Antifungal activity of plant extracts and Silver nano particles against Citrus brown spot pathogen (*Alternaria citri*).

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Abstract— *Citrus Brown spot caused by Alternaria citri. Different control strategies should be used for management of disease and for increasing potential yield of citrus. Hence in-vitro potential of Plant extract and Silver Nano particles were evaluated for control of Brown spot pathogen in Citrus mangroves of Pakistan. Four different plant extracts garlic, neem, mint and basil leaves extracts were used at concentration of 20%. 40% and 60 %. Their effect on radial mycelial growth was checked with reference to untreated or control petri plate. It was observed that almost all concentrations of plant extracts significantly reduce radial mycelia growth as compared to control. In all treatments of plant extracts it was observed that 60% concentration of neem extracts is effective with radial mycelia growth of 3.96 cm over control petri plate which is 7.73cm, garlic extract@ 60% gave least fungal mycelia growth 5.5cm followed by mint leaves @60% gave 3.16 cm radial growth and Basil leaves extract @60% reduces fungal mycelia growth upto 3.93 cm as compared to control petri plate. Nano particles were used for invitro control of brown spot pathogen .Ten different concentration of nanonparticles were used as 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppm. The result of study revealed that by increasing concentration of nano particles inhibit mycelial growth of pathogen more significantly and 100 ppm concentration of Silver nano particles reduce fungal mycelia growth upto 2.63cm as compared to untreated petri plate fungal growth 7.23cm.*

Keywords— Alternaria citri, green fungicides, nano particles, plant extracts.

I. INTRODUCTION

Pakistan has wide range of ecological climate, more than thirty one types of fruits has been produced in Pakistan like mangoes, guava, apple, peach, pear that comprises more than 73% of Gross annual production. From all fruits Citrus (Citrus reticulate) share one third in export[1].It has great export potential almost 250,000 tons of kinnow is exported to Russia, South East Asia and Middle East from Pakistan with foreign exchange of 120 million dollars. Punjab is major citrus growing province and 49% of citrus is produced here in Pakistan [2]. A large number of export & processing units have been established in last two decades in Sargodha. Citrus is attacked by wide range of pathogens include fungi, nematode, bacteria, viruses. Fungi play an important role in decreasing quality and quantity among all pathogens. Citrus is attacked a large number of diseases that includes Anthracnose, citrus scab, citrus decline, citrus canker, citrus scab and blemishes. Anthracnose, melanose, greasy spot and fruit blemishes are important diseases that are caused by fungi. The quality, quantity and market value greatly affected by fruits blemishes [3].

Alternaria citri is the casual organism of brown spot of citrus. Different pathotypes are associated with the diseases that are characterized on the bases of host specificity. Almost all species of citrus is attacked by different pathotypes oranges are not affected by the disease but considerable spotting develops on grape fruit [4]. Spores of *Alternaria* are found on twigs, leaves and root lesions on the tree and on the ground. Spore germination favored by presence of moisture, spores are disseminated by air and rain splashes. Disease symptoms appear on young leaves after 36 and 48 hours after infection. Fruit is the most susceptible part that continues to develop symptoms even after four months of petal fall [5]. Thousands of phyto chemicals like phenols, phenolic acid, quinonels, flavanoides, flavanoles, pannins have inhibitory activity against wide range of pathogens. Plant extract have anti microbial properties. Brown spot is managed biologically by application of plant extracts,

these extracts have antifungal activity and are used as botanical fungicides and pesticides. Proper agronomic practices also reduces source of inoculum from vicinity/orchard. Timely irrigation and adequate fertlization plays an important role in controlling disease causing pathogens [6]. Nano particles consists of nano silver combined with water soluble polymer and manufactured by exposing silver salt solution and silicate radioactive radiations[7]. Nano sized silica silver in concentration of 3.0 ppm has strong antifungal activity against wide range of phytopathogenic fungi however many useful bacterial species were not affected at 10ppm concentration but at 100ppm concentration of nano sized silica silver completely inhibit the activity of plant pathogenic fugi[8]. Now a day's whole worlds were thinking about debasement of the usage of botanicals as innovative chemotherapeutant in plant protection. Plant products had been widely used as pesticides now a days and some botanicals were used as green pesticide throughout the world. Some plant products like neem oil , pyrethriods and some essential oil has been used of some trees were used as antimicrobial agent against stored grains pests because they were easy to handle and they have no harmful effect fungal hyphal growth is greatly inhibited by using silver nano particles in those dependent manner. Efficacy of silver nano particles was observed among fungi on the hyphal growth in the following manner *Rhizoctonia R.solanae* ≥ *S.sclerotium* ≥ *S.minor*. *Sclerotium* germination growth proves that silver nano particles significantly inhibit its growth. High concentration of silver nano particles was effective in inhibiting sclerotial germination growth of s.sclerotia[9]. When hyphae of fungi that were exposed to silver nano particles was observed under microscope shows that nano particles severely damage the hyphal and leads to collapse and separation of layers of hyphae [10]. This research suggests using silver nano particles and plant extracts as an alternative to fungicides for management of *Alternariacitri*.

II. MATERIALS AND METHODS

2.1 Evaluation of Bio extracts

Four different plant extracts were used to inhibit the growth of test fungus. Mint, neem oil, garlic and basil leaves. Leaves were dried under shade and grinded to make fine powder. Required amount of powder was dissolved in distill water and passed through muslin cloth to make the desire concentration. Poison food technique was used for evaluation of plant extracts [11]. PDA(potato dextrose agar) was poisoned with plant extracts at concentration of 20 % , 40 % and 60 % test fungus was inoculated on it and petri plates were incubated at 25^0 Cfor 7 days. Radial mycelial growth was measured to check the inhibition of fungal growth. The petri plates were randomized into three replications using LSD.

TABLE 1
EVALUATION OF DIFFERENT CONCENTRATIONS OF BASIL LEAVES

Concentrations of Plant extracts (%)				
Treatments	**Neem**	**Garlic**	**Basil**	**Mint**
T1	20	20	20	20
T2	40	40	40	40
T3	60	60	60	60
T4	Control	Control	Control	Control

2.2 Evaluation of Nano particles against *Alternaria citri*

Stock solution of 1M of nano particles were prepared by dissolving silver nitrate and reductase in a proper concentration. The reaction was carried at room temperature 25^0C and constantly stirs the solution [12]. Poison food technique was used for evaluation of nano particles. We prepare 10 different concentration of nanoparticles 10 ppm, 20 ppm, 30 ppm, 40 ppm 50 ppm, 60 ppm, 70 ppm, 80 ppm 90 ppm and 100 ppm by diluting the stock solution of silver nano particles with the help of distilled water in a specific amount. PDA was prepared and solution of required concentrations of nano particles were added in the liquid medium in petri plate than mix the media containing nano particles solution poisoned media was allowed to solidify after solidification pure culture of *Alternaria citri* was inoculated on the poisoned media and incubated at 25^0C for seven days . In order to check inhibition of fungal growth by silver nano particles the radial mycelia growth was measured daily for 7 days. Three replication of each concentration of nano particles were made and compared with growth in control plates. Percentage of inhibition was measured with the help of mean colony diameter and calculated by following formula [13].

$$\text{Percent inhibition} = X - Y / X$$

X= colony diameter in check

Y = colony diameter on nano particles treated plate

TABLE 2
EVALUATION OF NANO PARTICLES AGAINST *ALTERNARIA CITRI*

Nano particles Concentration	
1 mMnano particles	T1 10 ppm
	T2 20 ppm
	T3 30 ppm
	T4 40 ppm
	T5 50 ppm
	T6 60 ppm
	T7 70 ppm
	T8 80 ppm
	T9 90 ppm
	T10 100 ppm
	T11 Control

2.3 Statistical Analysis

The data recorded was collected regarding the growth of fungus and was subjected to stastical analysis by using MSTAT-C. Means of the treatments were analysed by applying LSD (Fisher least significance difference) [14] at confidence interval 95%.

III. RESULTS AND DISCUSSION

3.1 Basil Leaves

Statistical analysis of treatments were shown in table basil extract were used at concentration of 20 ,40 and 60 % followed by 32.1, 39.82 and 44.096 % average decrease in colony growth over control. All means are significantly different are not significant.

TABLE 3
COMPARISON OF MEANS OF COLONY GROWTH OF *A. CITRI AT* DIFFERENT CONCENTRATIONS OF BASIL LEAVES.

Treatments	Means of colony in diameter (Cm)	Av. Decrease in Colony growth over Control (%)
T1 20 %	4.66 [c]	32.71
T2 40 %	4.23 [b]	39.82
T3 60 %	3.93 [a]	44.096
T4 Control	7.03 [d]	_
LSD Value	0.9	

LSD at 0.05 probability level

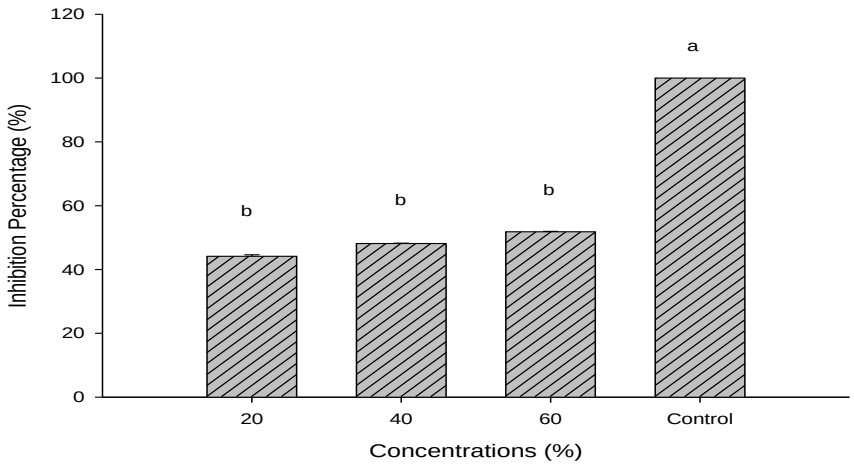

FIG 1: INHIBITION % AGE OF *A.CITRI* AT DIFFERENT CONCENTRATIONS OF BASIL LEAVES.

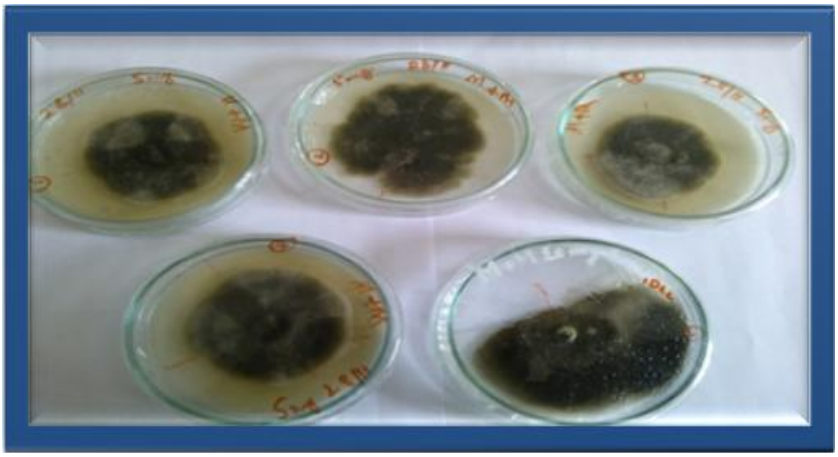

FIG 2: EFFECT OF BASIL LEAVES ON GROWTH OF *A. CITRI*

Plant extract have fungicidal properties basil leave were used at 20%, 40% and 60% concentrations and they inhibit the growth of test fungus. Maximum inhibition zone was formed at 60%.

3.2 Garlic Extract:

Statistical analysis of treatments were shown in table garlic extract was used at concentration of 20 ,40 and 60 % followed by 9.72, 24.8 and 26.66 % average decrease in colony growth over control . All means are significantly different are not significant.

TABLE 4
COMPARISON OF MEANS OF COLONY GROWTH OF *A. CITRI AT* DIFFERENT CONCENTRATIONS OF GARLIC EXTRACT.

Treatments	Means of colony in diameter (Cm)	Av. Decrease in Colony growth over Control (%)
T1 20 %	6.8 ^{c}	9.72
T2 40 %	5.7 ^{bc}	24.8
T3 60 %	5.$^{5\ a}$	26.66
T4 Control	7.5 ^{d}	-
LSD Value	0.9	

LSD at 0.05 probability level all means are different as not showing common letter

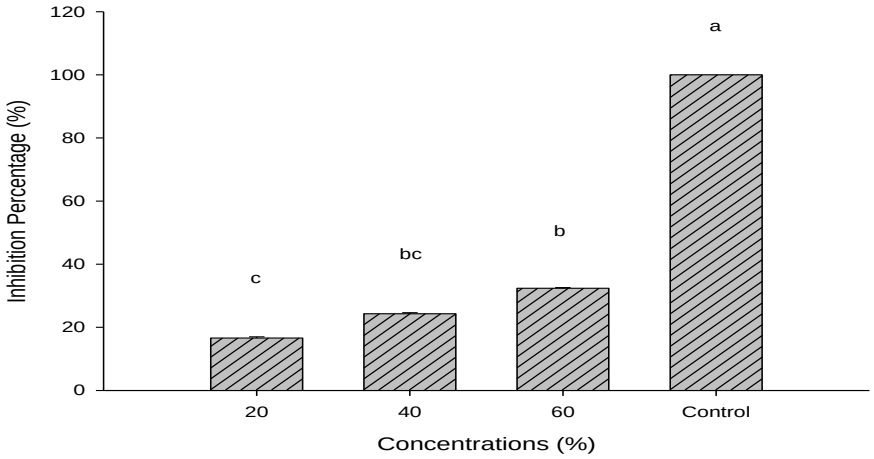

FIG 3: INHIBITION %AGE OF *A. CITRI AT* VARIOUS CONCENTRATIONS OF GARLIC EXTRACT

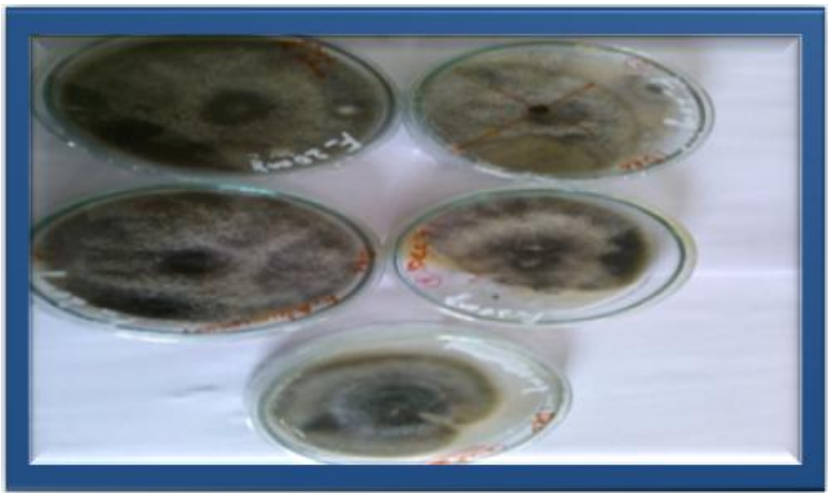

FIG 4: EFFECT OF GARLIC EXTRACT ON *A.CITRI*

Garlic extract was used at 20,40 and 60% concentrations the above graphs indicates that garlic extract have maximum antifungal activity at 6o% concentration. It inhibit spore germination of *A.citri.*

3.3 Mint Leaves

Statistical analysis of treatments were shown in table mint extract was used at concentration of 20 ,40 and 60 % followed by, 48.96, 57.98 and 59.27 % average decrease in colony growth over control . All means are significantly different are not significant.

TABLE 5

COMPARISON OF MEANS OF COLONY GROWTH OF *A. CITRI AT* DIFFERENT CONCENTRATIONS OF MINT LEAVES.

Treatments	Means of colony in diameter (Cm)	Av. Decrease in Colony growth over Control (%)
T1 20 %	3.96 [cd]	48.96
T2 40 %	3.26 [bc]	57.98
T3 60 %	3.16 [a]	59.27
T4 Control	7.76 [e]	_
LSD Value	0.5	

LSD at 0.05 probability level .Means are significantly different

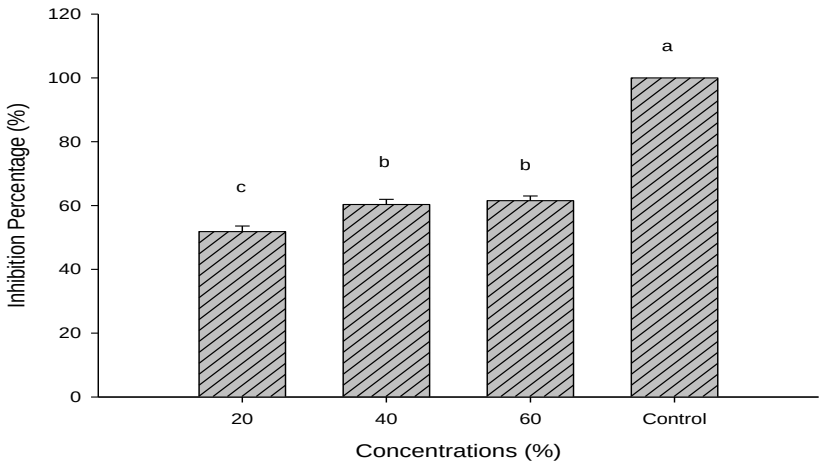

FIG 5: INHIBITION % AGE OF *A. CITRI AT* VARIOUS CONCENTRATIONS OF MINT LEAVES

Mint also have antifungal activity 20, 40 and 60% concentration were used in the experiment and it would supressed the growth of *A.citri* at 40 and 60% and forms maximum inhibition zone at these concentrations.

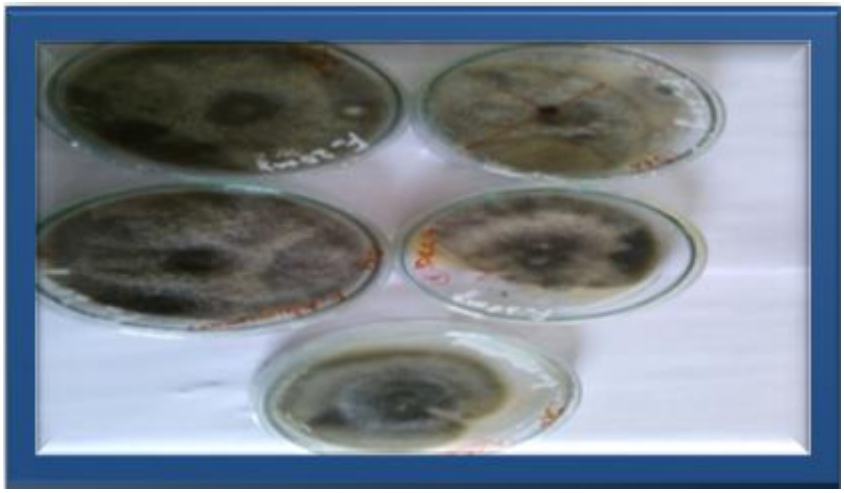

FIG 6: EFFECT OF MINT ON GROWTH OF *A.CITRI*

3.4 Neem Extract

Statistical analysis of treatments were shown in table neem extract was used at concentration of 20 ,40 and 60 % followed by 40.49, 44.76 and 49.54 % average decrease in colony growth over control . All means are significantly different are not significant.

TABLE 6
COMPARISON OF MEANS OF COLONY GROWTH OF *A. CITRI AT* DIFFERENT CONCENTRATIONS OF NEEM EXTRACT.

Treatments	Means of colony in diameter (Cm)	Av. Decrease in Colony growth over Control (%)
T1 20 %	4.6 [bc]	40.49
T2 40 %	4.27 [b]	44.76
T3 60 %	3.96 [a]	49.54
T4 Control	7.73 [d]	_
LSD Value	0.09	

LSD at 0.05 probability level all means are significantly different because not sharing common letter.

Neem was used as a antifungal agent and it greatly inhibit growth of *A. citri* . The above graph indicates that conidial germnation was inhibited at all concentration of neem and maximum inhibition zone was formed at 60 %.

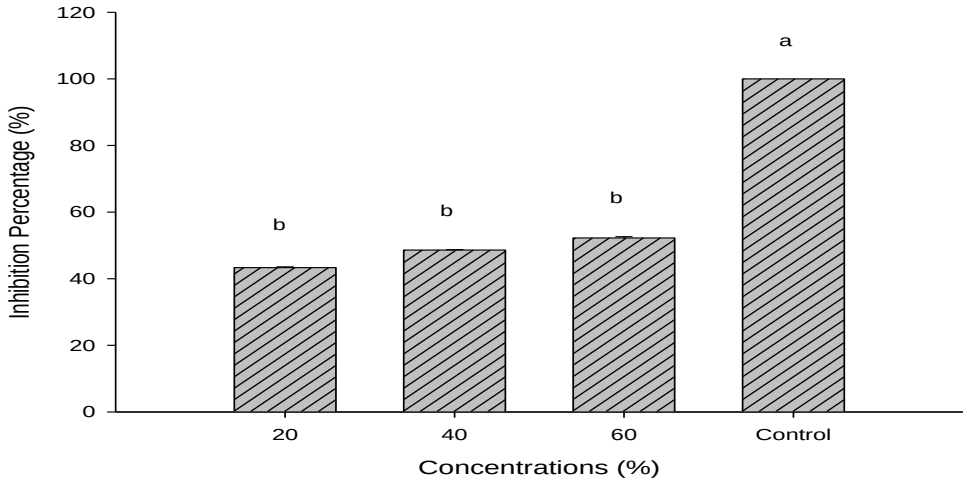

FIG 7: INHIBITION % AGE OF *A. CITRI AT* VARIOUS CONCENTRATIONS OF NEEM LEAVES

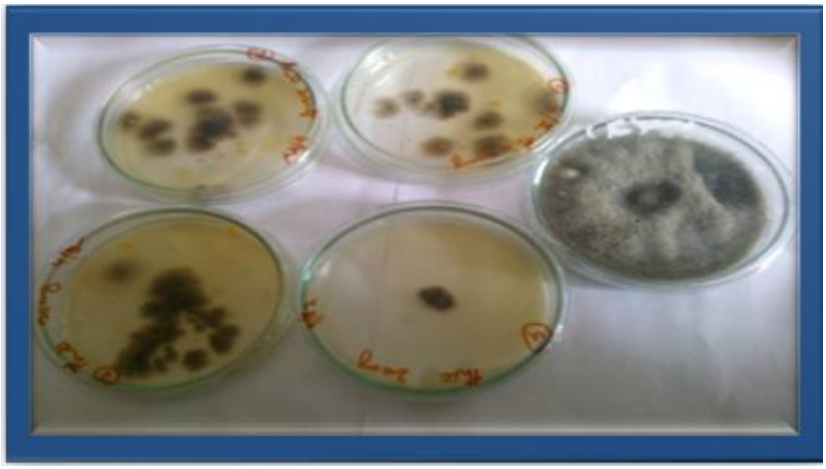

FIG 8 : EFFECT OF NEEM EXTRACT ON GROWTH OF *A. CITRI*

IV. DISCUSSION

Citrus is facing a lot of problems in Pakistan that reduce market value of fruit. Blemishes are major problem that also include citrus brown spot. Different control strategies should be used for management of disease and for increasing potential. Plant extracts and Silver nano particles were used in our study for management of diseases basically four plant extracts garlic, neem, mint and basil leaves extracts were used at concentration of 20%. 40% and 60 % and all concentrations reduces mycelial growth of associated pathogen .60 % concentration gave more significant results. Colony growth of *A. citri* was suppressed to 59 % by using plant extracts in vitro over control plate in our study so plant extracts were used as organic green pesticides for control of diseases. The effect of eleven botanicals on growth of different fungi also *A. citri* was studied and proved that 40% concentration of neem extract suppressed the colony growth of *A.citri* [15].The effect of botanicals on growth of *A. citri* and proved that growth of *A. citri* was inhibited by garlic, neem, mint basal pat 60% concentration [16].Garlic, basil and neem extracts against *A. citri* gave ideal control against the pathogen at 40% concentration of these extracts [17]. Neem was also used in another experiment and it gave effective control of *A. citri*[18]. Efficacy of neem extract against *Alternariacitri* showed that 30% concentration is ideal for inhibition of mycelial growth of *A.citri*[19].Botanicals are green pesticides they are cheap to handle and also eco friendly and has no harmful effects on ecosystem.

Nano particles were also used in our study for management of brown spot in-vitro. We used ten different concentration of nanonparticles that was 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 ppm. The result of our study revealed that by increasing concentration of nano particles inhibit mycelial growth of pathogen more significantly our study concides with Mohamed et al., 2014[20] who investigated on efficacy of nano particles against *A. citri*100 ppm concentration of nano particles was more effective against pathogen and it can supresesmycelial growth upto 63.77 %. Nano particles are modern technology in IPM and it is gaining popularity day by day. Synthesis of nano particles by *A.alternata* they suggested that nano particles had strong antifungal activity against all isolates of *A.citri*[21] [22]. Nano particles consists of nano silver combined with water soluble polymer and manufactured by exposing silver salt solution and silicate radioactive radiations. Nano sized silica silver in concentration of 3.0 ppm has strong antifungal activity against wide range of phytopathogenic fungi however many useful bacterial species were not affected at 10 ppm concentration but at 100 ppm concentration of nano sized silica silver completely inhibit the activity of plant pathogenic bacteria.

V. CONCLUSIONS

In the present study antifungal activity of plant extracts and Nano particles were evaluated against citrus brown spot pathogen *Alternaria citri.* Neem, garlic, basal, and mint leaves extracts were used in the study for in vitro control of disease it was found that these botanicals are green pesticides for inhibiting the mycelial growth of *Alternaria citri*. 20 %, 40 % and 60 % concentrations of these extracts were made for mycelial inhibition of pathogen all plant extracts @60% were effective for management of citrus brown spot pathogen. Nano particles were also used in study and different concentration of nano sized silver particles which were used includes 10, 20, 30,40,50, 60, 70,80, 90 and 100 ppm. Average decrease in colony growth was maximum at 100 ppm concentration over control at 100 ppm concentration maximum inhibition was up to 63.77 %. Nano particles are also used in place of synthetic chemicals for disease management and amount of inoculums in the field is reduced by using plant extracts and nano particles as an alternative to chemical control and moreover this is eco friendly method for management of fungal plant pathogens.

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