

Different Methods used to Control Early Blight of Potato in Laboratory Conditions

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ABSTRACT

The present study was designed with the objective “to assess the efficacy of various fungicides, salts and natural plant extracts with different doses against *A. solani*”. Results revealed that lowest mycelial growth and highest inhibition percentage of *A. solani* at highest concentration (2000 ppm) were observed in the fungicide treatments Bravo (2.2mm and 91%) followed by Dithane M-45 (3.65mm and 85.53%), Maneb-80 (7.55mm and 70%) while lowest was observed in case of Metalaxyl (11.29mm and 55.23%). Among inorganic salts, at the concentration of 5%, sodium bicarbonate inhibited maximum mycelial growth with maximum percentage inhibition (5.96mm and 77.82%) followed by potassium carbonate (9.1mm and 66.13%). In case of Plant extracts, *P. hysterothorus* was most effective to inhibit mycelia growth at the concentration 20%, while mycelial growth inhibition and inhibition percentage by *P. hysterothorus* at the concentrations of 10, 15 and 20% were (9.33mm and 56.26%), (11.58mm and 48.87%) and (10.53mm and 53%), respectively. Thus, the present study revealed that plant extracts and inorganic salts have shown significant inhibition and proved to be cost effective and eco-friendly for the management of *A. solani* and was comparable with fungicides.

Key words: Potato, Blight, Plant extract, eco-friendly, Fungicide, Management.

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INTRODUCTION

Potato is one of the World's most important crops. Potato ranks fourth in World production and ranks fifth in area.^[1] Tuber of potato plant is an excellent source of carbohydrates, proteins and vitamins.^[2] Mostly potato is grown in countries of northern temperate zones of the World. But in developing countries, potato production is increasing faster than any other food crop. In Pakistan, potato has become an important crop for both farmers and consumers and is widely cultivated on large areas all over the country.^[3]

Potato production has increased many folds in Pakistan but its per unit area production is very low as compared to other countries of the World.^[4,3] The main reason behind low productivity is many biotic factors. Among them, early blight is the most important and destructive disease caused by *Alternaria solani*.^[5,6] Spore abundance in the atmosphere and in the soil while the fungus is always a serious threat under favorable conditions to potato crop.^[7] Dark and sunken concentric ring-like lesions are usually a characteristic feature of this disease. Some reports show that in the absence of late blight, early blight is the most predominant factor of defoliation in potato crop.^[7] The spores remain on the soil surface and leaves and penetrate into the potato tubers during harvest.^[8,9] When this disease is left untreated, disease loss occur from 5-78%.^[8] High temperature, humidity and rainfall are the most promising factors for spreading of potato early blight disease.^[10] Potato early blight is polycyclic disease and spores survive in infected seeds and crop debris for longer period of times. Favorable

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temperature for spore germination is 20-30°C with free moisture. *A. solani* is imperfect fungus having no sexual stage in life cycle and is produced by the conidia.^[11] The spores remain on the soil surface and leaves and penetrate into the potato tubers during harvest.^[8,9] When this disease is left untreated, disease loss occurs from 5-78%.^[6] Keeping in mind its devastating nature, the preventive measures are the best way, but if this disease is established once, then are difficult to control.^[12] The pathogen, *A. solani* have both the air and soil borne stages in life cycle that makes difficult to control this fungus.^[13]

Currently the management of this disease is done with the help of fungicides which have adverse effects on the environment. Fungicides can be replaced with eco-friendly alternatives like plant extracts which can be drawn from any plant part and this is considered as the safest disease control measure. Plant extracts can inhibit the mycelial growth of the fungus *A. solani*.^[14,15] Natural products like plant extracts, the salts are good alternatives in disease management of potato early blight.^[16] Sodium carbonate and potassium carbonate have been used in the management of various fungal diseases of plants.^[17,18] Management strategy including salts and natural plant extracts could prevent the excessive employment of fungicides. *In vitro* studies help in choosing the most effective fungicides, salts and plant extracts; thus, save input costs. Hence, present study was designed for the selection of best treatments, so that, recommendation for the control of early blight disease of potato may be recommended.

MATERIALS AND METHODS

The present research work was carried out in laboratory of Plant Pathology, College of Agriculture, University of Sargodha, during 2015-2016 to evaluate the efficacy of fungicides, botanical extracts and inorganic salts against the fungus *A. solani* causing agent of potato early blight.

Sample Collection

Diseased samples of potato crop were collected from Sargodha District. After sample collection, samples were placed in polythene plastic bags and carried out to laboratory of Plant Pathology, College of Agriculture, University of Sargodha. The samples were stored in refrigerator at 4°C for further use.

Isolation and Identification of the Pathogen

The fungus was isolated from infected samples of potato and then cut into small pieces along with growing margins of about 1.5-2 cm, surface sterilized them with

1% sodium hypochlorite (bleach) for approximately 2 min then washed three times with distilled water and placed on prepared PDA containing petri-plates of 90 mm. These petri-plates were incubated at 24±2°C for 7 days to check the sporulation for further studies.

Preparation of Pure Culture

Colony of fungus from infected samples was observed after five to seven days. Pure culture was obtained with the help of single spore technique as used by incubating at about 24°C for seven days and observed it daily to get rid of contamination from bacteria and other fungal saprophytes.^[6]

Management Strategies of Potato Early Blight Evaluation of Fungicides

Evaluation of five different fungicides was done at 1000 ppm, 1500 ppm and 2000 ppm, with four replications, using poisoned food technique against *A. solani* causes of early blight of potato (Table 1).

Preparation of Fungicide Formulation

Potato dextrose agar in molten form was used and required quantity of each fungicide was added separately to get a required concentration of fungicides. The fungicides were thoroughly mixed well and about 20ml poisoned media was poured in each petri plates and permitted to solidification. The actively grown seven days old culture of *A. solani* was carefully cut using gel cutter and transferred aseptically to the center of each Petri-dish containing the poisoned media. Appropriate control was maintained by growing the cultures on PDA media without the any fungicide, which served as control. The plates were incubated at 25 ± 1°C for seven days and then colony diameter was recorded after three, five and seven days of fungus growth. After the incubation, data was taken starting from 72 hr, for period of seven days and calculated the mycelial mean growth per plate.

In vitro Evaluation of Inorganic Salts

Evaluation of four inorganic salts at 3%, 4% and 5% against *A. solani* was performed using poisoned food technique with three replications (Table 2).

Table 1: Description of fungicides used for evaluation against *A. solani*.

Sr.no	Trade name	Common name	Formulations
1	Bravo	Chlorothalonil	75WP
2	Dithane M- 45	Mancozeb	80 WP
3	Metalaxyl	Ridomil Gold	65 WP
4	Topsin-M	Thiophanate methyl	70WP
5	Maneb80	Maneb	80WP

Table 2: Description of inorganic salts used for evaluation against *A. solani*.

Sr. No	Chemical name	Common name	Chemical Formula
1	Potassium Carbonate	Pearl ash	K ₂ CO ₃
2	Potassium Bicarbonate	Salt of tartar	KHCO ₃
3	Sodium Carbonate	Washing soda	Na ₂ CO ₃
4	Sodium Bicarbonate	Baking soda	NaHCO ₃

Preparation of Salts Concentration

Salts concentrations 3%, 4% and 5% of inorganic salts (potassium carbonate, potassium bicarbonate, sodium carbonate and sodium bicarbonate) were prepared by weight over volume method.

Evaluation of Inorganic Salts

The efficacy of inorganic salts against *A. solani* was evaluated by using food poisoned technique. Inorganic salts at concentrations 3%, 4% and 5% were amended into molten PDA media at the rate of 1:4 (v/v) and poured into petri plates. A 5-mm actively growing disc from pure culture of *A. solani* was placed at the center of each plate and incubated at 24°C. PDA medium without salt (0.00%) served as control.

Evaluation of Plant Extracts

Efficacy of plant extracts was evaluated in the laboratory through poisoned food technique against *A. solani*, using concentrations 10%, 15% and 20%, with three replications of each plant extract (Table 3).

Preparation of Plant Extracts

Extracts of garlic bulb, sufeda, Datura, Chatak chandni and Neem leaves were prepared in laboratory of Department of Plant Pathology, College of Agriculture, Sargodha. Leaves of neem, Datura, sufeda and chatak chandni were washed with distilled water and then surface sterilized with 1% sodium hypochlorite solution. For preparation of fresh Garlic extract, the outer dry peel of cloves was removed, surface sterilized for 2 min in ethanol and washed in three changes of sterile distilled water. Cloves were crushed in blender with water 1:1 (w/v) and then filtered through muslin cloth to produce a 100% crude plant extract. Leaves of neem were homogenized in blender with water 1:1 (w/v) and then filtered through muslin cloth. Similarly, leaves of Datura, sufeda and chatak chandni were homogenized in blender with water 1:1 (w/v) separately and then filtered through muslin cloth to produce a 100%

Table 3: Description of plant extracts used for evaluation against *A. solani*.

Sr.no	Botanical name	Common name	Family
1	<i>Azadirachta indica</i>	Neem	Meliaceae
2	<i>Allium sativum</i>	Garlic	Liliaceae
3	<i>Datura stramonium</i>	Datura	Solanaceae
4	<i>Eucalyptus camaldulensis</i>	Sufeda	Myrtaceae
5	<i>Parthenium hysterophorus</i>	Chatak Chandni	Astraceae

crude plant extract. All the plant extracts were heated at 40°C for 15 min to avoid contamination and then further dilutions were made to get required concentrations (10%, 15% and 20%) by volume over volume method.

The efficacy of plant extracts against *A. solani* was evaluated by using poisoned food technique. Plant extracts at different concentrations (10%, 15% and 20%) were amended into molten PDA medium at the rate of 1:4 (v/v) and poured into petri plates. A 5-mm agar disc from seven days old pure culture of *A. solani* was placed at the center of each plate and incubated at 25°C. PDA media without plant extract (0.00%) served as control. The experiment was repeated three times for each plant extract.

Statistical Analysis

The data was analyzed using statistical software package Statistix-8. The data was subjected to Analysis of Variance (ANOVA) to determine the main and interactive effects of treatments. For comparison of means, Least Significant Difference (LSD) test at $P < 0.05$ was used. Graphs were plotted to show the means of mycelial growth inhibition and inhibition percentage. Inhibition percentage of mycelial growth was calculated by the formula.^[19]

$$\text{Inhibition Percentage} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

RESULTS

Evaluation of fungicides

Results showed that all tested fungicides at all concentrations significantly ($P \leq 0.05\%$) inhibited the mycelial growth of *A. solani* compared to control.

Data regarding mycelial growth revealed that Bravo was the most effective fungicide in reducing the mycelial growth of *A. solani* (91.45%) followed by Dithane M-45 (84.62%), Maneb-80 (70.17%) and Topsin-M (52.91%).

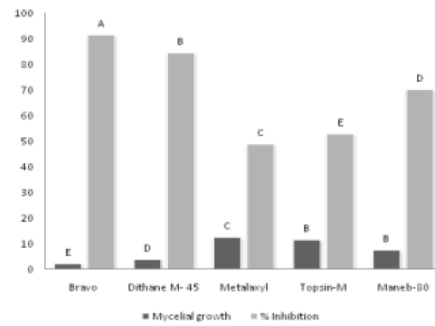
While Metalaxyl was the least effective (48.92%). Results also indicated that the inhibition percentage was increased by increasing the concentrations of fungicides.

The lowest mycelial growth and highest inhibition percentage of *A. solani* at 1000 ppm was observed in the treatment Bravo (2.1mm and 91.45%) followed by Dithane M-45 (3.75mm and 84.64), Maneb-80 (7.33mm and 70.17%), Topsin-M (11.57mm and 52.91%), while at the same concentration, fungicide Metalaxyl (12.55mm and 48.92%) found least effective. At 1500ppm concentration, the lowest growth and highest inhibition percentage was in the treatment Bravo (2.00mm and 91.7%) followed by Dithane M-45 (3.57mm and 85.33%), Maneb-80 (6.44mm and 73.53%) and Topsin-M (10.20mm and 58.08%) with least effective results (11.56mm and 52.49%). Similarly, growth of *A. solani* varied significantly ($P \leq 0.05$) at 2000 ppm of different fungicides. Highest growth was observed in untreated treatment (25.22mm) while lowest in treatment Bravo (2.20mm and 91.28%), followed by Dithane M-45 (3.65mm and 85.53%), Maneb-80 (7.55mm and 70.06%), Topsin-M (10.98mm and 56.46%), while metalaxyl was least effective (11.29mm and 55.23%) (Figure 1).

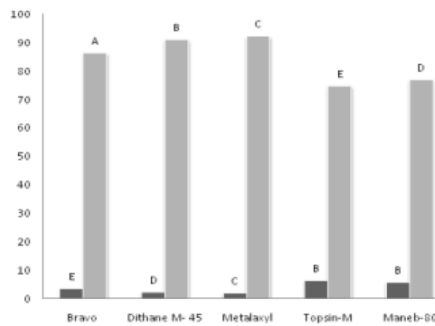
Evaluation of inorganic salts

Inorganic salts, potassium carbonate, potassium bicarbonate, sodium carbonate and sodium bicarbonate were evaluated against *A. solani* at three concentrations (3%, 4% and 5%). Growth of *A. solani* was suppressed significantly by all salts as compared to control. The results showed that sodium carbonate showed maximum mycelial growth and percentage inhibition of *A. solani* at 3% concentration (14.5mm and 42%) followed by potassium carbonate (16.3mm and 34.80%), while sodium carbonate and potassium bicarbonate showed the same growth and percentage inhibition of *A. solani* (16.5mm and 34.00%).

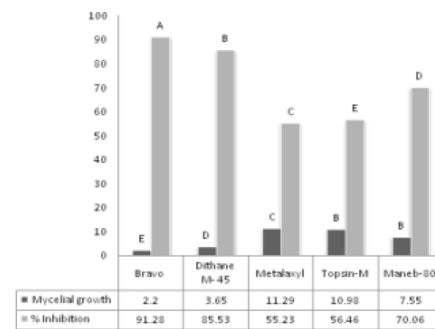
Inorganic salts at the concentration of 4% showed that, sodium bicarbonate was very effective inorganic salt that reduced the mycelial growth and showed highest inhibition percentage (13.4mm and 43.75%), followed by Potassium carbonate (14.35mm and 40.21%), Sodium carbonate (17.5mm and 27.08%) and Potassium bicarbonate (20.5mm and 14.58). The inhibition activity of inorganic salts against *A. solani* at the concentration of 5% showed that, sodium bicarbonate inhibited maximum mycelial growth with maximum percentage inhibition (5.96mm and 77.82%) followed by potassium carbonate (9.1mm and 66.13%), potassium bicarbonate (11.35mm and 57.76%) and sodium carbonate (13.03mm and 51.15%). These results showed that sodium



Fungicides at 1000 ppm concentration



Fungicides at 1500 ppm concentration



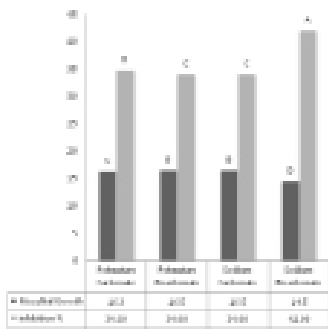
Fungicides at 2000 ppm concentration

Figure 1: Mycelial growth inhibition and inhibition percentage of *A. solani* by fungicides at different concentration.

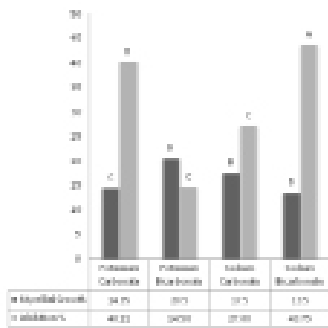
bicarbonate had very good antifungal activity against *A. solani* (Figure 2).

Evaluation of plant extracts

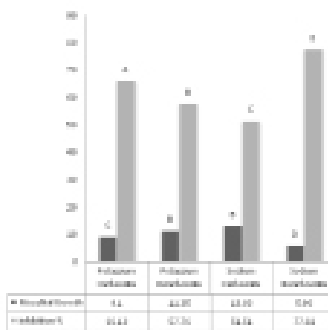
Mycelial and inhibition percentage revealed that all tested plant extracts at different concentrations significantly ($P \leq 0.05$) controlled *A. solani* compared to control. At the concentration of 10%, *Parthenium hysterophorus* was the most effective and showed maximum mycelial growth inhibition and inhibition percentage (9.33mm and 56.26%), followed by *Eucalyptus camaldulensis* (10.4mm and 51.24%), *Azadirachta indica* (10.44mm



Salts at 3% concentration



Salts at 4% concentration



Salts at 5% concentration

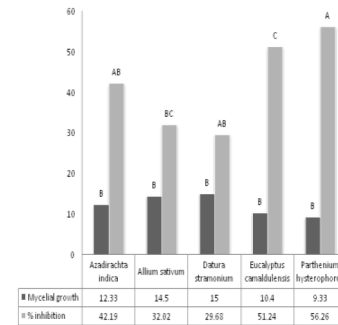
Figure 2: Mycelial growth inhibition and inhibition percentage of *A. solani* by inorganic salts at different concentrations.

and 50.00%), *Allium sativum* (14.5mm and 32.02%) and *Datura stramonium* (15.00mm and 29.68%). At the concentration of 15%, *Azadirachta indica* showed maximum mycelial growth inhibition and inhibition percentage against *A. solani* (10.50mm and 50.00%), followed by *Parthenium hysterophorus* (11.58mm and 48.87%), *Eucalyptus camaldulensis* (11.89mm and 47.51%), *Allium sativum* (12.56mm and 39.85%) and *Datura stramonium* (14.35mm and 31.27%). At the concentration of 20%, *Parthenium hysterophorus* displayed maximum mycelial growth inhibition and inhibition percentage (10.53mm and 53%) followed by *Azadirachta indica* (11.58mm and 48.87%),

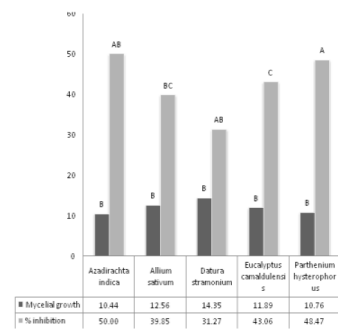
Eucalyptus camaldulensis (11.89mm and 47.51%), *Datura stramonium* (12.46mm and 44.99%) and *Allium sativum* (13.77mm and 39.21%) (Figure 3).

DISCUSSION

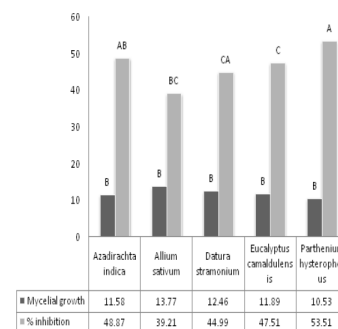
Potato is very important food commodity and is the fourth important crop worldwide. Early blight is the most prevalent and destructive disease of potato in all potato growing areas of the world and in Pakistan, also one of the most yield limiting factor in potato crop. During this study, the symptoms in the field of potato were first identified as small to irregular lesions developed as distinctive bull eye pattern with concentric rings. Symptoms first appeared on older leaves, the most



Plant Extracts at 10% concentration



Plant Extracts at 15% concentration



Plant Extracts at 20% concentration

Figure 3: Mycelial growth inhibition and inhibition percentage of *A. solani* by plant extracts at different concentrations.

susceptible plants were those which were weak, wounded by insects and malnourished.^[11]

During current research, efficacy of five fungicides, Bravo (Chlorothalonil), Dithane M-45 (Mancozeb), Topsin-M (Thiophanate-methyl), Maneb-80 (Maneb) and Metalaxyl (Ridomil gold) was evaluated *in vitro* against *A. solani* using food poison technique. Chlorothalonil at concentration 1000ppm was highly effective in reducing mycelial growth (2.1mm) with (91.45%) inhibition percentage, while metalaxyl showed the least mycelial growth (12.55mm) with (48.92%) inhibition percentage. Current findings are in line with the findings of many researchers who evaluated different fungicides against *Alternaria* blight and reported that Tetra Methyl Thiram Disulphide (TMTA), Dithane M-45, Bavistin, Dithane Z-78, Difoltan, Blitox, Captafol and Bordeaux mixture were effective against *A. solani*.^[20-22] reported that fungicides were the best efficient method for the management of early blight disease and observed high yields.^[23] observed that hexaconazole (0.05%) and azoxytrobin (0.2%) was very effective for the control of early blight. It is apparent from present work that all the fungicides significantly inhibited the mycelial growth of the fungus; however, Bravo (Chlorothalonil) significantly superior to all tested fungicides. All four inorganic salts (Potassium carbonate, Sodium bicarbonate, Potassium bicarbonate and Sodium carbonate) were evaluated *in vitro* at three different concentrations, 3%, 4% and 5% through food poison technique. All salts reduced the mycelial growth significantly. Among all the salts sodium bicarbonate gave best inhibition percentage (77.87%) at concentration of 5%. The reduction in fungal growth of *A. solani* may be due to antifungal activity of inorganic salts. Similar results were also observed by^[18] who conducted an experiment to check the efficacy of inorganic salts (sodium bicarbonate and potassium bicarbonate) and its results proved to be effective against many other plant fungal pathogens when used in integrated manner. There are different ways through which inorganic salts control fungal diseases, these include, inhibition of spore germination, inhibition of mycelial growth and by hindering aspersoria and spore production^[17,24,25]

Current findings showed that plant extracts were also effective. Plant extracts, *A. indica*, *A. sativum*, *D. stramonium*, *E. camaldulensis* and *P. hysterophorus* were evaluated *in vitro* at three different concentrations through food poison technique for their inhibitory effect on growth of mycelium. Among all extracts *P. hysterophorus* was found highly effective in inhibition of fungal growth (56.26%) at 10% concentration while least inhibition was observed in extracts of *D. stramo-*

nium (29.68%). The efficacy of plant extracts belongs to different plant species on the growth of the plant pathogen have been reported by several researchers.^[19-23] The inhibitory effect of these extracts may be due to their direct lethal effect on fungus growth or *in vitro* activity against fungal pathogens.^[26] The extract of *A. indica* has shown antifungal, antibacterial and antiviral properties.^[27] The antifungal activity of *A. sativum* may be due to presence of “Allicin” which inhibit mycelium growth.^[28] Thus there is possibility that in same fashion these salts have inhibited mycelial growth of *pathogen*.

CONCLUSION

Preliminary data suggested that the tested fungicides, plant extracts and inorganic salts at all concentrations were capable of *A. solani* mycelial growth inhibition. Fungicides, Chlorothalonil and Dithane M-45 were most effective. Among inorganic salts, sodium bicarbonate and potassium carbonate showed the most promising results. While in case of plant extracts, *P. hysterophorus*, *A. indica* and *E. camaldulensis* significantly controlled mycelial growth of pathogen.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUMMARY

More studies related to alternative management strategies and their mode of action might contribute to expand their application in the field of Plant Pathology.

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