nanoparticles*:** In vitro, purified silver nanoparticles of different concentration (50ppm,100ppm,150ppm) were prepared. Dilute the purified silver nanoparticles. Antifungal activity of silver nanoparticles was evaluated by using poisoned food technique. Prepared silver nanoparticles were mixed with PDA media, pour into petri plates then inoculated with 5mm disc of tested culture by using a cork borer. Inoculated plates were raped with foil paper and labeled the plates. Control was maintained as PDA plates without silver nanoparticles. Raped plates were incubated at 26°C.Record the mycelial growth after 3, 5, 10 day of inoculation and measured the percentage of growth inhibition of treatments with growth control.

***Data Record*:** (Taskeen-Un-Nisa et al., 2011) calculated inhibition zone by using formula

Percentage of mycelial growth inhibition = (dc-dt)/dc×100

where dc = Average diameter (in mm) of fungal colony in control, dt = Average diameter (in mm) of fungal colony in treatment.

Table 1. Silver nanoparticle treatments against confirmed pathogen to inhibit the growth of fungal isolates.

Treatments	Concentrations
T1	Silver nanoparticles(50ppm)
T2	Silver nanoparticles (100ppm)
T3	Silver nanoparticles (150ppm)
T4	Control

***Applications of chemical on alfalfa crop*:** Antifungal potential of different fungicides were evaluated *in vitro*.

***Checked the efficacy of fungicide by poisoned food technique*:** *In vitro* evaluation of antifungal potentaion of fungicides including (Menacoxeb + Propineb) (Trifloxystrobin), copper oxychloride was done against fungal isolate confirm as a pathogen of alfalfa leaf spot by using poisoned food technique. Different concentration of selected fungicides (100ppm,150ppm,200ppm) were prepared. Each selected fungicide was added in PDA media bottle with the help of injection needle. After that shaking the PDA bottle thoroughly to mixed the fungicide in the medium. The medium was poured into petri plates at 15 ml/plate. For control, same amount of distilled water was added in plates instead of fungicides. Took the fungal pathogen with the help of a cork borer and inoculated at the centre of the Petri plates. Raped the plates with foil paper and incubated at 25°C for 7 days. After 7 days of incubation, the diameter of the mycelial growth (mm) of pathogens was measured and compared with control plates (Haq et al., 2017).

***Data recording*:** Antifungal efficacy of fungicide was calculated by using formula by (Gupta and Tripathi, 2011).

% inhibition zone = C-T/C * 100

Where, C= Growth of control plate T= Growth of fungicide treated plate

Treatments	Fungicide
T1	Mancozeb
T2	Propineb
T3	Thiram
T4	Copper oxychloride
T5	Iprodion
T6	Control

***Statistical analysis*:** Randomized Complete Block Design (RCBD) will be use. Data were recorded and analyzed statistically by using Statistics 8.1 software i.e Fisher analysis of variance (Steel et al., 1997) and least significance Difference (LSD) test (p~0.05) was used to compare with treatment means at 5% of probability level under Randomized Complete Block Design (RCBD) for the significance and non-significance of treatment in disease management.

RESULTS

In vitro evaluation of nanoparticles against pathogenic isolates

ANOVA table for nanoparticles and fungicides

Analysis of variance table for GRW

Source	DF	MS	F	P
Replication	2	0.0020		
Concentration (C)	2	2.5387	239.86	0.0000
Treatments (T)	1	44.8996	4242.13	0.0000
Days	2	1.7524	165.56	0.0000
C * T	2	3.0096	284.35	0.0000
C * Days	4	0.0053	0.51	0.7320
T * Days	2	0.7033	66.45	0.0000
C * T * Days	4	0.0057	0.54	0.7092
Error	34	0.0106		

Grand Mean = 2.5093 ; CV = 4.10

LSD All-Pairwise comparisons test of growth for concentration

CONC	Mean
C1	2.8083A
C2	2.6317B
C3	2.0878C

Alpha 0.05
Standard Error for Comparison 0.0343
Critical T Value 2.032

Critical Value for Comparison 0.0697
Error term used: Replication * Concentration * Treatments * Days, 34 DF
All 3 means are significantly different from one another.

LSD All-Pairwise comparisons test of growth for treatment

Treatment	Mean
C	3.4211A
T1	1.5974B

Alpha 0.05
Standard Error for Comparison 0.0280
Critical T Value 2.032
Critical Value for Comparison 0.0569
Error term used: Replication * Concentration * Treatments * Days, 34 DF
All 2 means are significantly different from one another.

LSD All-Pairwise comparisons test of growth for days

Days	Mean
7	2.8006A
5	2.5472B
3	2.1800C

Alpha 0.05
Standard Error for Comparison 0.0343
Critical T Value 2.032
Critical Value for Comparison 0.0697
Error term used: Replication * Concentration * Treatment * Days, 34 DF
All 3 means are significantly different from one another.

LSD All-Pairwise Comparisons Test of Growth for Concentration*Treatment

Concentration	Treatment	Mean
C3	C	3.4500A
C2	C	3.4411A
C1	C	3.3722A
C1	T1	2.2444B
C2	T1	1.8222C
C3	T1	0.7256D

Alpha 0.05
Standard Error for Comparison 0.0485
Critical T Value 2.032
Critical Value for Comparison 0.0986
Error term used: Replication * Concentration * Treatment * Days, 34 DF
There are 4 groups (A, B, etc.) in which the meansare not significantly different from one another.

LSD All-Pairwise comparisons test of growth for treatment*days

Treatment	Days	Mean
C	7	3.8900A
C	5	3.4944B
C	3	2.8789C
T1	7	1.7111D
T1	5	1.6000E
T1	3	1.4811F

Alpha 0.05
Standard Error for Comparison 0.0485
Critical T Value 2.032
Critical Value for Comparison 0.0986
Error term used:
Replication*Concentration*Treatment*Days, 34 DF
All 6 means are significantly different from one another.

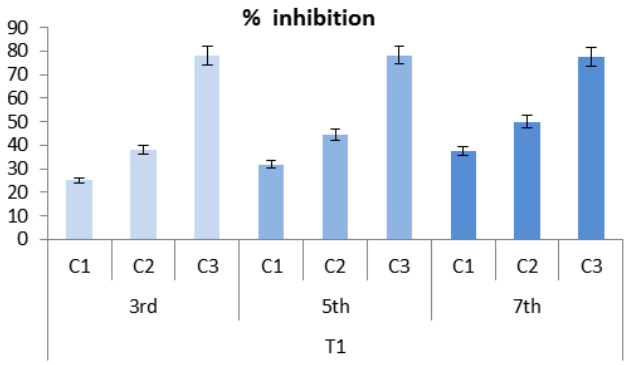

Figure 1. Graphical representation % of inhibition at different concentration.

ANOVA table for fungicides after 3rd day
Analysis of variance table for growth

Source	DF	MS	F	P
Replication	2	0.00540		
Concentrations (C)	3	0.18591	69.42	0.0000
Fungicide (F)	5	6.18656	2310.07	0.0000
C * F	15	0.01340	5.00	0.0000
Error	46	0.00268		
Total	71			

Grand Mean= 0.9983 ; CV= 5.18

LSD All-Pairwise comparisons test of growth for concentration

Concentrations	Mean
C1	1.1161A
C2	1.0300B
C3	0.9733C
C4	0.8739D

Alpha 0.05
Standard Error for Comparison 0.0173
Critical T Value 2.013
Critical Value for Comparison 0.0347
Error term used: Replication*Concentration*Fungicide, 46 DF
All 4 means are significantly different from one another.

LSD All-Pairwise comparisons test of growth for fungicide

Fungicide	Mean
C	2.4042A

F5	1.0133B
F4	0.8350C
F3	0.7292D
F2	0.5867E
F1	0.4217F

Alpha 0.05
Standard Error for Comparison 0.0211
Critical T Value 2.013
Critical Value for Comparison 0.0425
Error term used: Replication*Concentration*Fungicide, 46 DF
All 6 means are significantly different from one another.

LSD All-Pairwise Comparisons Test of Growth for Concentration*Fungicide

Concentration	Fungicide	Mean
C3	C	2.4833A
C4	C	2.4000A-B
C1	C	2.3667B
C2	C	2.3667B
C1	F5	1.1500C
C2	F5	1.0500D
C1	F4	0.9767D
C3	F5	0.9767D
C1	F3	0.8767E
C2	F4	0.8767E
C4	F5	0.8767E
C3	F4	0.7900F
C2	F3	0.7767F-G
C1	F2	0.7500F-H
C4	F4	0.6967G-I
C3	F3	0.6867H-I
C2	F2	0.6267I-J
C1	F1	0.5767J-K
C4	F3	0.5767J-K
C3	F2	0.5267K-L
C2	F1	0.4833L
C4	F2	0.4433L-M
C3	F1	0.3767M
C4	F1	0.2500N

Alpha 0.05
Standard Error for Comparison 0.0423
Critical T Value 2.013
Critical Value for Comparison 0.0851
Error term used: Replication*Concentration*Fungicide, 46 DF
There are 14 groups (A, B, etc.) in which the meansare not significantly different from one another.

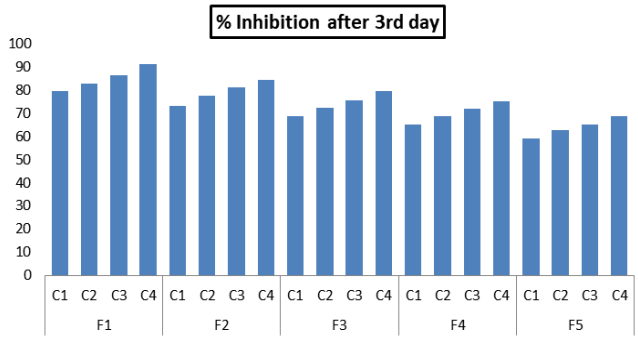

Figure 2. Graphical representation of % inhibition.

ANOVA table for 5th day
Analysis of variance table for growth

Source	DF	MS	F	P
Replication	2	0.0098		
Concentrations (C)	3	0.1411	42.15	0.0000
Fungicide (F)	5	10.250	3061.91	0.0000
C * F	15	0.0308	9.19	0.0000
Error	46	0.0033		
Total	71			

Grand Mean =1.1322 ; CV= 5.11

LSD All-Pairwise comparisons test of growth for concentrations

Concentrations	Mean
C1	1.2283A
C2	1.1639B
C3	1.1189C
C4	1.0178D

Alpha 0.05
Standard Error for Comparison 0.0193
Critical T Value2.013
Critical Value for Comparison 0.0388
Error term used: Replication*Concentration*Fungicide, 46 DF
All 4 means are significantly different from one another.

LSD All-Pairwise comparisons test of growth for fungicide

Fungicide	Mean
C	2.9692A
F5	1.0783B
F4	0.8875C
F3	0.7650D
F2	0.6275E
F1	0.4658F

Alpha 0.05
Standard Error for Comparison 0.0236
Critical T Value 2.013
Critical Value for Comparison 0.0475
Error term used: Replication*Concentra*Fungicide, 46 DF
All 6 means are significantly different from one another.

LSD All-Pairwise comparisons test of growth for concentration*fungicide

Concentration	Fungicide	Mean
C3	C	3.1000A
C4	C	3.0267A-B
C2	C	2.9500B
C1	C	2.8000C
C1	F5	1.2033D
C2	F5	1.1000E
C3	F5	1.0667E
C1	F4	1.0267E-F
C4	F5	0.9433F-G
C2	F4	0.9267G
C1	F3	0.9033G-H
C3	F4	0.8500G-I
C2	F3	0.8300H-J
C1	F2	0.8000I-K
C4	F4	0.7467J-L
C3	F3	0.7167K-M
C2	F2	0.6533L-N
C1	F1	0.6367M-N
C4	F3	0.6100N-O
C3	F2	0.5667N-P
C2	F1	0.5233O-P
C4	F2	0.4900P-Q
C3	F1	0.4133Q
C4	F1	0.2900R

Alpha 0.05
Standard Error for Comparison 0.0472
Critical T Value 2.013
Critical Value for Comparison 0.0951
Error term used: Replication*Concentration*Fungicide, 46 DF
There are 18 groups (A, B, etc.) in which the means are not significantly different from one another.

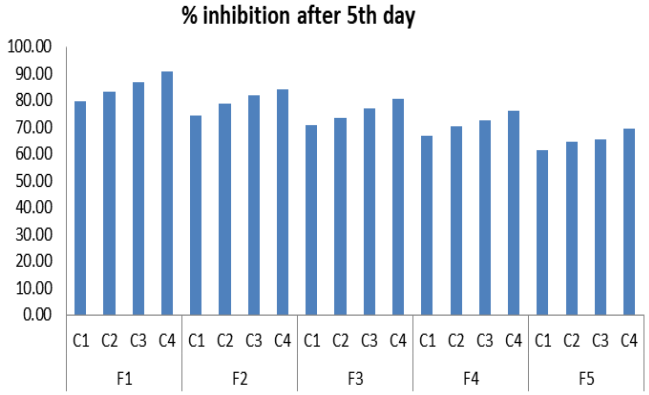

Figure 3. Graphical representation of % inhibition.

ANOVA TABLE FOR 7TH Day Fungicides
Analysis of variance table for growth

Source	DF	MS	F	P
Replication	2	0.0094		
Concentrations (C)	3	0.1576	44.19	0.0000
Fungicide (F)	5	13.6535	3828.29	0.0000
C * F	15	0.0197	5.54	0.0000
Error	46	0.0036		
Total	71			

Grand Mean =1.2454 ; CV =4.80

LSD All-Pairwise comparisons test of growth for concentration

Concentrations	Mean
C1	1.3478A
C2	1.2850B
C3	1.2200C
C4	1.1289D

Alpha 0.05
Standard Error for Comparison 0.0199
Critical T Value 2.013
Critical Value for Comparison 0.0401
Error term used: Replication*Concentration*Fungicide, 46 DF
All 4 means are significantly different from one another.

LSD All-Pairwise comparisons test of growth for fungicide

Fungicide	Mean
C	3.3750A
F5	1.1542B
F4	0.9500C
F3	0.8100D
F2	0.6750E
F1	0.5083F

Alpha 0.05
Standard Error for Comparison 0.0244
Critical T Value 2.013
Critical Value for Comparison 0.0491
Error term used: Replication*Concentration*Fungicide, 46 DF
All 6 means are significantly different from one another.

LSD All-Pairwise comparisons test of growth for concentration*fungicide

Concentration	Fungicide	Mean
C2	C	3.4167A
C4	C	3.4167A
C3	C	3.4000A
C1	C	3.2667B
C1	F5	1.2600C
C3	F5	1.1667C-D
C2	F5	1.1500D
C1	F4	1.0833D-E
C4	F5	1.0400E-F
C2	F4	1.0100E-G

C1	F3	0.9500F-H
C3	F4	0.9167G-I
C2	F3	0.8767H-J
C1	F2	0.8467I-K
C4	F4	0.7900J-K
C3	F3	0.7600K-L
C2	F2	0.6900L-M
C1	F1	0.6800L-M
C4	F3	0.6533M-N
C3	F2	0.6267M-O
C2	F1	0.5667N-O
C4	F2	0.5367O-P
C3	F1	0.4500P
C4	F1	0.3367Q

Alpha 0.05
Standard Error for Comparison 0.0488
Critical T Value 2.013
Critical Value for Comparison 0.0982
Error term used: Replication*Concentration*Fungicide, 46 DF
There are 17 groups (A, B, etc.) in which the meansare not significantly different from one another.

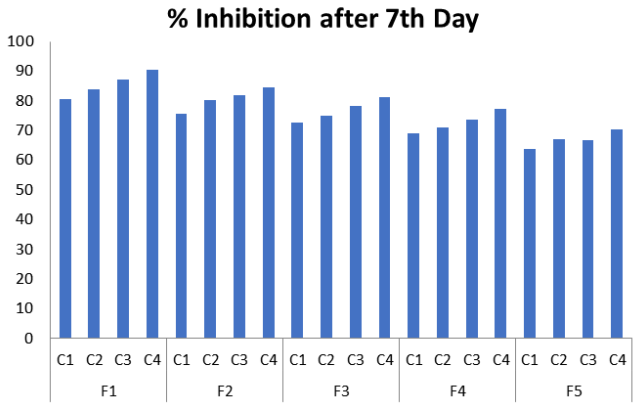

Figure 4. Graphical representation of % inhibition.

DISCUSSION

Alfalfa *(Medicago sativa)* is widely cultivated fodder crop in Pakistan and USA. However, its production is severely affected by several biotic stresses, with leaf spot disease caused by *Curvularia buchloes* emerging as a major threat. (Faraz et al., 2021) described that Curvularia leaf spot causesd severe loss in fodder crop. . The main purpose of this research was a management of leaf spot in alfalfa crop. Nanotechnology offers some green and eco-friendly solutions for plant disease management and also can be used as bio-manufacturing units, which will provide an added benefit in being easy to use, as compared to other. Some fungal species are non-pathogenic and combined with the simplicity of production and handling will improve the mass production of nanoparticles. Mycosynthesis of gold, silver, gold-silver alloy, selenium, tellurium, platinum, palladium, silica, titania, zirconia, quan-tum dots, usnic acid, magnetite, cadmium sulphide and uraninite nanoparticles has also been reported by various researchers (Alghuthaymi et al., 2015) Silver has both ionic and nanoparticle types of antimicrobial activity. It is believed that enzyme inactivation induces the great anti-microbial effect of silver primarily in unicellular microorganisms (Sadeghi et al., 2016). Michalecka et al. (2011) demonstrated the beneficial use of SNPs instead of commercial fungicides. Application of nanotechnology in plant pathology is still in the early stages. For example, nanofungicides, nanopesticides and nanoherbicides are being used exten-sively in agriculture practices (Prasad et al., 2014). Many kinds of nanoparticles were present but silver nanoparticle was most effective in disease management. That's way silver nanoparticles were used. Silver nanoparticles were synthesized by using Fungal/Bacterial strain to analyze their antifungal potential. Silver nano-particles was used to find out the optimum concentration which reduces the growth of fungal pathogens. As far as my results concerned different concentration (50ppm, 100ppm, 150ppm) of silver nanoparticles were used for the management of *Curvularia* leaf spot of alfalfa but 150ppm concentration was most effective in controlling the disease as compared to other concentrations. Instead of others, at 150ppm concentration growth of inhibition was grater. Silver nanoparticles at 150ppm concentration were proved most effective control agents for the management of curvularia spp. This observation aligns with the findings of other studies, conducted by Nisar et al. (2020), who demonstrated high antimicrobial activity of AgNPs, causing fungal cell membranes as well as enzyme systems disruption. In addition, Ivanišević, (2023) highlighted that biogenic AgNPs are very environmental friendly and can be potential choices in sustainable agricultural practice. Through a parallel examination of the tested fungicides, Iprodione showed the best antifungal activity against the fungal pathogen, while Copper oxychloride and Thiram showed comparable effects. These findings provide supports the research by Haq et al. (2021), which reported that Iprodione was effective in managing Curvularia species in alfalfa. Nevertheless, environmental concerns due to excessive use of chemical fungicides do exist, such as development of resistance and residue components in both the soil and products (Russell, 1995). This study provides strong evidence for the potential of biogenic silver nanoparticles as an effective component of integrated disease management strategies against Curvularia-induced leaf spot in alfalfa. Future research should explore the long-term effects of AgNPs in field conditions, their interaction with plant physiology, and their potential integration into large-scale crop protection programs.

***Conclusion*:** The present study was conducted to address the emerging threat of leaf spot disease in alfalfa (*Medicago sativa*), caused by *Curvularia buchloes*, which poses a serious challenge to forage crop productivity in Pakistan. Given the economic significance of alfalfa and the increasing prevalence of fungal pathogens, this research evaluated the efficacy of integrated disease management strategies, including the application of fungicides and silver nanoparticles (AgNPs), in both in vitro and in vivo experiment.

The isolated Curvularia pathogen was confirmed through pathogenicity tests and morphological identification. Silver nanoparticles were biosynthesized and applied at different concentrations (50 ppm, 100 ppm, and 150 ppm) using the poisoned food technique. Among these, 150 ppm showed the highest antifungal activity by significantly inhibiting the mycelial growth of the pathogen. This suggests that biosynthesized silver nanoparticles can serve as effective and eco-friendly alternatives to synthetic fungicides.

***CRediT author statement*:** Sidra Saleem performed the experiments, collected and analyzed data, and drafted the manuscript.Muhammad Tauseef Tariq Kisana provided resources, and critically reviewed the manuscript. Iftikhar Ahmad assisted with statistical analysis and contributed to data interpretation and manuscript revision.

***Conflict of interest*:** We declare manuscript is our original Research work and No conflict of interest.

***Ethical statement*:** All experiments included in this research paper have been planned keeping in the mind of ethics of research.

***Availability of data*:** We declare that the work we have submitted is original, has never been published and is not being considered for publication anywhere.

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