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EFFICIENCY OF SELECTED BOTANICALS AGAINST (*ALTERNARIA SOLANI*) CAUSING EARLY BLIGHT DISEASE ON TOMATO IN AZAD JAMMU AND KASHMIR

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ABSTRACT

Botanical extracts had proved efficient, cost effective and environment friendly means of plant disease management. *Alternaria solani* is a fungal pathogen responsible for early blight resulting in huge economic losses. There is dire need for effective cheap alternatives of conventional health hazardous and expensive chemical control. In this study *in-vitro* evaluation of four plant extracts (*Melia azedarach*, *Cannabis sativa*, *Achillia millefolium* and *Datura stramonium*) were used against early blight disease on tomato at 3 different concentrations (10, 15 and 20%) in complete randomized design. Pathogen was isolated from different tomato growing areas of Rawalakot, purified and multiplied on potato dextrose agar (PDA) followed by hyphal tip method. Food poisoned technique was used for fungal growth inhibition. *M. azedarach* was found most efficient with 73.18% inhibition at 20%, followed by *C. sativa*, *D. stramonium* and *A. millefolium* (55.79%, 37.12% and 31.9% respectively) as compared to control (88.2%). Tomato variety (Roma) was used as experimental material with Completely Randomized Design in the greenhouse experiment. *M. azedarach* showed highest reduction of early blight disease severity 67.93% at 20% concentration followed by *C. sativa*, *D. stramonium* and *A. millefolium* (53.63%, 43.28%, and 31.78%) respectively while it was 87.3% in control. Study showed that plant extracts could be used as alternatives of highly expensive, health hazardous chemicals to control early blight disease on tomato.

Keywords: *Alternaria solani*; Fungicides; Plant extracts, Growth inhibition, Disease control.

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is an obligatory enthusiastic crop of family Solanaceae, originated from Western coastal plains of South America. Tomato is predominant fruit vegetable ranks next to potato on world ranking whereas, as a processing crop it ranks first in the world. Tomato is one of the most popular vegetable of Pakistan, grown on an area of 6,0307 hectares with an average production of 95499 kg/ha (FAO, 2016). It contains 90% water, sugars, vitamins mainly A and B, dietary fibers, essential amino acids, minerals, antioxidants such as lycopene, carotenoids and

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phytochemical profile (Davey *et al.*, 2000). Due to its succulent nature tomato is prone to various diseases. Most of the diseases that are prevalent in Azad Jammu and Kashmir are of fungal origin like early, late blight and plant parasitic nematodes like root-knot nematodes (Tariq-Khan *et al.*, 2017; 2020, 2020a) responsible for huge monetary and quality losses. *Alternaria solani* is responsible for early blight in the area and target the crop during humid summer. Disease is usually characterized by its appearance of brown to dark brown necrotic spots having concentric rings on foliage, stem and fruits. The overzealous and indiscriminate use of most of the synthetic fungicides has created different types of environmental and toxicological problems (Babu *et al.*, 2001) and leads to resistance build-up among the pests as well as pathogens (Engindeniz *et al.*, 2013). Best control is by using a combination of various preventive measures like use of early blight resistant vegetable varieties, destruction of infested plants, and use of fungicides. Alternate method

economically sustainable, efficient and environment friendly is imperative (Ganie *et al.*, 2013) to be sort out. Many researchers introduced different management strategies and investigated that botanicals are sources of antioxidants which protect the plants against many diseases (Mohana and Raveesha, 2013). Botanical extracts have antimicrobial properties due to secondary metabolites like tannins, terpenoids, alkaloids and flavonoids and antibiotic constituents (Pretorius *et al.*, 2002). They are being used to accomplish management of plant diseases and are successfully documented well adopted. Yadav *et al.*, (2018) compared different treatments of plant extracts with chemicals for radial mycelial growth inhibition of *Alternaria solani* under *in-vitro* and *in-vivo* conditions showing their efficiency to minimize the fungal radial growth. Rawalakot is a temperate area with high rainfall and tomato is grown on every household, and early blight is usual malady to be addressed. Due to low number of plants at household level in kitchen gardening, it was found imperative to evaluate localized botanical extracts to manage the disease. The study has been designed to evaluate the efficiency of the selected botanical extracts against early blight (*Alternaria solani*) isolates in greenhouse conditions in Rawalakot conditions with moderate temperature and high humidity.

MATERIAL AND METHODS

Isolation and purification and Identification of fungal isolates:

An extensive survey of tomato growing areas of Rawalakot was done for the collection of early blight infected plants with symptoms on leaves. Isolation of fungus was done by direct placing of infected leaf portions on PDA after disinfection with 0.5% sodium hypochlorite solution followed by 4-5 washing and blot drying. Purification of fungal colonies was done using single spore and hyphal tip techniques on PDA plates and were incubated at 28°C for 3-7 days. Isolates were identified based on their morphology. Colony morphology with visual observation and using stereo and compound microscope. Spore color and size, presence and absence of septa are recorded under stereo and compound microscope from fully grown *A. solani* culture plates.

Pathogenicity Test: Pathogenicity test of *A. solani* was carried out under greenhouse conditions. The inoculum was prepared by growing each of tested isolates on PDA medium and incubated at 27 °C for seven days. Then 10 ml of sterile distilled water was added to each plate and colonies were carefully scraped with a sterile needle to make conidial suspension with concentration 2×10^4 spores/mL (Ganie *et al.*, 2013). To confirm Koch's postulates 3-5 tomato seeds

were sown in plastic pots having sterilized soil. Four weeks after the emergence of seedlings, fungal suspension was sprayed using atomizer on tomato leaves with three replications. Distilled water was used as control. After inoculation plants were covered with polyethylene bags to maintain high humidity conditions. After 48 hours bags were removed, and plants were kept under greenhouse conditions and were monitored regularly for the development of disease symptoms. Two weeks after inoculation disease severity was recorded in each treatment and disease intensity was recorded using scale 0-5 (Mayee and Datar, 1986) and found effective (Kumar and Kartikeya, 2013).

Collection of Plant material and Preparation of Botanical extracts:

Four plant species, Hemp (*Cannabis sativa*), Datura (*Datura stramonium*), Drake (*Melia azedarach*) and Yarrow (*Achillia millefolium*) were selected based on their antimicrobial properties, medicinal values and collected from Rawalakot localities. Leaves were used to prepare the extracts and only hemp seed was included along with leaves. The collected plant material was disinfected with 30% ethanol followed by washing with distilled water and drying then ground until formation of fine powder. Different concentrations (10, 15 and 20%) of plant extracts were prepared by dissolving 10g, 15g and 20g powder in 100ml distilled water respectively by shaking the suspension overnight in a shaker. After 24 hours, prepared suspension was filtered using whatman No.1 filter paper and stored for further use.

In-vitro evaluation of plant extracts: Antifungal bioassay was done using poisoned food technique. PDA amended with antibiotics (streptomycin) to avoid bacterial contamination and 3ml of each plant extract. Four-millimeter mycelial disk of 7 days old mature fungal colony was placed at middle of each petri plate. Fungicide (+tive control) and distilled water (-tive control). Inoculated plates were incubated at 27°C and were regularly monitored. As soon as, negative control plates were filled up, results were collated and analyzed to calculate the percentage inhibition of mycelia of pathogen using formula (Nisa *et al.*, 2011). Experiment was repeated thrice. Formula for estimation is given under;

$$PGI = \frac{C - T}{T} \times 100$$

Where: PGI = Percentage growth inhibition; C = Average colony growth of fungal colonies obtained from control plates; T = Average colony growth of fungal colonies obtained from treated plates.

Tomato Germplasm: Tomato variety (Roma) was used as experimental material; seeds were collected from Gene Bank NARC, Islamabad. One month-old tomato seedlings were

transplanted to pots containing sterilized soil with 1:1:1 proportion of sand, soil and compost. All pots were placed on a benchtop in green house at 30 °C with 65-80% RH and watered as required.

Disease severity assessment of treated plants:

Efficacy of plant extracts against early blight was estimated in pots. Treatments with 10, 15 and 20% of different plant extracts concentrations were evaluated as foliar treatments on 45 days old plants and repeated after 15 days. Extracts were applied at the rate of 33.3 ml/plant, and negative control with application of distilled water and standard fungicide (Mencozeb) at the rate of 2.5 g L⁻¹ of water for comparison with efficiency of plant extracts. In each treatment all tomato plants were inoculated with 20 ml inoculum density having 5×10⁶ spores/ml after two days of last extracts spraying. After inoculation, plants were covered with polyethylene bags for 48 hours to maintain humidity conditions and establishment of infection. Randomly six leaves from each replication were assessed for disease severity measurement. 0-5

Table 1. Description of samples collected from different locations of tehsil Rawalakot.

Locations	Sample Description
Char	leaf spots, dark brownish leaf spots
Dhamni	leaf lesions, elliptical concentric rings
Drake	lesions with dark concentric rings
Hussian Kot	brown to black lesions
Khrick	chlorotic leaves and lesions
Paniola	bull eyed target spot lesions
Khaigala	leaf spots, lesions with concentric rings
Singola	bright yellow lesions and dark spots
Chak	chlorotic leaves with concentric rings
Trar	dark brownish spots

Isolation and identification: From the samples collected, fourteen fungal isolates were observed have grey to olive green and black aerial mycelia growth. Conidiophores have dark colored conidia with an average 8-10 transverse and

disease rating scale was used for disease intensity measurement (Mayee and Datar, 1986) as previously described. Disease severity index from the pot experiment was calculated by using PSI formula (Percent Severity Index) described as Wheeler (1969).

$$PSI = \frac{SIR}{NoLo} \times 100$$

Where; SIR = Sum of individual ratings; NoLo = No of leaves observed; MDS = Maximum Disease scale

STATISTICAL ANALYSIS

The data collected during laboratory and pot experiment was analyzed using CRD two factor factorial design and treatment means were compared by employing HSD Tukey test at LSD 5% alpha (Steel *et al.*, 1997).

RESULTS

Survey and samples collection: Survey of ten different locations of tehsil Rawalakot was done and samples were collected on the basis of symptoms as described in table 1. Samples with spots, lesions and having concentric rings were collected and were further subjected to isolation and purification of fungal isolates.

1-2 longitudinal septa with long beak. Conidia were measured length/width having average range between 101.6-113.5 × 16.3-24.5 μm. All the isolates were identified morphologically as *A. solani* (Figure 1).



Figure 1. Cultural, microscopic representation, conidia showing beak and number of septa.

Pathogenicity Test: Pathogenicity results (Figure 2) showed that all tested isolates of *A. solani* were found able to cause early blight disease with varying degrees of disease severity. Isolate ALT-5 showed highest disease

severity (71.5%) while isolate ALT-1 exhibited lowest disease severity (11.78%) on tomato plants (Figure 2; 3). ALT-5 was found most virulent and used for further studies.

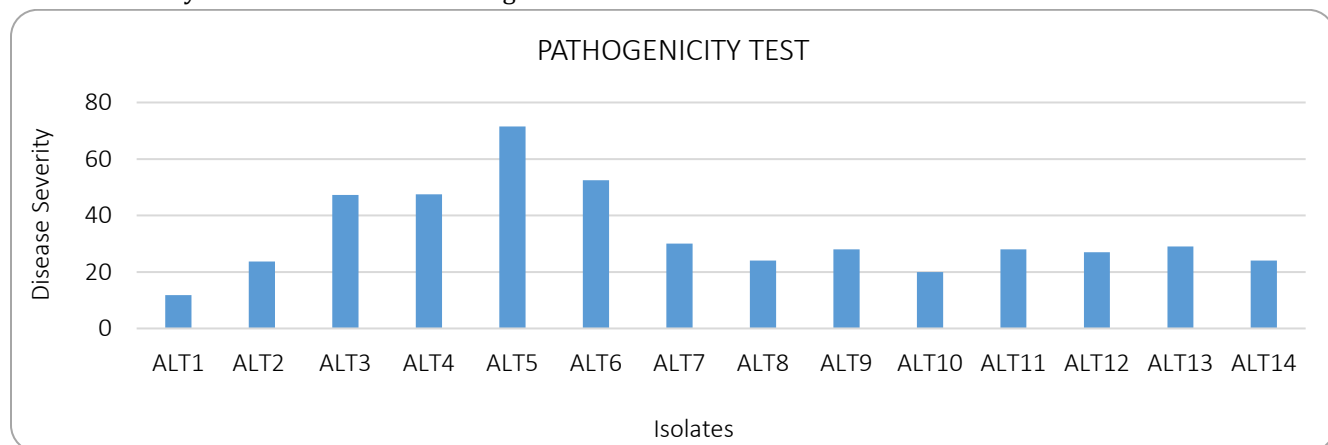


Figure 2. Pathogenicity test of *Alternaria solani* on tomato plants under greenhouse conditions.



Figure 3. Pathogenicity test plants having symptoms of early blight.

In- vitro effect of plant extracts on radial mycelial growth: Radial mycelial growth (RMG) means showed that growth of fungus decreased with increase in application of plant extracts. *M. azedarach* was found most effective and efficient against fungal mycelial growth reduction at all concentrations where PDA treated with *M. azedarach* showed 32.16, 28.00 mm and 24.00 mm growth at

10, 15, and 20% respectively followed by *C. sativa* 44.56, 42.76, 39.73 mm (Figure 4) while in negative control it was 89.66 mm growth in colony and in positive control it was 10.10 mm. Results confirmed findings that *A. indica* extract suppressed the growth of fungus (Hassanein *et al.*, 2008). A compound Azadirachtin cause suppression of growth and effective against fungi (Gujar *et al.*, 2012).

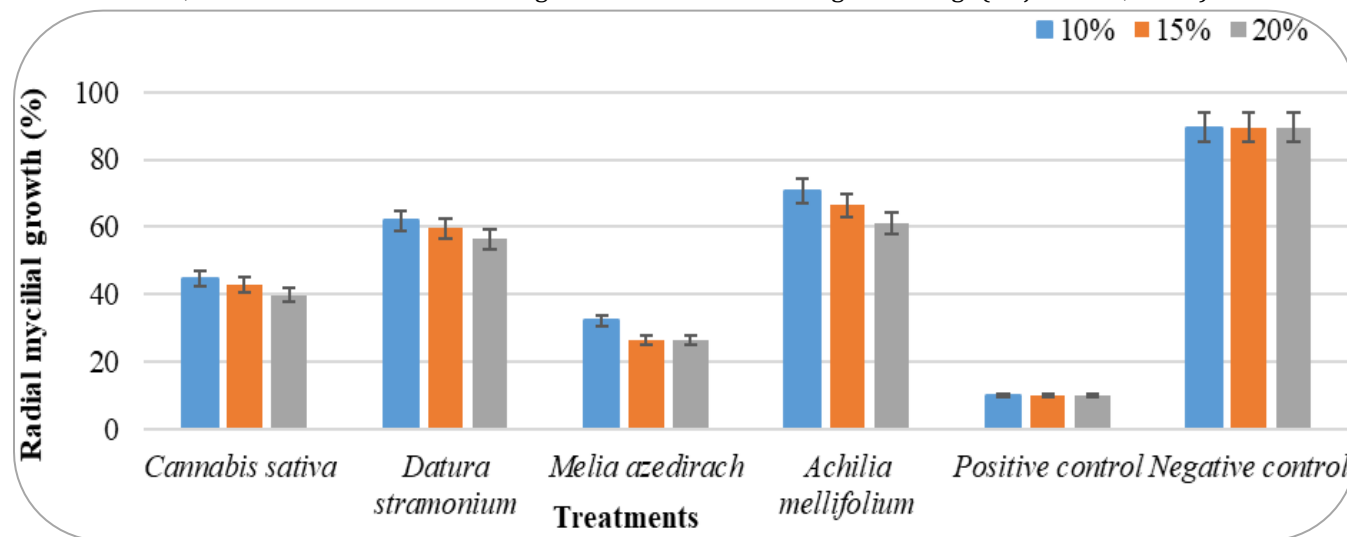


Figure 4. Antifungal activity of plant extracts against *Alternaria solani* isolate based on radial mycelial growth (mm) respectively (Figure 5). *D. stramonium* and *A. millefolium* did not show impressive inhibition for disease control. Unamaended media did not show inhibition while media treated with Mencozeb showed 88.2% inhibition.

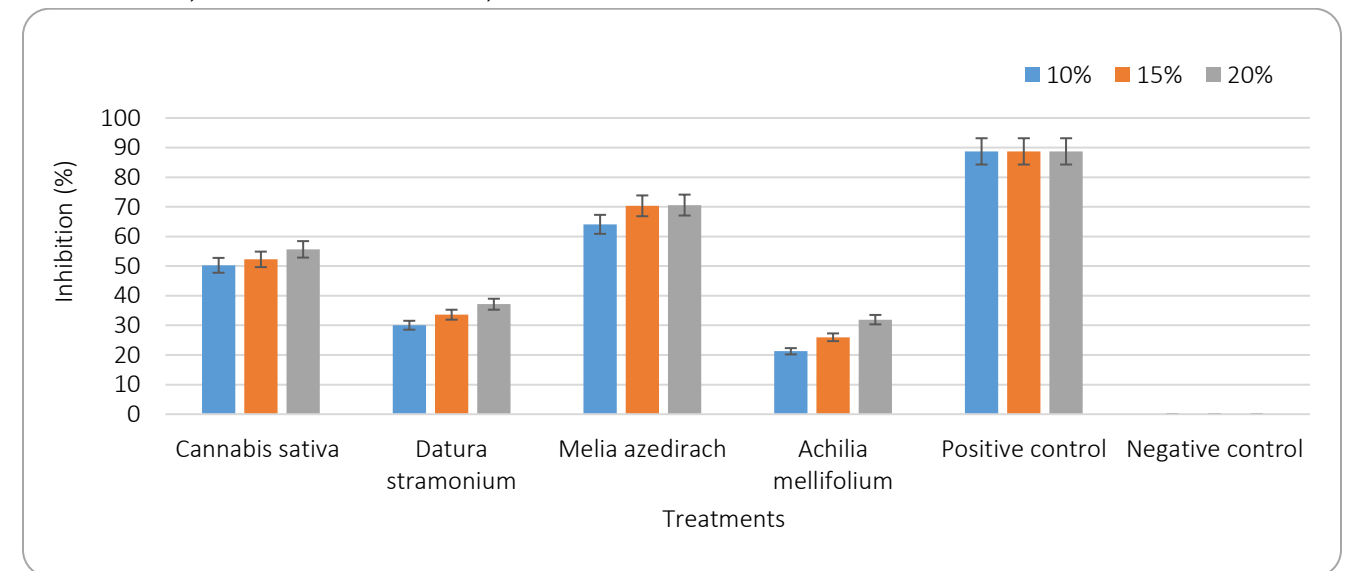


Figure 5. Impact of plant extracts against early blight pathogen (*A. solani*) in laboratory conditions in petri-plates and colony inhibition

Green House Experimentation and Disease Severity: Concentrations of plant extracts *C. sativa* and *M. azedarach*

ominously reduced the early blight disease in pot experiment. Most effective treatment was *M. azedarach* at

15 and 20% concentration, followed by *C. sativa* at 15 and 20% concentration. *A. millefolium* was found with least disease suppression impact on early blight while 80-90% reduction in disease severity as under fungicide impact. Among four plant extract tested, most effective plant extract was *Melia azedarach* which exhibited maximum

efficacy 67.77, 62.93, 56.32% at concentration of 10, 15 and 20% respectively followed by *C. sativa* 43.86, 47.84 and 53.63% while minimum reduction shown by *A. mellifolium* 31.33% (Figure 6). Positive control exhibit 87% efficacy using fungicide (Mencozeb) against early blight disease in *in-vivo* pot experiment.

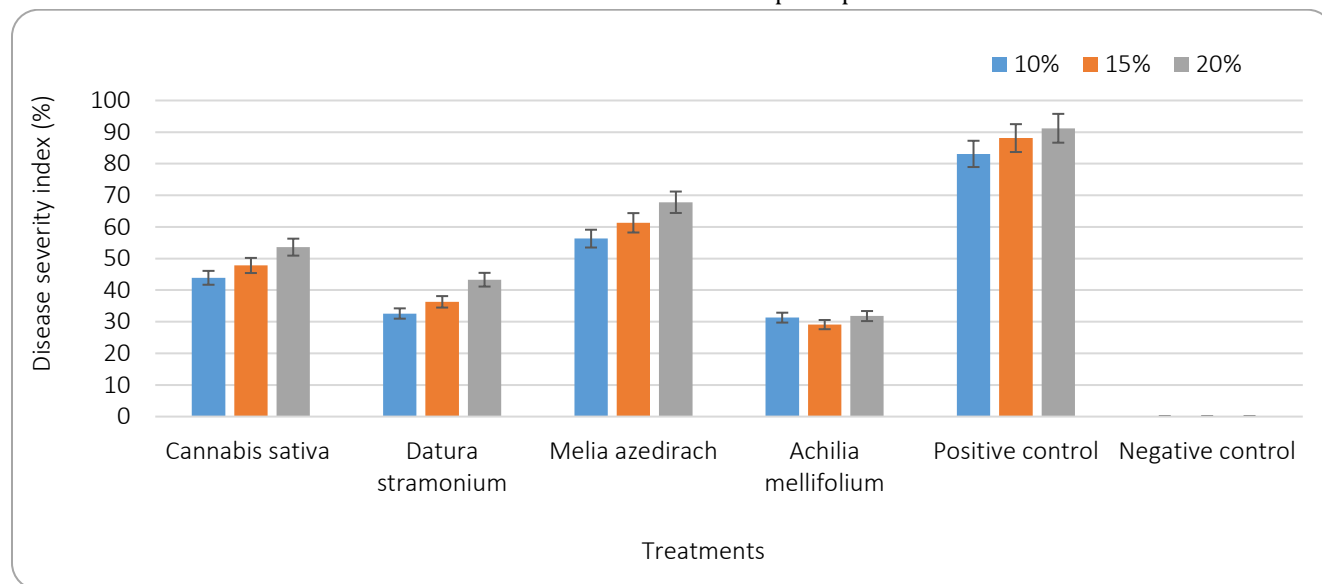


Figure 6. Effect of Four botanical extracts with 3 level concentrations on tomato early blight disease severity

DISCUSSION

Early blight caused by the fungus *Alternaria solani* (Ellis and Martin, 2014) is a major disease of tomatoes and potato. Disease is one of the most prevalent and catastrophic to tomato crop, reducing its yield and results in huge losses (Goufo *et al.*, 2008). Use of fungicides is considered as most impressive method for plant disease management but these chemicals are costly and have adverse impacts on environment (Bhandari, 2014). Unjustified and underdose applications due to financial constraints of the poor farmers leads to development of resistance in pathogen against chemicals (Hahn, 2014). It has compelled researchers to look for effective and different approach to reduce reliance on commercial fungicides. Plant extracts are considered as a worthy substitute to fungicides, as they showed antifungal capacity and are also ecofriendly. Certain plants originated secondary metabolites that displayed antifungal action are phenolics, terpenes, tannins, flavonoids, essential oil, alkaloids, polypeptide and lecithin. Such groups of compounds play a substantial role in physiology of plants and thus resulted in reduction of early blight in some of the previous studies (Kagale *et al.*, 2004).

In present research, efficiency of selected plant diffusates *C. sativa*, *D. stramonium*, *M. azedarach* and *A. millefolium* against *A. solani* were studied. Plant extracts tested at three concentration 10, 15 and 20% each by using food poison technique. All treatments showed significant difference for inhibiting the radial mycelial growth of fungi. Maximum radial mycelium growth was shown by *A. mellifolium* 70mm at 20% concentration and minimum radial mycelial growth was 24.00mm by *M. azedarach* at 20% concentration as compared to distilled water 89mm as negative control and Mancozeb 10.1mm positive control. In case of inhibition maximum inhibition 73% was showed by *M. azedarach* at 20% concentration and minimum percent inhibition was 31% by *A. mellifolium* at 20% as compared to control (Figure 5). It reveals that *Melia azedarach* might have improved concentration of ursolic acid, benzoic acid and 3,5 dimethoxy benzoic acid providing antifungal ingredients responsible for early blight control which is needed to be confirmed and also found effective in pervious *in-vitro* studies against *Alternaria* leaf blight of tomato with efficiency of 63.52% (Kumar *et al.*, 2017). It reveals that plant extracts naturally have antifungal properties against early blight. In pot experiment foliar application

of 33.3ml plant extracts at 20% concentration ominously suppressed the disease severity. At 20% concentration minimum disease severity index was 28% by *M. azedarach* while maximum severity index was 64% by *A. millefolium*. Application of *M. azedarach* at 20% concentration on tomato plants in pots significantly ($P < 0.05\%$) suppressed the growth of *A. solani* (Figures 4; 5; 6). Plant extracts (*M. azedarach*) are easy to handle plant ailments and different plants extract application on fungal pathogens revealed their highest efficiency against fungal growth. *In-vivo* results of experiments revealed hexaconazole found in plant leaves are effective (Yadav *et al.*, 2018). Therefore, from the foregoing argument it may accomplished that *M. azedarach*, a common medicinal plant could be used as effective bio-fungicide that has vast fungi toxic properties and effective against *A. solani*.

CONCLUSION

Synthetic fungicides applications are expensive and hazardous to human and animals can be reduced by the applications of botanical extracts, which is environment friendly approach. Plant disease can be reduced with the applications of such botanicals which are easily available to farmers even in their fields. Study confirmed their efficiency and availability to farmers. As in this research, one could confidently suggest that plant extract of *Melia azedarach* and *D. stramonium* at 20% concentration are effective against *A. solani* and can be used to breakdown the resistance in pathogen against conventional synthetic fungicides and reduce the farmer cost as well. These plants can be exploited as alternative used in control of early blight disease under Rawalakot conditions.

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Basharat Mehmood	: Provide technical input in writing manuscript
Muhammad T. Younas	: Designing of survey format and compilation and interpretation of data
Hafiz M. U. Rehman	: Draw graphs in Microsoft excel
Shabir Hussain	: Helped in statistical analysis