

**ANTI-FUNGAL PROPERTIES OF PLANT EXTRACTS IN VITRO AGAINST
Alternaria solani, THE CAUSE OF EARLY BLIGHT IN POTATO**

**Birendra Bahadur Rana^{1,*}, Sampada Wagle^{1,*}, Ashmita Kumari Singh²,
Yankee Sherpa², Abisha Suwal², Seema Khatri² and Giridhari Subedi¹**

¹National Potato Research Program, Nepal Agricultural Research Council

²Kantipur Valley College, Purbanchal University

*Correspondence: biru.deep25@gmail.com, sampadawagle254@gmail.com

ABSTRACT

Early blight, caused by *Alternaria solani* is one of the destructive diseases of potato. An in vitro experiment was conducted using the poisoned food technique to evaluate the efficacy of acetone extracts of six plant species on the mycelial growth of *A. solani*. Plant extracts of *Artemisia vulgaris*, *Melia azedarach*, *Parthenium hysterophorus*, *Datura stramonium*, and *Justicia adhatoda* at concentrations of 0.1%, 0.5% and 5% prepared from Soxhlet extraction were mixed well in the molten potato dextrose agar (PDA) media and poured into petri plates (90 mm Ø). PDA media treated with a fungicide mancozeb was positive control while the untreated media was negative control. The poisoned plates were inoculated with a 4 mm diameter agar plug of seven days old actively growing *A. solani* and incubated at 26 ± 2 °C. The radial mycelial growth was recorded after 3rd, 5th, 7th and 9th days of incubation and percentage inhibition growth was calculated by comparing it with negative control plates. *Artemisia vulgaris* significantly inhibited colonial growth at 0.1% and 0.5% concentrations by 36.07% and 12.48%, respectively, at 5th day of inoculation (dai) as compared to negative control. *M. azedarach* (0.72%), *P. hysterophorus* (10.17%) and *D. stramonium* (10.20%) were moderately inhibitory in effect at 1% concentration while *J. adhatoda* did not possess inhibitory effect on the mycelial growth of *A. solani* at 5th dai. All the tested extracts were found with some anti-fungal activity at 5% concentration. *A. vulgaris* can be exploited as a potent alternative to fungicides for controlling *A. solani*.

Key words : Mycelial growth, percent growth inhibition, poisoned food, Soxhlet extraction

INTRODUCTION

Potato (*Solanum tuberosum*) is a major vegetable in the terai and mid-hills, and a staple food in the high hills of Nepal. In 2022, the productivity of potatoes was 16.73 t/ha which is very low as compared to the other countries of world (MoALD, 2022). Diseases are the major factors responsible for the lower yield of potatoes in Nepal. After deadly late blight caused by *Phytophthora infestans*, early blight is a devastating foliar disease of potatoes caused by a fungus *Alternaria solani* (Abuley *et al.*, 2019; Christ, 1990). Early blight attacks on potato leaves, stems, and tubers, with foliar damage being the deadliest. It is responsible for premature defoliation resulting in significant impact on tuber yield and quality loss of up to 50% annually (Adhikari *et al.*, 2017; Landschoot, *et al.*, 2017; Leiminger *et al.*, 2014; Neergaard, 1945; Shtienberg *et al.*, 1990; Van der Waals *et al.*, 2004). It is particularly more prevalent in tropical and temperate zones of Nepal. Yield losses due to foliar damage depend on the disease severity (Van der Waals *et al.*, 2001). Yield declines by 1.36% with

1% increase in disease severity, and complete crop failure can result in times of serious illness (Pandey and Pandey, 2002). It is also stated that free water initiates sporulation and subsequent disease development and severity can be altered by 90% based on the duration of leaf wetness (Holley *et al.*, 1985; Van Cutsem *et al.*, 1994). Early blight is also enhanced in closely spaced plants, heavy fruit load, increased leaf maturity, above average rainfall or dew and shading (Horsfall & Diamond, 1957; Horsfall & Heuberger, 1942).

Symptoms of early blight first develop as a small, circular or irregular, dark-brown spot in mature and senescing foliage and in early maturing cultivars (Jones, 1893; Pscheidt, 1985; Shuman & Christ, 2005).

In traditional potato farming systems of hilly areas of Nepal such as Lhose and Bung system in eastern high-hills, Navo system in mid and western hills, Khorja in marginal slopy lands, seeding with ploughing in highlands, potato blight is a major disease (Upadhyay & Timilsina, 2020). Rural people of these areas assume that the chemical pesticides change the taste of potato, and therefore no chemicals are sprayed in potato fields and they are marketed as organic potatoes to fetch higher prices. Several studies have reported that the secondary metabolites such as terpenoids, polyphenols, flavonoids, alkaloids, tannins, glycosides and other volatile compounds derived from plants are important sources of phytochemicals to control plant diseases (Khan & Nasreen, 2010; Shalini & Sampathkumar, 2012). Alternative methods including the use of such useful plants for the control of early blight with reduced risk to environment and health have been investigated and adopted to assist those farmers in blight management. The major objective of this study is to evaluate the antifungal activity of selected locally available plants extract against the causative agent of early blight of potato, *A. solani* under in vitro conditions and screen for the presence of secondary metabolites responsible for their anti-fungal activity. It is anticipated that the research findings will enlighten the knowledge of plant protection personnel and pesticide manufacturers on the locally available plants that can be exploited to develop alternative plant disease control products which are much safer and affordable to control early blight disease.

MATERIALS AND METHODS

Collection and Preparation of Botanical Extract

Potential botanicals possessing botanical pesticidal properties were identified through a review of published papers, and a field visit was carried out to evaluate their habitat, status, and availability at the farmer's level. Six locally available plants, namely *Artemisia vulgaris* (Mugwort), *Datura stramonium* (Jimsonweed), *Lantana camara* (Lantana), *Justicia adhatoda* (Malabar nut), *Melia azedarach* (Chinaberry) and *Parthenium hysterophorus* (Santa Maria feverfew) were selected for the experiment. Fresh leaves of the experimental plants were collected, washed and shade dried at 24 ± 2 °C for 3 weeks. The leaf samples were then chopped into small pieces and air dried using hot air oven for several days at 25 °C (Tegelberg, 2018). The dried samples were manually ground, sieved, packed and stored at 4 °C until use.

Plant extracts were prepared using Soxhlet extraction method which continuously extracted the components using the condensed vapours of the solvent (Kamil Hussain *et al.*, 2019). Soxhlet assembly consisted of a collecting flask containing extraction solvent (200 ml acetone), Soxhlet extractor in which 20 g of ground plant sample was packed in filter paper and loaded into the thimble, and a condenser in which vapours of the solvent condense back into solvent. In Soxhlet extractor, there was a side tube which carried the vapour of the solvent from the flask to condenser and a siphon tube which siphoned

off the extract from the Soxhlet extractor to the flask. Soxhlet assembly was placed in a heating mantle which was set according to the boiling point of the solvent (56 °C) to transform extraction solvent into vapour. The extraction process was carried out until the refluxing solvent became clear. The excess solvent was evaporated using the rotary evaporator at 56 °C until the extract becomes fluid like. The extract was poured into falcon tubes and stored in the refrigerator at 4 °C.

Collection, Isolation, Culture and Maintenance of *A. solani*

Early blight was characterized by the visualization of leaf lesions and leaf samples with typical symptoms of *A. solani* were collected from Potato Crop Development Centre (PCDC) Nigale, Sindhupalchowk. Samples were surface sterilized with 1% Sodium hypochlorite for 5 minutes (Yu *et al.*, 2022) and washed three times with distilled water. They were then dried and incubated under a 5 mm thick potato tuber slice on top of a moistened filter paper in petri plates (9 mm Ø) at 24 ± 2 °C. After 24 hrs, fresh sporulation (whitish cottony fungal growth) was visible on abaxial surface of leaves. Pathogen was identified and recorded as *A. solani* by cultural characterization of the cottony fungal growth (Fig. 1) and morphological characterization of their conidia under microscope (Fig. 2). Freshly sporulated mycelia were then carefully inoculated on potato dextrose agar (PDA) media in petri plates (9 mm Ø) and incubated at 24 ± 2 °C for seven days. Pure culture of *A. solani* was maintained in the laboratory by regular transfer of culture to the new PDA.



Fig. 1. Morphological characterization of conidia of *A. solani*



Fig. 2. Cultural characterization of colony of *A. solani*

Experiment Details

The laboratory experiment was carried out at the Plant Pathology Laboratory of National Potato Research Programme, Nepal Agricultural Research Council (NARC), Khumaltar, Lalitpur during 2022 in completely randomized design (CRD) with five replications. Plant extracts, 0.5 and 1 ml of final stock solution per 100 ml of PDA were dispensed in petri plates to prepare the concentration of 0.5% and 1%, respectively.

Treatments

T1: Control, T2: Melia 1%, T3: Melia 0.5%, T4: Justicia 1%, T5: Justicia 0.5%, T6: Lantana 1%, T7: Lantana 0.5%, T8: Parthenium 1%, T9: Parthenium 0.5%, T10: Datura 1%, T11: Datura 0.5%, T12: Artemisia 1%, T13: Artemisia 0.5%, T14: Mancozeb 1000 ppm, T15: Mancozeb 2000 ppm.

Efficacy Testing

Efficacy of plant extracts against *A. solani* was evaluated using poisoned food technique as described by (Farooq & Nasreen, 2015). PDA media was used as a nutrient medium. Plant extracts, 0.5 and 1

ml of final stock solution per 100 ml and mancozeb @ 1 g/100 ml and 2 g/100 ml of PDA were dispensed in petri plates. PDA media untreated with fungicides and plant extracts were used as a negative control while PDA media treated with mancozeb was used as a positive control. Actively growing mycelial plug of 4 mm diameter from 7-day old culture of *A. solani* was inoculated at the center of sterilized petri plates. The plates were then incubated at 25 ± 1 °C. Radial colonial growth of *A. solani* was measured on 3rd, 5th, 7th and 9th days after incubation using Vernier caliper. Percentage inhibition of fungal growth was estimated by using the following formula (Vincent, 1947).

$$I = \frac{C - T}{C} * 100 \quad \dots\dots\dots(1)$$

where,

I = percent growth inhibition

C= colony diameter of fungal mycelium in negative control plates (mm)

T= colony diameter of fungal mycelium in plates amended with extracts and mancozeb (mm)

Table 1. Testing procedures for secondary metabolites

| Secondary Metabolites | Test Procedure |
|-----------------------|---|
| Flavonoid | 1 ml of aqueous extract was mixed with 1 ml of 10% lead acetate solution. Result: Yellow color formation indicates the presence of flavonoid. |
| Alkaloid | 1.5 ml of 1% HCL was mixed with 2 ml of plant extract. The mixture was heated for few minutes to which was added six drops of Mayer’s reagent. Result: Orange precipitate indicates the presence of alkaloid. |
| Saponin | 2 ml of plant aqueous extract was mixed with 5 ml of distilled water and agitated for 3 minutes. Result: Formation of frothing indicates the presence of saponin. |
| Diterpene | 2 ml of plant extract was dissolved in distilled water to which was added 3 drops of copper acetate solution. Result: Formation of emerald green color indicates the presence of diterpene. |

Phytochemical Screening of Botanical Extracts

Based on the observed anti-fungal activity against *A. solani*, in vitro phytochemical screening was performed to test the presence of secondary metabolites in the aqueous extracts of experimental plants using the method adopted by Harbourne (1998), and Trease & Evans (1989). For the preparation of aqueous extracts, dried powder of the plant leaf samples was boiled in water for 15-20 minutes and strained through four layers of muslin cloth and required volume of the stock solution was maintained.

Table 2. Phytochemical analysis of aqueous extracts

| Secondary metabolites | <i>Artemisia</i> extract | <i>Datura</i> extract | <i>Justicia</i> extract | <i>Lantana</i> extract | <i>Melia</i> extract | <i>Parthenium</i> extract |
|-----------------------|--------------------------|-----------------------|-------------------------|------------------------|----------------------|---------------------------|
| Flavonoids | + | + | + | + | + | - |
| Alkaloids | - | - | + | - | - | + |
| Saponins | + | + | + | + | + | + |
| Diterpenes | + | + | + | + | + | + |

+, - indicates the presence or absence of metabolites

Statistical Analysis

The collected data were entered in MS Excel (2019) and analyzed statistically in R-Studio software (version 4.2.1) by using Analysis of Variance (ANOVA). The mean of the treatments was compared using Duncan’s Multiple Range Test at 0.05 level of significance.

RESULTS AND DISCUSSION

In Vitro Efficacy of Different Plant Extracts Against *Alternaria solani*

The radial mycelial growth of *A. solani* in different treatments after 3rd, 5th, 7th and 9th day of incubation are presented in Table 3. Compared to negative control, *Artemisia* @ 1% and 0.5% concentration showed considerable reduction in radial mycelial growth of *A. solani* on all days of observation. On the other hand, *Melia*, *Lantana*, and *Justicia* at both concentrations (1% and 0.5%), and *Parthenium* and *Datura* at their lower concentrations (0.5%) were found to be ineffective in their effects on the *A. solani* growth. *Datura* and *Parthenium* at their higher concentrations (1%) somewhat exhibited moderate control over the growth of *A. solani* *in vitro*. Compared to positive control, none of the botanical extracts tested showed significant reduction in the radial mycelial growth of *A. solani*. The colony diameter in the treatments ranged from 4.634 mm to 58.31 mm.

Table 3. Effect of different treatments on the colony diameter/growth (mm) of *A. solani*

| Treatments | Mean Colony diameter (mm) of <i>A.solani</i> | | | |
|------------------------|--|----------------------|-----------------------|----------------------|
| | 3 rd dai | 5 th dai | 7 th dai | 9 th dai |
| Control | 26.346 ^{ab} | 35.794 ^{ab} | 41.398 ^{abc} | 47.706 ^{ab} |
| <i>Melia</i> 1% | 25.956 ^{ab} | 35.534 ^{ab} | 44.442 ^{abc} | 49.098 ^{ab} |
| <i>Melia</i> 0.5% | 26.958 ^{ab} | 33.932 ^{ab} | 41.146 ^{abc} | 46.944 ^{ab} |
| <i>Justicia</i> 1% | 26.966 ^{ab} | 35.990 ^{ab} | 44.794 ^{abc} | 51.120 ^{ab} |
| <i>Justicia</i> 0.5% | 28.486 ^a | 39.314 ^{ab} | 47.860 ^{ab} | 54.738 ^{ab} |
| <i>Lantana</i> 1% | 26.034 ^{ab} | 35.154 ^{ab} | 43.232 ^{abc} | 50.478 ^{ab} |
| <i>Lantana</i> 0.5% | 26.044 ^{ab} | 38.772 ^{ab} | 49.566 ^a | 58.310 ^a |
| <i>Parthenium</i> 1% | 23.514 ^{bc} | 32.150 ^{ab} | 38.950 ^{abc} | 45.618 ^{ab} |
| <i>Parthenium</i> 0.5% | 26.184 ^{ab} | 39.598 ^{ab} | 48.974 ^a | 51.082 ^{ab} |
| <i>Datura</i> 1% | 22.540 ^c | 32.144 ^{ab} | 40.632 ^{abc} | 45.386 ^{ab} |
| <i>Datura</i> 0.5% | 27.880 ^a | 40.488 ^a | 42.370 ^{abc} | 48.286 ^{ab} |
| <i>Artemisia</i> 1% | 13.592 ^d | 22.882 ^c | 32.808 ^c | 41.034 ^{ab} |
| <i>Artemisia</i> 0.5% | 21.098 ^c | 31.324 ^b | 35.034 ^{bc} | 37.372 ^b |
| Mancozeb 1000 ppm | 4.744 ^e | 9.134 ^d | 13.002 ^d | 16.584 ^c |
| Mancozeb 2000ppm | 4.634 ^e | 8.194 ^d | 10.922 ^d | 13.332 ^c |
| F-probability | *** | *** | *** | *** |
| SEM (±) | 0.273 | 0.650 | 1.032 | 1.388 |
| LSD (p=0.05) | 2.998 | 7.131 | 11.309 | 15.213 |

Note: Values are mean of five replications. The mean values with the common letter(s) within the column indicate a non-significant difference based on the Duncan multiple range test (DMRT) at 0.05 level of significance. dai = days after incubation, LSD = Least Significant Difference, SEM =Standard error of mean, CV = Coefficient of variation, ***= significant at 0.001 level of significance

The percent growth inhibition of *A. solani* compared to negative control in different treatments after 3rd, 5th, 7th and 9th days of incubation are illustrated in Table 4. Of all the plant extracts tested, *Artemisia* showed significant inhibition at both 0.5% and 1% concentration while *Parthenium* and *Datura* were moderately inhibitory in effect at 1% concentration at all days of observation. After 3 days of incubation, *Artemisia* 1% showed the highest inhibition (48.40%) whereas *Justicia* showed the least inhibition (-8.12%). Similar results were obtained in 5th, 7th and 9th days after incubation with *Artemisia* recording the highest inhibition rate at each of the observation.

Table 4. Percent inhibition of mycelial growth of *Alternaria solani* under in vitro condition

| Treatments | Percent growth inhibition of <i>A. solani</i> | | | |
|------------------------|---|---------------------|---------------------|---------------------|
| | 3 rd dai | 5 th dai | 7 th dai | 9 th dai |
| Control | 0 | 0 | 0 | 0 |
| <i>Melia</i> 1% | 1.48 | 0.72 | -7.53 | -2.92 |
| <i>Melia</i> 0.5% | -2.32 | 5.19 | 0.60 | 1.59 |
| <i>Justicia</i> 1% | -2.35 | -0.54 | -8.20 | -7.16 |
| <i>Justicia</i> 0.5% | -8.12 | -9.83 | -15.60 | -14.74 |
| <i>Lantana</i> 1% | 1.18 | 1.78 | -4.43 | -5.81 |
| <i>Lantana</i> 0.5% | 1.14 | -8.32 | -19.73 | -22.23 |
| <i>Parthenium</i> 1% | 10.74 | 10.17 | 18.64 | 18.43 |
| <i>Parthenium</i> 0.5% | 0.61 | -10.62 | 3.10 | 11.83 |
| <i>Datura</i> 1% | 14.44 | 10.20 | 38.86 | 34.87 |
| <i>Datura</i> 0.5% | -5.82 | -13.11 | -2.34 | -1.21 |
| <i>Artemisia</i> 1% | 48.40 | 36.07 | 20.74 | 13.98 |
| <i>Artemisia</i> 0.5% | 19.91 | 12.48 | 31.19 | 36.06 |
| Mancozeb 1000 ppm | 81.99 | 74.48 | 68.59 | 65.23 |
| Mancozeb 2000 ppm | 82.41 | 77.11 | 73.61 | 72.05 |

Owing to its considerable reduction in the radial mycelial growth of *A. solani* on all days of observation compared to negative control, *Artemisia* was supposed to carry the most potent and effective chemical compound to suppress fungal activity. None of the extracts proved more effective than the widely used commercial fungicide mancozeb in its effect on the growth of the fungi. The anti-fungal nature of *A. vulgaris* was also documented on the late blight pathogen, *P. infestans* (Shrestha & Ashley, 2007) and on dry rot pathogen, *Fusarium* sp. of potatoes (Zaker, 2014). Moreover, methanol extracts of *A. annua* was reported to have antifungal activities against root rot pathogens, *F. oxysporum* and *F. solani* (Ma et al., 2019). Similar findings on negative effect of *M. azedarach* on *A. solani* was reported by Singh et al. (2018) who found that *M. azedarach* at low concentration of 2% prompted the growth of *A. solani* significantly more than negative control.

A repetition of experiment following the same procedure, but with the higher concentration (5%) of six plant extracts evaluated before and 2000 ppm of chemical fungicide mancozeb was further carried out to evaluate their anti-fungal efficacy. This experiment was conducted to establish the relation of concentration of extracts in their anti-fungal potential if any.

The radial mycelial growth observations of *A. solani* in different treatments after 5th, 7th and 9th day of incubation are presented in Table 5. All of the botanical extracts tested were effective in inhibiting

the radial mycelial growth of the test pathogen as compared to negative control on all days of observation, however, the treatments differed significantly in their efficacy against test pathogen *A. solani*. After 5 days of incubation, the minimum colony diameter was recorded on *Artemisia* extract treated plates (4.91 mm) followed by *Melia* extract treated plates (6.49 mm) while the largest colony diameter (18.50 mm) was observed in *Lantana* extract treated plates. The efficacy of *Artemisia* and *Melia* extract tested against *A. solani* were significantly similar until the end of the study, however, *Artemisia* consistently performed better than *Melia* throughout the study. Similarly, *Lantana* followed by *Datura* was found with the least inhibitory effect on the colony growth of *A. solani* among the extracts tested. Meanwhile, remaining two plants extracts *Parthenium* and *Justicia* showed moderate inhibitory effect on the mycelial growth of test pathogen compared to negative control.

Table 5. Effect of different treatments on the colony diameter/growth (mm) of *A. solani*

| Treatments | Mean colony diameter of <i>A. solani</i> (mm) | | |
|----------------------|---|----------------------|----------------------|
| | 5 th dai | 7 th dai | 9 th dai |
| <i>Melia</i> 5% | 6.490 ^{de} | 10.250 ^e | 17.545 ^{de} |
| <i>Justicia</i> 5% | 10.983 ^{ed} | 20.800 ^{cd} | 24.458 ^{ed} |
| <i>Lantana</i> 5% | 18.500 ^b | 27.450 ^b | 40.943 ^b |
| <i>Parthenium</i> 5% | 9.066 ^{de} | 17.700 ^d | 28.595 ^{ed} |
| <i>Datura</i> 5% | 14.490 ^{bc} | 24.486 ^{bc} | 34.328 ^{bc} |
| <i>Artemisia</i> 5% | 4.913 ^e | 5.326 ^e | 6.163 ^e |
| Mancozeb 5% | 5.723 ^e | 7.043 ^e | 11.145 ^e |
| Control | 29.556 ^a | 43.950 ^a | 59.408 ^a |
| F-probability | *** | *** | *** |
| SEM (±) | 0.571 | 0.702 | 1.374 |
| LSD (p=0.05) | 4.849 | 5.953 | 11.653 |

Note: Values are mean of three replications. The mean values with the common letter(s) within the column indicate a non-significant difference based on the Duncan multiple range test (DMRT) at 0.05 level of significance. dai = days after incubation, LSD = Least Significant Difference, SEM =Standard error of mean, CV = Coefficient of variation, *** = significant at 0.001 level of significance

The percent growth inhibition of *A. solani* compared to negative control in different treatments after 5th, 7th and 9th days of incubation are illustrated in Table 6. Inhibitory activities of all the treatments were significantly different at p<0.001 level of significance. The data revealed that all of the plant extracts tested showed significant inhibition as compared to negative control at 5% concentration. After 5 days of incubation, *Artemisia* showed the highest inhibition (83.228%) which is statistically similar to positive control mancozeb (80.46%) whereas *Lantana* showed the least inhibition (36.76%). Similar results were obtained in 7th and 9th days after incubation. Inhibition rate of above 50% was shown by four extracts namely: *Justicia*, *Melia*, *Parthenium*, and *Artemisia* throughout the experiment, with *Artemisia* recording the highest inhibition. The results of the experiment are further illustrated in Fig. 3. Of all the plant extracts tested, *Artemisia* showed statistically similar effect on the mycelial growth of *A. solani* as that of positive control.

Table 6. Percent inhibition of mycelial growth of *Alternaria solani* under in vitro condition

| Treatments | Mean Growth Inhibition (%) | | |
|----------------------|----------------------------|---------------------|---------------------|
| | 5 th dai | 7 th dai | 9 th dai |
| <i>Melia</i> 5% | 78.30 | 77.02 | 70.72 |
| <i>Justicia</i> 5% | 61.08 | 52.17 | 57.65 |
| <i>Lantana</i> 5% | 36.77 | 36.88 | 30.53 |
| <i>Parthenium</i> 5% | 68.68 | 59.31 | 51.84 |
| <i>Datura</i> 5% | 49.55 | 43.20 | 41.66 |
| <i>Artemisia</i> 5% | 83.23 | 87.85 | 89.72 |
| Mancozeb | 80.46 | 83.90 | 81.18 |
| Control | 0.00 | 0.00 | 0.00 |

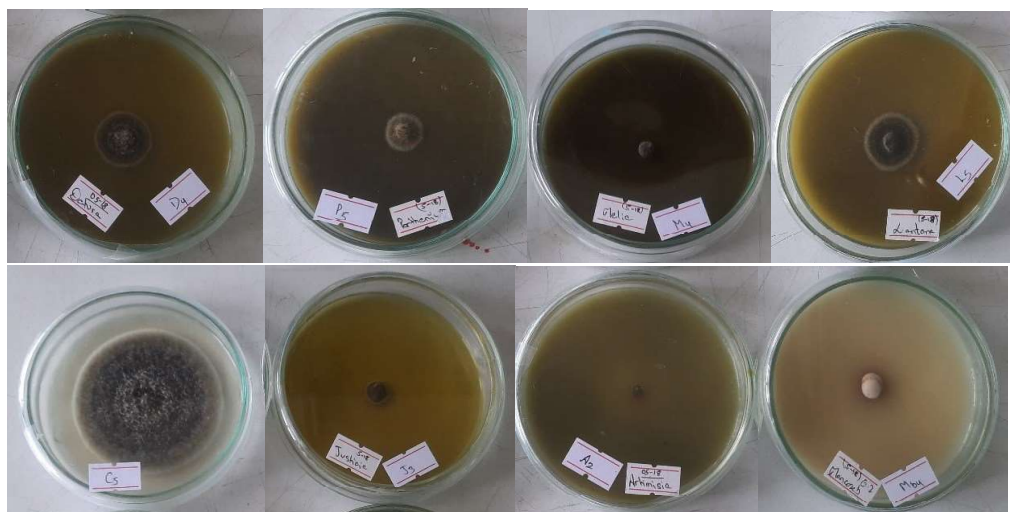


Fig. 3. Growth of *A. solani* in different treatments in vitro

(From left to right: 1st row: *Datura* 5%, *Parthenium* 5%, *Melia* 5%, *Lantana* 5%,
2nd row: Negative Control, *Justicia* 5%, *Artemisia* 5%, Positive Control (Mancozeb @ 2000 ppm))

Several previous researchers have demonstrated similar anti-fungal properties of tested plant species on test pathogen and several other pathogens (Subedi et al., 2015; Subedi et al., 2019). Jabeen et al. (2014) reported the effectiveness of *Melia* leaf extract @5% in retarding the colony diameter of *A. solani* by 90%. Sasode et al. (2012) showed the inhibitory effect of 10% crude extract of *D. stramonium* in the mycelial growth of *A. brassicae* by 39.22%. Kavita and Dalbeer (2015) demonstrated that leaf extract of (*Datura stramonium* at 10% concentration reduced mycelial growth of *A. brassicae* by 53.6%. and leaf extract of *L. camara* at 10% concentration inhibited mycelial growth of *A. brassicae* by 49.67%. Likewise, Bhanage et al. (2019) reported that *Lantana* extract at 5% concentration inhibited *A. solani* mycelial growth by 32.78%. Singh and Srivastava (2012) reported 100% inhibition of *A. alternata* of potatoes and tomatoes in vitro with acetone extract of

L. camara. Tapwal *et al.* (2011) recorded 8% and 50% growth inhibition of *A. solani* by *Parthenium* extract at 5% and 20% concentration, respectively. Raza *et al.* (2016) reported that *Parthenium* extract @ 15% reduced linear growth of *A. solani* and increased inhibition percentage by 59.94% by 7th day of inoculation. Sharma & Kumar (2016) and Shinwari *et al.* (2020) also demonstrated and reported the anti-fungal potential of *J. adhatoda*.

All the tested plant species were found to possess anti-fungal potential and can be explored for the production of biopesticides. Moreover, the selected concentration of plant extracts was not effective in completely limiting *A. solani*, but it can definitely be used in conjunction with fungicides as an IPM strategy to reduce the use of harmful and expensive synthetic fungicides. The research findings will enlighten the knowledge of plant protection personnel, pesticide manufacturers and farmers on the economic potential of such neglected plant species for disease management and fungicides cost reduction.

Phytochemical Screening of Secondary Metabolites in Aqueous Extracts of Selected Plants

The results of preliminary phytochemical analysis of aqueous extracts of experimental plants are interpreted and illustrated (Fig. 4). The presence of major secondary metabolites, namely flavonoids, alkaloids, saponins and diterpenes found to be the reason behind the tested plant extracts valued for the anti-fungal potential.

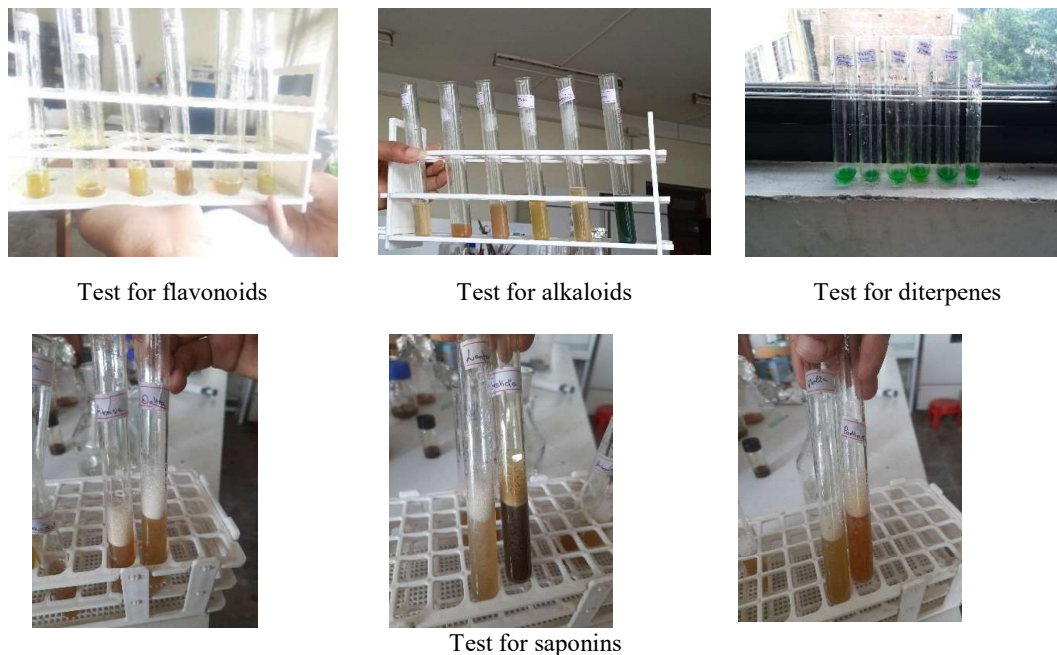


Fig. 4. Detection of various secondary metabolites in plant extracts.

The active compounds observed in the tested plant species were reported to possess anti-microbial potential in several previous studies (Dhanuskar *et al.*, 2000; Cowan, 1999; Din *et al.*, 2016; Ribera & Zuñiga, 2012).

CONCLUSIONS

This study revealed significant inhibition effect of all the tested plant extracts over negative control and *Artemisia* extract over positive control, mancozeb at higher concentration of 5%. At low concentration of 0.5% and 1%, *Artemisia* alone was found effective in inhibiting the mycelial growth of *A. solani*. *Parthenium* and *Datura* were found moderately effective at 1% concentration. *Justicia* and *Lantana* were found with the minimum inhibitory effect at all concentrations. From the findings of this study, it was evident that all plant extracts possessed anti-fungal properties against *A. solani* at higher concentrations, *Artemisia* being the most potent. An *in vivo* experiment in green house and field experiments needed to be carried out to verify the in-vitro efficacy of *Artemisia* extract relative to mancozeb in the management of early blight. The tested plant species should also be assayed for their efficacy on a wide range of pathogens in order to expand their antimicrobial scope.

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