

RESEARCH ARTICLE

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Assessment of Plant Extracts and their *In vitro* Efficacy against Potato Early Blight Incited by *Alternaria solani*

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Abstract

Botanicals obtained from the plants are well known for the suppression of inimical plant pathogens. The present study explores the efficacy of five locally available plant extracts for their antifungal activity against the early blight of potato incited by *Alternaria solani*. The extracts include *Datura stramonium*, *Allium sativum*, *Azadirachta indica*, *Eucalyptus globulus*, and *Lantana camara*. All extracts reduced mycelial growth and conidial germination of *A. solani*. *In vitro* studies showed that extracts obtained from *A. sativum* and *A. indica* have significant inhibition of mycelial growth of *A. solani* (88.80 and 86.62 percent) at 20 percent concentration. Higher concentrations of *A. sativum* extract caused a higher reduction of *A. solani* radial growth on potato dextrose agar medium. Extracts obtained from *A. sativum* and *A. indica* at 20 percent concentration, were found most effective for inhibition of conidial germination (85.50 and 80.04 percent) respectively of *A. solani*. Observations by scanning electron microscope (SEM) showed dramatic alteration in *A. solani* hyphae collapsed and spores shrunk when treated with extract of *A. sativum* at a 20 per cent concentration. The qualitative and quantitative analysis of various phytochemicals like flavonoids, alkaloids, saponins, tannins, steroids, terpenoids, glycosides, and phenols was showed *A. sativum* extract better than all the other plant extracts. Observation also revealed that 20 percent concentration of garlic extract has potential to inhibit to *A. solani*.

Keywords: *Alternaria solani*, Plant extracts, Scanning Electron Microscope, Mycelial deformation

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Abbreviations: ITCC- Indian Type Culture Collection, PDA- Potato Dextrose Agar, CRD- Completely Randomized Design, mm- milli meter, ml- milli litre, µl- micro litre, LCB- Lactophenol Cotton Blue, SEM- Scanning Electron Microscopy, M- molar, h- hour, nm- nano meter, mins- minutes, g- gram, mg- milligram, N- normal, °C- Degree Celsius, ANOVA- Analysis of Variance, CD- Critical Difference.

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INTRODUCTION

The potato (*Solanum tuberosum* L.), belong to family *Solanaceae*, is important tuber crops in sub-tropical and temperate regions also called as "King of vegetables"¹. Potato is excellent source of vitamins, amino acids, carbohydrate, proteins, minerals and phytochemicals such as phenolic compound and carotenoids². Presence of these nutrients reduces risk of several diseases such as cancer, diabetes, cataract and aging. This crop subjected to attack by more than hundred diseases including fungi, bacteria, virus and viroid. Among fungal diseases, early blight is one of the most detrimental fungal disease caused by *Alternaria solani* (Ell. and Mart.), present all over the world particularly potato growing Asian countries³. Primary infection by *Alternaria solani* can cause premature defoliation and small, irregular to circular dark brown spots on potato leaves. Lesions surrounded by a dark brown border resulting shallow, dry, corky rot produce on the older leaves considered as a foliar symptom. The disease can cause 70 to 80 percent yield loss in India^{4,5} and 80- 100 % worldwide⁶. Several fungicides are reported for the control of early blight, that is, chlorothalonil and triphenyl tin hydroxide^{7,8}. In current scenario use of eco-friendly methods for management of plant diseases, to minimize synthetic chemicals and fungicides, caused environmental hazards to human and animals^{9,10}. Resistant cultivars are an environment friendly method against this disease but resistant cultivars not durable for long time. Development of resistant cultivars is time consuming and expensive so there is need to develop holistic model to management early blight of potato¹¹. Current study aimed to develop eco-friendly methods including botanicals for control early blight of potato. Use of botanicals or plant extract in plant disease management because they are eco-friendly, nonpolluting, cheaper, cost effective strategy, easily biodegradable and effective biocontrol methods, can be used in integration with other strategies for protection of crop plants¹². The uses of plant extracts or phytochemicals in plant defence system against pathogen are not hazardous to environment, human and animals' health. Numerous plant pathogenic microorganisms are controlled by

the application of various plant extracts such as *Azadirachta indica*, *Allium sativum*, *Urtica spp.*, *Ruta graveolens* and *Melaleuca alternifolia*¹². Many studies have shown that effects of plant extract for controlling the plant pathogens under *in vitro* conditions^{13,14}. Chapagain et al.¹⁵ reported the use of saponin rich extract of *Balanites aegyptica* fruit against *A. solani*. Another study by Muto et al.¹⁶ tested 14 natural plant species against *A. solani*. Neem (*Azadirachta indica*) extract have antifungal¹⁷, antibacterial¹⁸, and antiviral¹⁹ properties. Plants possess numerous secondary metabolites compounds showing plant defense mechanisms against pathogen¹⁹. Aromatic secondary metabolites like terpenoids, flavonoids, tannins, glycosides, saponins, sterols and alkaloids are synthesized by the natural plant species²⁰. Availability of such bioactive compounds in plant species which are easily biodegradable and non-phytotoxic^{22,23}. Current study explores plant extracts against *A. solani* under *in vitro* conditions.

MATERIALS AND METHODS

Plant extracts used in the study were obtained from *Datura stramonium*, *Allium sativum*, *Azadirachta indica*, *Eucalyptus globulus* and *Lantana camera*.

Plant materials and preparation of plant extracts

D. stramonium, *A. indica*, *E. globulus* and *L. camera* were collected from the agricultural research farm. *Allium sativum* was obtained from local vegetable market. Apparently fresh leaf material was collected and washed with distilled water and then surface sterilized with sodium hypochlorite (NaClO) 1 percent solution for 2 mins then rinsed thrice with sterilized distilled water. Surface sterilized plant leaves were air dried in room temperature (35°C) and homogenized to make 1 to 2 mm diameter fine powder using a grinder. Aqueous extracts were prepared by decoction method²⁴. In brief 20g dried fine powder of every plant sample was gently mixed with 80 ml of sterile distilled water and heated for 10 to 15 mins to remove contamination. Subsequently solution was filtrated through double layered muslin cloth, and finally centrifuged at 8,000 rpm for 25 mins. The obtained supernatant from previous step was filtered through Whatman filter paper No. 1 and sterilized in autoclaved. Sterilized

solution considered as 100 percent concentration and preserved aseptically in separate sterile beakers at 4°C until experiment completion.

Collection of pure culture of pathogen

The authentic culture of *A. solani* was borrowed from the Indian Type Culture Collection, New Delhi, with the ITCC No. 3640. The fungus was sub-cultured and maintained on potato dextrose agar medium (PDA) at 4°C. The pathogenicity was checked under *in vitro* condition by detached leaf assay (Fig.1).

Evaluation of different plants extracts under *in vitro* condition

To check the efficacy of five plant extracts against test pathogen under the *in vitro* condition was done with the help of poisoned food technique²⁵. Different concentrations of test extracts, including 5, 10, 15 and 20 percent was added in PDA medium, mixed gently and 20 ml media poured in Petri dishes. PDA medium without plant extract was taken as control. Petri dishes containing different test extracts with PDA were placed at the centre of each plate with 5 mm diameter mycelial disk from 10 days old *A. solani* culture and plates were incubated at 27°C for one week and radial growth of mycelium was measured at 7 days after inoculation. This experiment was done with three replications for each treatment. To measure diameter of test pathogen colony by using two perpendicular lines in each Petri plates. The percent inhibition of test pathogen was assessed using the formula²⁶.

$$I = C - T/C \times 100$$

Where, I= Per cent inhibition of mycelia growth
C = Growth of mycelium in control (mm)
T = Growth of mycelium in treatment (mm)

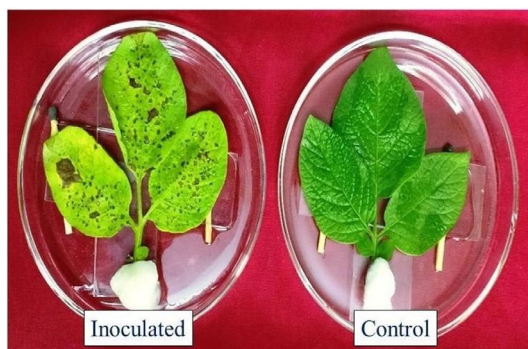


Fig. 1. Pathogenicity test: Leaves detached from plants 1 week after inoculation

Preparation of conidial suspension

Conidial suspension was prepared by following Stammler et al.²⁷ with slight modification. 10 ml sterilized distilled water was added to every Petri plate and 7-day old spore colonies were scraped with the help of rubber spatula. 10 ml spore suspensions were made from each plate and conidia density was adjusted to 1.8×10^4 spores ml⁻¹ with the help of haemocytometer.

Inhibition of conidial germination of test pathogens

Five plant extracts were used to check the antifungal activity of aqueous extract on conidial germination of *A. solani* through microscopic slide technique with slight modification²⁸. The plant extracts at four different concentrations (5, 10, 15 and 20%) was added as films on dried clean microscopic slide. 50 µl conidial suspension was spread over these films and sterile distilled water slide served as a control. Every slide was transfer on a glass rod in a Petri dish under moist conditions and incubated for 3 days at 27°C²⁹. Five slides were replicated three times for each concentration. Lactophenol cotton blue (LCB) was added to each slide to arrest conidia germination after incubation. Each slide was observed under a light microscope at ×400 magnification to determine the germination rates of conidia. The experiment was repeated four times, with three replications every time. The percent inhibition of conidial germination for each treatment was assessed by the given formula:

$$\text{Percent inhibition} = C - T/C \times 100$$

Where, C = Germinated conidia in control
T = Germinated conidia in treatment,

Mycelial deformation study by Scanning electron microscopy (SEM)

Mycelial disks treated with the plant extracts (*Datura stramonium*, *Allium sativum*, *Azadirachta indica*, *Eucalyptus globulus* and *Lantana camera*), among them *A. sativum* (20 %) and mycelial growth of *A. solani* was prepared by following procedure described by 30 with slight modifications. Mycelial disks were fixed with Karnovsky solution containing 2.5 percent glutaraldehyde, 2 percent paraformaldehyde in a 0.05 M sodium cacodylate buffer (pH 7.2) for two days for SEM study. The mycelial disk gently washed 3 times by same buffer solution for 35 mins, followed by fixation done with 1 per cent

osmium tetroxide solution for 2 h further washed thrice using sterilized distilled water and then dehydrated in a crescent acetone solution series that is, 30, 50, 70, 85, 90 and 100 percent. The samples were then dried in a critical point dryer (Bal-tec CPD 030) with carbon dioxide. Finally, the specimens were spotted on aluminium stubs and fixed with double sided black tape and coated with 20nm gold layer with the help of vacuum evaporator (Bal-tec SCD 050). All specimens were visualized under Joel SEM- 6400JSM- 6360LV microscope (JEOL Ltd., Japan).

Qualitative analysis of phytochemical screening of different aqueous plant extract

The aqueous extract of five selected plants (*D. stramonium*, *A. sativum*, *A. indica*, *E. globulus*, and *L. camera*) was tested for antimicrobial secondary metabolites properties against test pathogen.

Alkaloids

1.5 ml extract was taken for alkaloid testing. 2 ml of 1 per cent HCL solution thoroughly mixed with test extract. After mixing, solution was heated for to 2 mins and Mayer's reagent (6 drops) was added. Orange colour formation indicates the presence of alkaloids³¹.

Flavonoids

1 ml of aqueous solution of test extract was gently mixed with 1 ml of 10 percent lead acetone. Develop yellow colour that showed the presence of flavonoids³².

Saponins

The procedure described by Saidulu et al³³ was followed for the assay 2 ml of aqueous test extract was taken and mixed with 6 ml sterilized distilled water. Formation of froth was observed after 3 mins.

Steroids

0.5 ml test extract mixed with 2 ml of acetic anhydride solution and further 2 ml H₂SO₄ was added to the reaction. The colour change violet to green or blue expressed the presence of steroids³⁴.

Tannins

Few drops of ferric chloride solution was mixed with test extract. Development of blackish colour expressed the presence of tannins in the extract³³.

Terpenoids

2 ml test extract was mixed with 5 ml

of chloroform and heated for 5 mins and then allowed to dry. Further 2 ml concentrated H₂SO₄ was added and again heated on low flame for 2 mins. Greyish colour develops to show presence of terpenoids³³.

Glycosides

Each test extract was hydrolysed with hydrochloric acid and then neutralized with NaOH solution. Red colour developed after Fehling's solution A and B were mixed and this revealed that presence of glycosides³⁵.

Phenols

1 ml of test extract was extracted in ethanol and then evaporated till dryness. After drying residue was dissolved in sterile distilled water and add 0.5 ml Folin-ciocalteu reagent followed by 5 ml of 20% Na₂CO₃ solution. Bluish colour developed reveals that presence of phenols³⁶.

Quantitative analysis of phytochemical screening of different aqueous plant extract

Alkaloids test extract weighed 2 ml in to 250 ml capacity sterile conical flask. 200 ml glacial acetic acid in methanol was added and mixed gently. Flask were covered with aluminium foil and incubated at 35°C for 4 h. Then filtered obtained from previous step was and concentrated in water bath. Further few drops of concentrated NH₄OH were added into test extract for precipitation. The precipitate was extracted, washed with dilute NH₄OH and again filtered. The remaining residue was calculated in term of percentage³⁷.

Flavonoids

10 g each test extract was extracted with the 100 ml of 80 percent ethanol solution at 37°C. The obtained solution was filtered by the help of Whatman filter paper No. 42 and then filtrate placed into water bath for dryness. The weight of solution and percentage quantity was calculated according³⁸.

Saponins

20 g each dried sample and 20 per cent ethanol was added. Sample was incubated in water bath for 1 h with constant shaking. The solution filtered and residue again extracted with 200 ml of 20 percent methanol. The extracts were allowed to be reduced up to 40 ml in water bath. Concentrated solution placed into a 250 ml capacity separatory funnel, 20 ml diethyl ether was added and then vigorous shaking. Ether layer was

Table 1. Mycelial growth of *Alternaria solani* influenced by different concentration of plant extracts

Plant extract	5 %		10%		15%		20%	
	Radial growth (mm)	Inhibition %	Radial growth (mm)	Inhibition %	Radial growth (mm)	Inhibition %	Radial growth (mm)	Inhibition %
Datura	31.66±3.06 ^c	64.90	23.96±1.81 ^c	73.47	18.73±1.38 ^d	79.23	12.76±1.84 ^c	85.85
Garlic	18.96±0.32 ^d	78.98	16.06±0.90 ^d	82.19	14.40±0.45 ^e	84.3	10.10±0.20 ^d	88.80
Neem	41.40±0.65 ^b	54.10	29.86±0.75 ^b	66.89	20.80±0.30 ^c	76.94	12.06±1.00 ^c	86.62
Eucalyptus	33.10±0.26 ^c	63.30	28.23±0.61 ^c	68.70	25.13±0.35 ^b	72.13	20.50±0.65 ^b	77.27
Lantana	31.30±1.31 ^c	65.29	24.86±0.91 ^c	72.43	20.40±0.43 ^c	77.38	19.26±0.50 ^b	78.64
Control	90.00±0.00 ^a	-	90.00±0.00 ^a	-	90.00±0.00 ^a	-	90.00±0.00 ^a	-
C.D. = (0.05%)	2.51		1.78		1.17		1.66	

Results are expressed as means of three replicates, ± indicate standard deviation of the means. Different letters showed significant differences in treatments results according to Duncan's multiple rang test at P≤0.05.

formed and discarded, and purification process was repeated. After purification, 60 ml n-butanol was added and further extract were washed thoroughly twice with 15 ml of 5 percent NaCl. The solution was heated for evaporation, and then dried in hot air oven. Weighed and percentage was calculated of saponin³⁹.

Steroids

1 gm sample was crushed in 20 ml of methanol and filtered. 2 ml filtrate was taken and add 5 ml of chromagen solution. The solution was incubated at 35°C for 30 mins. Absorbance was recorded at 550 nm wavelength⁴⁰.

Tannins

500 mg samples were added in 50 ml distilled water. The solution allows to shaken for 60 mins and filtered. 10 ml filtrate was pipetted out into the new test tube and 2 ml 0.1 M ferric chloride in 0.1 N HCl was added along with 0.008 M potassium ferrocyanide. The final absorbance was recorded at 120 nm wavelength⁴¹.

Terpenoids

1 g of extracts were taken, crushed in 50 ml of methanol and filtered. 2.5 ml filtrate was taken and 2.5 ml of 5 % phosphomolybdic acid was added afterward 2.5 ml of concentrated H₂SO₄ were added. The mixture was incubated at 35°C for 30 mins. The final observance was recorded at 700 nm wavelength⁴⁰.

Glycosides

1 g test samples were taken and macerated with 50 ml of methanol and filtered. 1 ml supernatant was taken and 4 ml of alkaline pirate solution was added. The reaction mixture was heated for 5 mins and allowed to cool. The observance was recorded at 490 nm wavelength³⁴. Phenols: 2 g test samples were taken in 100 ml diethyl ether solution and allowed to defat with help of Soxhlet apparatus for 2 to 3 h. Defatted solution was heated with 50 ml of ether for 15 mins. 10 ml of reaction was pipetted out into 50 ml capacity flask and 15 ml sterile distilled water was added. Further, 2 ml of NH₄OH solution and 2 ml of concentrated amyl alcohol were added. The reaction was incubated at 35°C for 30 mins. Absorbance was recorded at 505 nm wavelength⁴¹.

Statistical analysis

The analysis of data with help of one-way analysis of variance (ANOVA) and compared means for significance at p level of ≤ 0.05 through

Table 2. Conidial germination of *Alternaria solani* exposed to different concentrations of plant extracts under *in vitro*

Plant extract	5 %		10%		15%		20%	
	Percent conidial germination	Inhibition %	Percent conidial germination	Inhibition %	Percent conidial germination	Inhibition %	Percent conidial germination	Inhibition %
Datura	54.97±19.67 ^b	39.23	38.76±13.22 ^b	57.13	31.08±20.58 ^b	65.62	24.77±5.74 ^{bc}	72.60
Garlic	30±9.35 ^b	64.60	21.95±4.37 ^b	75.72	18.08±2.45 ^b	80	13.11±2.45 ^c	85.50
Neem	39.94±9.12 ^b	55.82	30.32±4.49 ^b	66.46	29.16±3.65 ^b	67.75	18.04±3.34 ^{bc}	80.04
Eucalyptus	60.45±22.43 ^b	33.14	46.67±17.65 ^b	48.38	34.72±12.48 ^b	61.60	28.71±11.66 ^b	68.24
Lantana	43.21±19.93 ^b	52.21	34.57±18.83 ^b	61.76	25.53±11.25 ^b	71.76	21.60±8.38 ^{bc}	76.11
Control	98.28±0.51 ^a	-	98.28±0.51 ^a	-	98.28±0.51 ^a	-	98.28±0.51 ^a	-
C.D. = (0.05%)	33.97		26.69		23.96		14.26	

Results are expressed as means of three replicates, ± indicate standard deviation of the means. Different letters showed significant differences in treatments results according to Duncan's multiple rang test at P≤ 0.05.

Duncan's multiple-range test (DMRT) (IBM SPSS, Version 20).

RESULTS

Effects of plant extracts on mycelial growth of *A. solani*

All tested plant extracts were found effective against mycelial growth of *A. solani* as compared to control (Table 1). In general, proportional reduction of mycelia growth was showed with increasing the concentration of plant extracts from 5 to 20%. The minimum colony diameter (10.10 mm) was recorded on *A. sativum* amended media followed by *A. indica* (12.06 mm) and *D. stramonium* (12.76 mm) extracts at 20% concentration (Fig. 2). The full growth was recorded in control after seven days of inoculation. The maximum colony diameter (20.50 mm) was recorded on *E. globulus* extracts at 20% concentration compared to other plant extracts.

Effects of plant extracts on conidial germination of *A. solani*

The results from the conidial germination assay by various plant extracts are represented in Table 2. Conidial germination was decreased gradually with increase concentration of tested plant extracts from 5 to 20%. All plant extracts were significantly decreased the conidial germination of *A. solani* compared with the control. *A. sativum* and *A. indica* extracts were found best among all extracts for the inhibition for conidial germination. Percent inhibition of conidial germination was observed highest at 20% concentration of *A. sativum* (85.50%) and *A. indica* (82.04%) extracts. Least reduction of conidial germination was observed in *E. globulus* (68.24%) at 20% extract concentration. *E. globulus* and *D. stramonium* extracts were found less effective on inhibition conidial germination than the other three extracts. Inhibition started at low concentration (5%) with low values (33.14 and 39.23 for *E. globulus* and *D. stramonium*, respectively).

Qualitative screening of phytochemicals in aqueous extract

Phytochemical analysis for tested extract revealed that the presence of secondary metabolites showed anti-microbial properties against *Alternaria solani* (Table 3). *A. sativum* extract was containing all major secondary metabolites, except steroids. The *L. camara*

Table 3. Qualitative analysis of phytochemicals in aqueous plant extracts

No.	Phytochemicals	<i>Datura stramonium</i>	<i>Allium sativum</i>	<i>Azadirachta indica</i>	<i>Eucalyptus globulus</i>	<i>Lantana camara</i>
1.	Flavonoids	+	+	+	+	+
2.	Alkaloids	+	+	+	-	-
3.	Saponins	+	+	+	+	+
4.	Tannins	-	+	+	-	+
5.	Steroids	+	+	+	+	+
6.	Terpenoids	+	+	+	-	+
7.	Glycosides	+	+	+	+	-
8.	Phenols	-	+	+	+	+

(+): Presence of phytochemicals, (-): Absence of phytochemicals

extract possesses flavonoids, saponins, tannins, steroids, terpenoids, phenols but alkaloids and glycosides were absent. *Eglobulus* and *D stramonium* contains the secondary metabolites like flavonoids, alkaloids, saponins, tannins, steroids, terpenoids, glycosides and phenols except alkaloids, terpenoids, tannins and phenols. Further, *A. indica* extract contains flavonoids, alkaloids, glycosides and phenols except other secondary metabolites like saponins, tannins, steroids and terpenoids.

Quantitative assessment of phytochemicals of different aqueous plant extract

The results represented in Table 4 for quantitative analysis of phytochemicals in tested

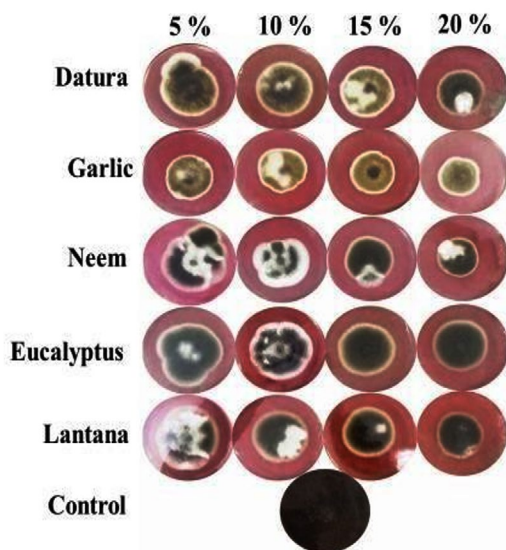


Fig. 2. Mycelial growth of *Alternaria solani* at different concentration of plant extracts after seven days of inoculation

plant extracts. The results revealed that maximum flavonoids content (1.12 %) was recorded in *A. sativum* followed by *A. indica* (0.90 %), and *L. camara* (0.82 %). *A. sativum* and *A. indica* extracts content huge amounts of secondary metabolites like alkaloids (0.93 and 0.89 %), tannins (12.14 and 8.23 %), phenols (0.81 and 0.64 %), steroids (0.85 and 0.81 %), terpenoids (2.31 and 2.22 %), and glycosides (2.76 and 2.61%) respectively. However, saponins content was higher in *A. sativum* and *D. stramonium* (3.45 and 2.25 %) respectively. The *E. globulus* extracts was recorded minimum concentration of secondary metabolites including flavonoids (0.49 %), alkaloids (0.21 %), saponins (1.45 %), and tannins (4.87 %). Phenols (0.37 %), steroids (0.53 %), terpenoids (1.89 %) and glycosides (2.21 %), were found low concentration in *D. stramonium* extracts.

Mycelium and conidia of *A. solani* under scanning electron microscopy

Based on mycelial growth assay results, *A. sativum* extract was further investigated by scanning electron microscopy (SEM) against *A. solani*. Results obtained from SEM revealed that the hyphae of the pathogen exposed to *A. sativum* plant extract were collapsed, short branching, and caused curling in the hyphae, whereas no such changes were observed in the hyphae developed in the control treatment. Moreover, *A. sativum* extract results revealed that no conidia formation, shorter germ tubes, and caused shrinking in conidia. The untreated control did not cause any spore shrinking (Fig.3).

Table 4. Quantitative analysis of phytochemical of different aqueous plant extract

No.	Plant extract	Flavonoids (%)	Alkaloids (%)	Saponins (%)	Tannins (%)	Phenols (%)	Steroids (%)	Terpenoids (%)	Glycosides (%)
1.	<i>Datura stramonium</i>	0.56±0.00 ^{ab}	0.78±0.01 ^b	2.25±0.11 ^b	5.11±0.11 ^b	0.37±0.00 ^{bc}	0.53±0.01 ^b	1.89±0.12 ^c	2.21±0.11 ^d
2.	<i>Allium sativum</i>	1.12±0.02 ^a	0.93±0.00 ^a	3.45±0.12 ^a	12.14±0.23 ^a	0.81±0.01 ^a	0.85±0.00 ^a	2.31±0.19 ^a	2.76±0.12 ^a
3.	<i>Azadirachta indica</i>	0.90±0.01 ^b	0.89±0.02 ^{ab}	2.08±0.03 ^b	8.23±0.21 ^a	0.64±0.02 ^a	0.81±0.03 ^a	2.22±0.01 ^b	2.61±0.17 ^b
4.	<i>Eucalyptus globulus</i>	0.49±0.01 ^{abc}	0.21±0.01 ^{bc}	1.45±0.04 ^d	4.87±0.10 ^{ab}	0.51±0.01 ^{bc}	0.62±0.05 ^b	1.97±0.16 ^{bc}	2.32±0.10 ^{bc}
5.	<i>Lantana camera</i>	0.82±0.01 ^c	0.32±0.02 ^{bc}	1.89±0.03 ^c	6.89±0.08 ^b	0.63±0.02 ^{ab}	0.79±0.02 ^a	2.01±0.15 ^{ab}	2.45±0.19 ^c

Results are expressed as means of three replicates, ± indicate standard deviation of the means. Different letters showed significant differences in treatments results according to Duncan's multiple rang test at P≤ 0.05.

DISCUSSION

In the current study we demonstrated that antimicrobial compounds were present in five plant extracts. The plant extracts inhibited mycelial growth of *A. solani* and conidial germination under *in vitro* condition. Various plants have been reported to comprise antifungal activity and they have shown encouraging results under *in vitro* conditions⁴²⁻⁴⁵. The current *in vitro* study revealed that among the five tested plant extracts, *A. sativum* and *A. indica* resulted significant reduction in conidial germination and growth of *A. solani*. Gradual increase in concentrations of plant extracts represents gradual decrease in conidial germination of the pathogen. Plant extracts evaluated against *A. solani* in the current study also showed significant results⁴⁶. Reports suggested antifungal activity of *A. sativum* against harmful phytopathogens^{47,48}. Significant inhibition was observed with *A. sativum* extract at higher concentration (20%) against the *A. solani*. Investigation of the efficacy of *A. sativum* by Scanning Electron Microscope also showed higher inhibition of test fungus and this finding is in agreement with that of Baka and Rashad⁴⁹. Plant extracts used at higher concentration have capability to reduce growth of phytopathogenic fungi⁵⁰. Plant extracts from *A. sativum* also comprised botanical compounds such as Allicin, which checked the growth of pathogen mycelium and also inhibited spore germination⁵¹.

Plant extracts were analyzed for potential antifungal phytochemicals. Various secondary metabolites were detected including alkaloids, flavonoids, saponins, tannins, phenols, terpenoids, glycosides and steroids. The extract from *A. sativum* was most potential in inhibiting the growth of *A. solani* compared to other plant extracts, because presence of phytochemicals in huge amounts. Plant derived secondary metabolites like flavonoids have antifungal, antibacterial and antiviral activity used in higher concentrations⁵². Most of these phytochemicals have antimicrobial activity in response to disease causing detrimental microbes like fungi, bacteria and virus⁵³. Various other studies have reported efficacy of phytochemicals against *A. solani*^{54,55,43}. Plants are huge in the array of secondary metabolites which have been implicated for their *in vitro* antifungal properties. Phytochemicals like substances play a pivotal role

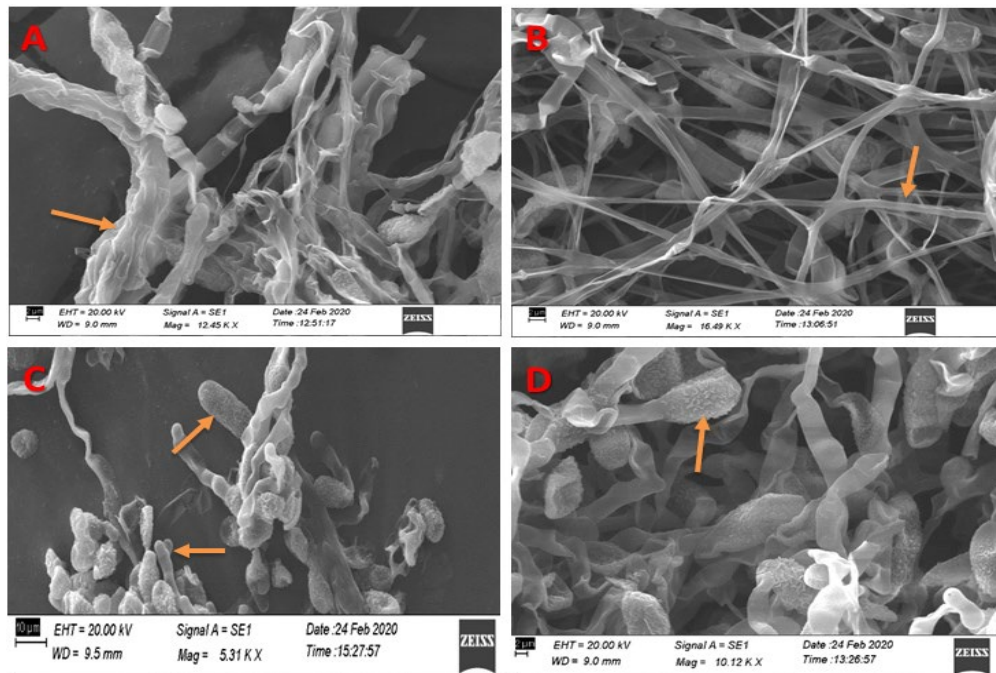


Fig. 3. SEM micrographs showing effects of *A. sativum* extract on the morphology and conidia of *Alternaria solani*. (A) Hyphae after treated with *A. sativum* extract (20%) observed collapsed hypha (arrow). (B) Hyphae of untreated treatment showing normal hypha (arrow). Bar = 2.0 μ m. (C) Continuation in the sentence shrinking and an arrow shows the small rings (septa) formation on conidia. (D) In control treatment showing no deformity.

in plant defense mechanism against invasion by harmful microorganism and insects⁵⁶.

CONCLUSION

The present investigation showed that among five tested plant extracts, *A. sativum* and *A. indica* resulted significant reduction in mycelial growth of *A. solani*, reduction in conidial germination or no conidia formation, formation of shorter germ tubes and shrinking in conidia. Various secondary metabolites were also detected in the plant extracts. The research concludes that plant extracts such as *Allium sativum*, *Azadirachta indica*, *Datura stramonium*, *Eucalyptus globulus*, and *Lantana camara* at higher concentrations have capability to reduce pathogen growth and spore germination of inimical plant pathogenic fungi because of presence of several antifungal secondary metabolites such as alkaloids, saponins, terpenoids, flavonoids and phenols. This biocontrol method is environmentally safe, eco-friendly, long lasting, and minimizing the use of hazardous

chemicals for management of *Alternaria solani* in potato.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

SK was responsible for methodology, investigation and wrote the manuscript. LB helped in performing data analysis. RC supervised the experiment and reviewed the manuscript and made suitable changes. The author(s) read and approved the final manuscript.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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