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EFFECT OF AQUEOUS LEAF AND BARK EXTRACTS OF *AZADIRACHTA INDICA* A. JUSS, *EUCALYPTUS CITRIODORA* HOOK AND *PINUS ROXBURGHII* SARG. ON LATE BLIGHT OF POTATO

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A B S T R A C T

Aqueous extracts of sundried powder of leaves and bark of three test plants *Eucalyptus citriodora* Hook., *Azadirachta indica* A. Juss and *Pinus roxburghii* Sarg. were evaluated for their efficacy on epidemiological components (disease severity, lesion size and area under disease progress curve) of late blight of potato caused by *Phytophthora infestans* (Mont.) de Bary in pot culture during 2008. Among the tested plants, aqueous extracts of *A. indica* and *P. roxburghii* significantly contributed in reduction of disease severity, lesion size and area under disease progress curve (AUDPC) after potato leaves were inoculated with *P. infestans*. Extracts of *E. citriodora* had no effect on the studied epidemiological parameters of late blight disease comparative to control. Results demonstrated that extracts of *A. indica* were more effective than *P. roxburghii* in controlling late blight disease. Similarly, leaf extracts were found to be more effective in minimizing disease severity, lesion size and AUDPC than bark extracts. Further research is needed as to whether these extracts can be used as commercial formulations for management of late blight disease.

Keywords: Disease severity, fungicides, natural products, *Phytophthora infestans*.

INTRODUCTION

Late blight of potato caused by *Phytophthora infestans* (Mont.) de Bary is a challenging pathogenic disease of potato and other members of *Solanaceae* which results in considerable production and monetary losses exceeding three billion dollars annually (CIP, 1997; Fry, 2008). The disease may become pandemic in environments with low temperatures (below 20ºC) and high humidity (above 90%) and may result in the destruction of whole potato crop in the field within five days (Haynes *et al*., 2002; Namanda *et al*., 2004). Generally, systemic and contact fungicides are used to control late blight of potato and to reduce yield losses, however, rigorous use of fungicides contribute to environmental pollutions, health risks and introduction of new and more aggressive strains of *P. infestans*; thus there is an increasing public demand for fungicides alternative compounds or natural products to tackle late blight disease (Kato *et al*., 1997; Daayf *et al*., 2003; Majeed *et al*., 2011). Application of natural products, plant extracts and biological antagonists for managing late blight of potato would be helpful in minimizing reliance on synthetic chemicals and pertinent issues attached with the use of such chemicals. However, pesticide market is dominated by synthetic chemicals (Daayf *et al*., 2003) which provide greater challenges for ecofriendly natural products to be opted as sole alternative to synthetic chemicals. One of the many reasons for farmers to mainly rely on fungicides application for controlling late blight disease is the high efficacy and quick results of synthetic chemicals despite of potential health and environmental risks they pose, in addition to playing a key role in the emergence of new resistant strains of *P. infestans*.

In recent decades, rigorous applications of chemical fungicides and other insecticides and pesticides have widely altered public attitude regarding the safety and

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health issues associated with the use of such chemicals for controlling various plant pathogens and insects/pest. Addressing public concerns about health risks and environmental pollution posed by the frequent application of fungicides and insecticides, an increasing tendency of replacing these chemicals with alternative plant extracts and other natural products have been observed in plant pathology research over the last 10 years. Regarding the control strategies implied for late blight of potato, several plant extracts have been previously documented with effective results (Wang *et al*., 2004; Stephan *et al*., 2005; Krebs *et al*., 2006). Efficacy of biological antagonist, natural products, plant extracts and compost tea for controlling late blight pathogen has been frequently reported in the literature. Leaf extracts of grass lemon (*Cymbopogon citratus* DC. Stapf.) at 10 % concentration was found effective in controlling spore germination and disease incidence of *P. infestans* and *A. solani* in field as well as in bioassay experiments (Abdelkhair and Haggag, 2007). Aqueous, acetone or n-hexane leaf extracts of *Inula viscose* L. significantly retarded mycelia growth and disease severity of *P. infestans* and other fungi (Wang *et al*., 2004).

Eucalyptus citriodora Hook (Myrtaceae), *Azadirachta indica* A. Juss (Meliaceae) and *Pinus roxburghii* Sarg. (Pinaceae) are important aromatic medicinal plants with potent allelopathic, antifungal, antimicrobial and pest repellent activities (Luqman *et al*., 2008; Batish *et al*., 2008; Wang *et al*., 2010; Shuaib *et al*., 2013). Important metabolites in *E. citriodora* are citronellal, isopulegol and citronellol (Olivero-verbel *et al*., 2010) which have been tested effectively against pathogenic fungi *Helminthosporium oryzae* (Breda de Haan) and *Rhizoctonia solani* Kuhn (Ramezani *et al*., 2002a,b), *Fusarium oxysporum* Schlecht*., Pythium ultimum* and *R. solani* (Lee *et al*., 2007) and *Phytophthora cactorum* (Leb. & Cohn.)*, Cryphonectria parasitica* (Murrill) Barr and *Fusarium circinatum* Niren. & O'Donnell (Lee *et al*., 2008). Major constituents of *A. indica* are alkaloids, flavonoids and phenolics with proven effects on phyto-pathogenic fungi (Rashid *et al*., 2004; Javed *et al*., 2008; Wang *et al*., 2010). Similarly, Leaves and bark of *P. roxburghii* possess alkaloids, glycosides, flavonoids, saponins, tanins and triterpines which may have potent antifungal actions (Bissa *et al*., 2008; Shuaib *et al*., 2013). The objective of this work was to evaluate sundried leaf and bark extracts of the test plants for their effects on late blight of potato caused by *Phytophthora infestans.*

MATERIALS AND METHODS

Plant materials: Leaves and barks of fully mature trees of *Eucalyptus citriodora*, *Azadirachta indica* and *Pinus roxburghii* were collected from Hazara University Campus, Mansehra during June 2008. Leaves and bark samples were washed with distilled water in order to remove dust and impurities; dried with bolting paper. Collected plant materials were dried under open sunny conditions for seven days.

Aqueous extracts preparation: For preparation of aqueous extracts, 10 g of each sundried leaves and barks were crushed with an electric blinder in 100 ml distilled water and stored for 12 hours following the method of Hussain *et al*. (2010) with minor modifications. Soaked materials were filtered through filter paper no. 1 and filtrate was stored as original stock solution at room temperature for further use.

Experimental: Potato seed tubers (cv. Desiree) were collected from Hazara Agricultural Research Station, Abbotabad and were sown in 28 plastic pots (40 × 20 cm) containing loamy soil. Two tubers were sown in each pot. Randomized complete block design (RCBD) was followed considering each pot a single unit which was further replicated four times. The experiment was conducted in open field conditions during September 2008 at Botany Department, Hazara University, Mansehra. Need bases irrigation was done during the experimental period. Fifty days after planting, four middle leaves of each potato plant were inoculated with 20 µl zoospore suspensions of *Phytophthora infestans*, previously cultured and obtained from naturally late blight infected leaves following the method of Pliakhnevich and Ivaniuk (2008). Zoospores suspensions were transferred to the midrib of each leaf on the adaxial side. Control plants were inoculated with distilled water. One day after inoculations, leave were sprayed with aqueous leaf and bark extracts of respective trees through a hand sprayer at two days interval. Late blight disease was measured as percent disease severity, lesion area and area under disease progress curve (AUDPC) as previously described by Majeed *et al*. (2011) and Chacon *et al*. (2007).

Statistical analysis: In order to determine significance of the plants or plant parts used as controlling agents against late blight disease, data were analyzed by Duncan's Multiple Range Test (DMRT). To separate set of means, least significant differences (LSD) test was used at $p \leq 0.05$.

RESULTS

Effect on disease severity: Data presented in Table 1 demonstrated significant (p≤0.05) effects of aqueous leaf and bark extracts of *A. indica* and *P. roxburghii* on disease severity determined as percent leaf infection. Maximum disease severity (74. 14%) was recorded in control plants. Almost similar results for disease severity (leaf extract, 73.97%; bark extract, 74.04%) were recorded for leaves treated with extracts of *E. citriodora* which had no significant effect on this parameter. Application of aqueous extracts of *A. indica* revealed significantly lowered disease severity 52.89% and 57.18% for leaf and bark extracts respectively in comparison to control. Leaf and bark extracts of *P. roxburghii* resulted in 61.83% and 63.97% disease severity respectively (Table 1). Results also indicated that leaf extracts were more effective than bark extracts of the tested plants for causing significant decrease in disease severity. Leaf and bark extracts of *A. indica* reduced disease severity by 40.17% and 29.66% respectively, whereas, decrease in disease severity under the influence of leaf and bark extracts of *P. roxburghii* was 19.9% and 15.91% respectively (Figure 1).

Table 1. Effect of aqueous leaf and bark extracts on disease severity, lesion size and AUDPC of *Phytophthora infestans*. *Significantly different from control (p≤0.05)

Plant extracts	Disease severity (%)	Lesion size (mm²)	AUDPC
Control	74.14	102.71	1456.12
Eucalyptus citriodora			
Leaf	73.97	103.11	1413.32
Bark	74.04	102.01	1409.50
Pinus roxburghii			
Leaf	61.83*	90.14*	1249.89*
Bark	63.96*	93.86*	1300.54*
Azadirachta indica			
Leaf	52.89*	71.87*	1100.12*
Bark	57.18*	75.53*	1136.10*
LSD values	1.923	2.12	42.754

Effect on lesion size: Table 1 shows that lesion size (mm²) on potato leaves were significantly lowered by aqueous extracts of *A. indica* and *P. roxburghii* when compared to control. Contrarily, extracts of *E. citriodora* completely failed to affect the studied parameter. Larger lesion sizes measuring in the range 102.01-103.11 mm² were recorded in control samples and leaves treated with aqueous extracts of *E. citriodora*. This was followed by significantly lowered lesion sizes in plants sprayed with aqueous extracts of *P. roxburghii* (90.14 mm² for leaf extracts and 93.86 mm² for bark extracts) and *A. indica* (71.87 mm² for leaf and 75.53 mm² for bark extracts).

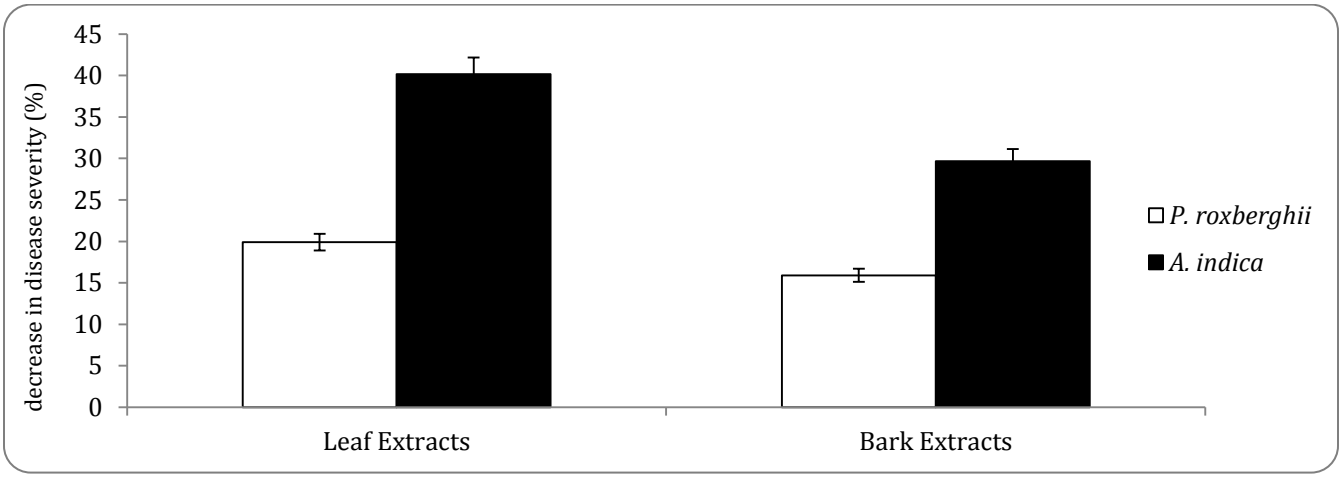

Figure 1. Effect of leaf and bark extracts of *P. roxburghii* and *A. indica* on percent decrease in disease severity of potato foliage.

Among the tested plants, *A. indica* caused pronounced effects on lesion size than *P. roxburghii*. Similarly, in both cases leaf extracts were superior to bark extracts by reducing lesion size to a significant extent. In general, effect of leaf extracts and bark of *A. indica* on lesion size was pronounced reducing the studied parameter by 42.91% and 36.31% respectively (Figure 2).

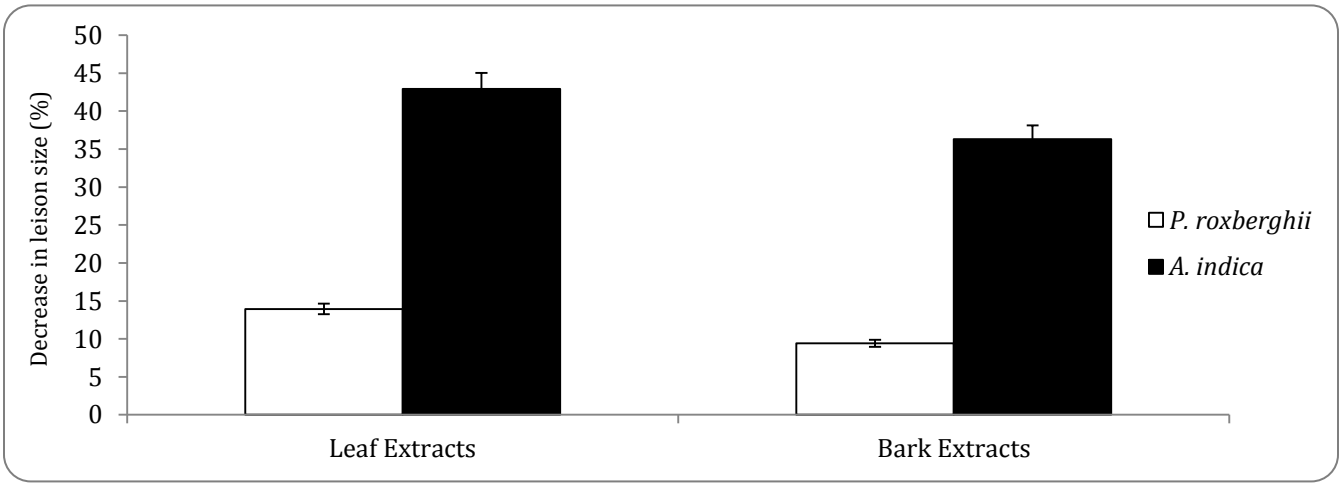

Figure 2. Effect of leaf and bark extracts of *A. indica* and *P. roxburghii* on decrease in lesion size.

Effect on area under disease progress curve (AUDPC): Like other disease components, AUDPC was differentially affected by extracts of the test plants. Plants treated with leaf and bark extracts of *E. citriodora* demonstrated slightly decrease in AUDPC values (1413.32 and 1409.50) than control (1456.12), however, these decreases were not significant (Table 1). Application of aqueous extracts of leaves and bark of *A. indica* and *P. roxburghii* resulted in significantly lower AUDPC values. Lowest AUDPC were recorded in plants sprayed with leaf and bark extracts of *A. indica* (1100.12 and 1136.10). AUDPC values for leaf and bark extracts of *P. roxburghii* were 1249.89 and 1300.54 respectively (Table 1). Results demonstrated that leaf extracts of *A. indica* and *P. roxburghii* were superior in their effects in minimizing AUDPC values than bark extracts. AUDPC was minimized to significant extent 32.36 and 16.49% by leaf extracts of *A. indica* and *P. roxburghii* followed by their respective bark extracts resulting in decrease in AUDPC values 28.16 and 11.96% respectively (Figure 3).

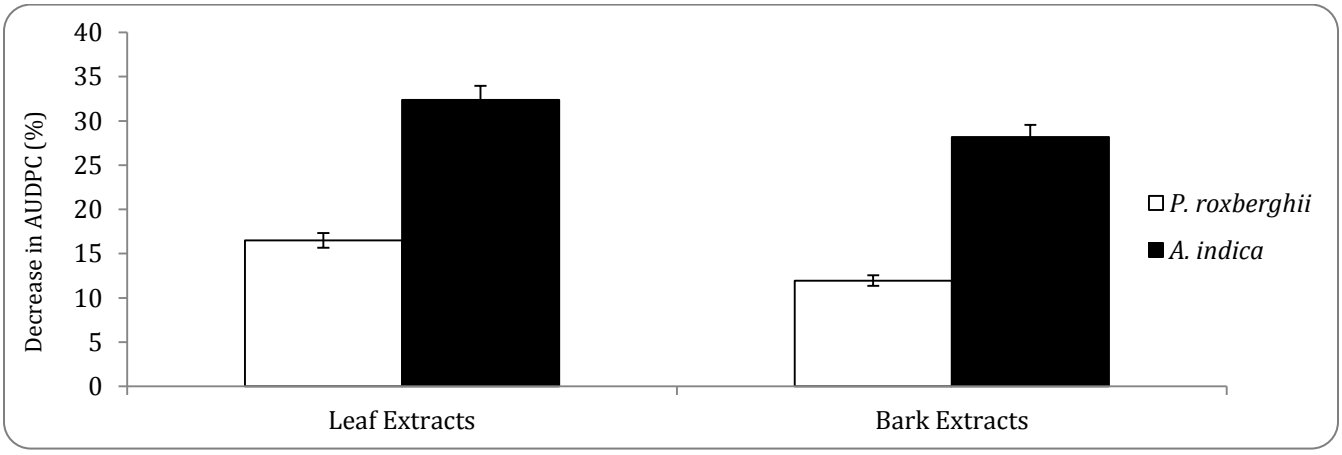

Figure 3. Effect of leaf and bark extracts on percent decrease in area under disease progress curve (AUDPC)a.

DISCUSSION

The objective of this study was to screen out leaf and bark extracts of *E. citriodora*, *A. indica* and *P. roxburghii* for possible effects on late blight of potato. Results demonstrated that leaf and bark extracts of *E. citriodora* had no significant effect on disease severity, lesion size

and AUDPC; however, significant differences were recorded for these attributes when potato leaves were treated with leaf and bark extracts of *P. roxburghii* and *A. indica*. Maximum disease severity (74.14%), lesion size (102.71 mm^2) and AUDPC (1456.12) was observed in control plants with almost consistent values in plants treated with aqueous leaf and bark extracts of *E. citriodora*. Contrarily, plants treated with *A. indica* revealed minimum disease severity, lesion size and AUDPC followed by plant treated with extracts of *P. roxburghii*. Overall inhibitory effects of tested plants on late blight disease were in the order of *A. indica* > *P. roxburghii*. Significant decrease in disease severity, lesion size and AUDPC under the effect of leaf and bark extracts of *A. indica* and *P. roxburghii* in our study could be due the presence of phytoalexins and secondary metabolites which could have inhibitory effects on mycelia growth and sporangial germination of the target pathogen resulting in lowering these epidemiological parameters when compared to control (Batish *et al*., 2008; Wang *et al*., 2010; Shuaib *et al*., 2013).

Different plant parts possess naturally occurring chemicals like alkaloids and phenolic compounds, categorized as secondary metabolites or phytoalexins, at different concentrations which apparently have roles in host defense against pathogens (Friedman, 2006). In the present study, extracts of *A. indica* and *P. roxburghii* showed phathotoxic potentials against the tested pathogen by reducing disease severity, lesion size and AUDPC significantly while *E. citriodora* had no effect on the studied parameters. Efficacy of aqueous leaf and bark extract of *A. indica* and *P. roxburghii* for causing significant reduction in epidemiological components may be attributed to the presence of phytoalexins present in their leaves and barks. Possible explanation for leaf extracts as more efficient than bark extracts of the tested trees in controlling late blight as reported here may be due to higher concentration of phytoalexins in leaves than barks. Several phytochemicals i.e., alkaloids, glycosides, flavonoids, saponins, tanins and triterpines have been isolated from leaves and barks of *P. roxburghii* with known activity against various microbes (Bissa *et al*., 2008; Shuaib *et al*., 2013). Similarly major bio-active constituents such as alkaloids, flavonoids and phenolics from *A. indica* with inhibitory action against a broad spectrum of pathogens including *Phytophthora infestans* and several other fungi have been documented elsewhere (Ahmad and Beg, 2001; Javed *et al*., 2008; Wang *et al*., 2010). In general, our results are supported by previous researchers who reported different plant extracts with increased efficiency against late blight of potato in laboratory as well as in field experiments (Abdelkhair and Haggag, 2007; Wang *et al*., 2004; Majeed *et al*., 2011).

CONCLUSION

Among the tested plants, leaf and bark extracts of *A. indica* and *P. roxburghii* contributed to significant decrease of late blight disease severity, lesion area and area under disease progress curve (AUDPC). Although our results clearly demonstrated that crude aqueous leaf and bark extracts of *A. indica* and *P. roxburghii* were effective in minimizing the epidemiological components of late blight of potato; however, further studies are needed to confirm the efficacy of these plants as commercial formulations against late blight of potato.

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