Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 28-31
Received: 17-03-2019
Accepted: 19-04-2019

Shridhar BP
Department of Plant Pathology, Dr. YS Parmar University of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh, India

Monica Sharma
Department of Plant Pathology, Dr. YS Parmar University of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh, India

Amit Sharma
Department of Basic Sciences, College of Horticulture and Forestry, Dr. YS Parmar University of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh, India

Correspondence
Monica Sharma
Department of Plant Pathology, Dr. YS Parmar University of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh, India

Efficacy of aqueous and cow-urine based biopesticides against *Phytophthora nicotianae* var. *parasitica* causing buckeye rot of tomato

Shridhar BP, Monica Sharma and Amit Sharma

Abstract
Buckeye rot of tomato, caused by *Phytophthora nicotianae* var. *parasitica*, is a serious threat to the crop production and has taken a heavy toll of the crop in India which affects mostly the fruits during both spring and winter season crops. In the present investigation, aqueous and cow-urine based leaf extracts of five plants viz., *Lantana camara*, *Artemisia vulgaris*, *Murraya koenigii*, *Eucalyptus tereticornis* and *Melia azaderach* were evaluated for their efficacy against the test pathogen under *in vitro* conditions. Out of five aqueous biopesticides, *Eucalyptus tereticornis* was found most effective with 51.48 per cent inhibition in mycelial growth of the pathogen followed by *Artemisia vulgaris* (48.52%), *Lantana camara* (44.81%) and *Murraya koenigii* (42.96%). Aqueous extract of *Melia azedarach* was least effective with 39.62 per cent mycelial growth inhibition. Among five cow-urine based biopesticides, *Eucalyptus tereticornis* was found most effective with 71.89 per cent inhibition in mycelial growth of the pathogen followed by *Artemisia vulgaris* (68.74%), *Lantana camara* (63.58%) and *Murraya koenigii* (59.51%). Cow-urine based botanical extract of *Melia azedarach* was least effective with 55.41 per cent mycelial growth inhibition.

Keywords: Plant extracts, antifungal, eco-friendly, indigenous, organic

Introduction
Tomato (*Solanum lycopersicum* L.), a member of family *Solanaceae*, is prone to a number of diseases among which, buckeye rot of unripe tomato is of serious concern to small and marginal hill farmers as it sometimes results in complete failure when fruiting coincides with the onset of rains. High relative humidity coupled with warm weather conditions causes high incidence of the disease. In India, losses due to this disease range between 35 and 40 per cent which may rise with the prevalence and severity of disease up to 90 per cent depending upon the favourable weather conditions. Under high humidity and good rainfall conditions, the incidence of buckeye rot alone may go up to 90 per cent (Gupta and Thind, 2006) [7].
The synthetic chemical fungicides are being used successfully for the management of diseases, but indiscriminate use of these chemicals led to have development of fungicide resistance in pathogen population and more important environmental pollution, posing a potential risk to animal and human health such as undesirable side effects due to their carcinogenic properties (Alkhail, 2005; Lyon *et al*., 1995) [1, 11]. Considering the adverse and alarming effects of synthetic pesticides on environment and natural habitats and the promotion of environmentally sustainable and organic agriculture, fungicide alternatives such as the use of natural plant products is needed (Slusarenko *et al*., 2008; Rice *et al*., 1998) [19, 15]. Plants contain antimicrobial compounds which can be toxic to pathogens and these natural plant products can be used to control plant diseases. Contrary to the problems associated with the use of synthetic chemicals, botanical extracts are environmentally non pollutive, renewable, inexhaustible, indigenously available, easily accessible, largely non phytotoxic, systemic ephemeral, thus readily biodegradable, relatively cost effective and hence constituted as suitable plant protecting agents in the strategy of disease management (Kuberan *et al*., 2012) [10].
The objective of this study was to screen leaf extracts of botanicals for antimicrobial activity against the *Phytophthora nicotianae var. parasitica,* a pathogen of tomato causing buckeye rot disease with the aim to develop alternative control strategy to reduce dependency on synthetic fungicides.

Materials and Methods
Source of pathogen
The pathogen was isolated from the infected fruits of tomato showing the typical symptoms of buckeye rot disease following the standard isolation methods and was used as inoculum.

The pathogen isolated was maintained on carrot-juice agar medium throughout the study at 25 ± 1°C in an incubator for 7 days.

Plant materials and extract preparation

Freshly harvested (200 g) leaves of plants *viz.*, *Lantana camara*, *Artemisia vulgaris*, *Murraya koenigii*, *Eucalyptus tereticornis* and *Melia azedarach* were taken and washed under tap water. Each sample was grinded in mixer and blender by adding small quantity of sterilized distilled water. After grinding, distilled water was added to make the final volume 200 ml. It was then homogenized in orbital shaker for half an hour to get 100 per cent (w/v) botanical extract of each plant. The extracts were then filtered through double-layered muslin cloth. Sterilization of botanical extracts was done through tyndallization in autoclave at pressure 5 psi for 30 minutes for three consecutive days. The extracts were kept in refrigerator for further use. In case of preparation of cow-urine based botanical extract, same procedure was followed as mentioned above but distilled water was replaced by ten days old cow-urine to make the final volume of 200 ml of the botanical extract to make 100 per cent (w/v) cow urine based botanical extract.

***In vitro* evaluation of aqueous biopesticides**

Botanical extracts of *Lantana camara*, *Artemisia vulgaris*, *Murraya koenigii*, *Eucalyptus tereticornis* and *Melia azedarach* were evaluated under *in vitro* conditions against the pathogen by following poisoned food technique (Falck, 1907; Nene and Thapliyal 2011) [6, 13]. Desired concentrations of botanical extracts, each at 5, 10 and 15 per cent was prepared in sterilized distilled water by mixing with equal quantity of double strength sterilized carrot-juice agar medium and poured aseptically in sterilized Petriplates. Medium mixed with equal quantities of sterilized distilled water without any treatment was taken as control. These plates were then inoculated with mycelia discs of 5 mm diameter taken from actively growing culture of *P. nicotianae* var. *parasitica* in the centre and incubated at 25±1 °C in BOD incubator. Each treatment was replicated thrice in completely randomized design (CRD). Regular observations were made and finally colony diameter was measured after the control plates were completely covered by the pathogen.

Per cent inhibition of mycelial growth was calculated by the formula (Vincent, 1947) [20]:

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

Where,

I = Per cent inhibition

C = Growth of test pathogen in absence of biopesticide (cm)

T = Growth of test pathogen in presence of biopesticide (cm)

***In vitro* evaluation of cow urine based bio-pesticides**

Cow-urine based leaf extracts of *Lantana camara*, *Artemisia vulgaris*, *Murraya koenigii*, *Eucalyptus tereticornis* and *Melia azedarach* were evaluated under *in vitro* conditions against the pathogen by following poisoned food technique (Falck, 1907; Nene and Thapliyal 2011) [6, 13]. Desired concentrations of cow-urine based botanical extracts, each at 2, 5 and 10 per cent was prepared in sterilized distilled water by mixing with equal quantity of double strength sterilized carrot-juice agar medium and poured aseptically in sterilized Petriplates. Medium mixed with cow urine only at 2, 5 and 10 per cent concentration was preapared along with medium mixed with equal quantities of sterilized distilled water without any treatment were taken as control. These plates were then inoculated with mycelial discs of 5 mm diameter taken from actively growing culture of *P. nicotianae* var. *parasitica* in the centre and incubated at 25±1 °C. Experiment was conducted in completely randomized design (CRD) and each treatment was replicated thrice. Regular observations were made and finally colony diameter was measured after the control plates were completely covered by the pathogen. Per cent inhibition of mycelia growth was calculated by using formula (Vincent, 1947) [20].

Results

Efficacy of aqueous biopesticides

The results show that all the aqueous extracts of botanicals inhibited the mycelial growth of *P. nicotianae* var. *parasitica* in comparison to control. Aqueous extracts of *Eucalyptus tereticorni*s was the most effective and significantly superior amongst all the treatments with 51.48 per cent inhibition in mycelial growth of the pathogen followed by *Artemisia vulgaris*, *Lantana camara* and *Murraya koenigii* with 48.52, 44.81 and 42.96 per cent inhibition, respectively. Aqueous extract of *Melia azedarach* was least effective with 39.62 per cent mycelial growth inhibition.

Table 1: *In vitro* effect of aqueous biopesticides on the mycelial growth of *P. nicotianae* var. *parasitica*

Treatment	Per cent mycelial growth inhibition at different concentrations (%)			
	5	10	15	Mean
Lantana camara	31.11 (33.87)	38.89 (38.56)	64.44 (53.38)	44.81 (41.93)^{c}
Artemisia vulgaris	26.67 (31.07)	46.67 (43.07)	72.22 (58.18)	48.52 (44.10)^{b}
Murraya koenigii	33.33 (35.24)	36.66 (37.24)	58.89 (50.10)	42.96 (40.86)^{d}
Eucalyptus tereticornis	33.33 (35.23)	43.33 (41.15)	77.78(61.86)	51.48 (46.08)^{a}
Melia azedarach	21.11 (27.33)	34.44 (35.92)	63.33 (52.72)	39.62 (38.65)^{e}
Mean	21.11 (27.12)^{c}	34.44 (32.65)^{b}	63.33 (46.04)^{a}	
$CD_{0.05}$				
Aqueous biopesticide				1.13
Concentration				0.80
Aqueous biopesticide × Concentration				1.96

Figures in the parentheses are arc sine transformed values

Efficacy of cow-urine based biopesticides

The perusal of results show that all the cow-urine based botanical extracts inhibited the mycelial growth of *P. nicotianae* var. *parasitica* in comparison to control. The inhibition rate was higher than the aqueous botanical extracts. Cow-urine based extracts of *Eucalyptus tereticorni*s was most effective and significantly superior amongst all the treatments with 71.89 per cent inhibition in mycelial growth of the

pathogen followed by *Artemisia vulgaris*, *Lantana camara* and *Murraya koenigii* with 68.74, 63.58 and 59.51 per cent inhibition, respectively. Cow-urine based botanical extract of *Melia azedarach* was least effective with 55.41 per cent mycelial growth inhibition.

Table 2: *In vitro* effect of cow-urine based biopesticides on the mycelial growth of *P. nicotianae* var. *parasitica.*

Treatment	Per cent mycelial growth inhibition at different concentrations (%)			
	2	5	10	Mean
Lantana camara	41.72 (40.22)	69.62 (56.54)	79.39 (62.97)	63.58 (53.24)[c]
Artemisia vulgaris	47.89 (43.77)	71.89 (57.95)	86.44 (68.36)	68.74 (56.69)[b]
Murraya koenigii	38.77 (38.49)	62.43 (52.17)	77.34 (61.55)	59.51 (50.74)
Eucalyptus tereticornis	52.72 (46.54)	74.16 (59.43)	88.79 (70.41)	71.89 (58.79)[a]
Melia azedarach	32.86 (34.96)	60.64 (51.12)	72.74 (58.50)	55.41 (48.19)[d]
Cow urine	15.06 (22.81)	22.10 (28.02)	30.13 (33.27)	22.43 (28.03)[e]
Mean	32.72 (32.40)[c]	51.55 (43.60)[b]	62.12 (50.72)[a]	
$CD_{0.05}$				
Cow urine based biopesticide			0.59	
Concentration			0.38	
Cow urine based biopesticide × Concentration			1.02	

Figures in the parentheses are arc sine transformed values

Discussion

Buckeye rot of tomato is the major disease affecting tomato production. The development of disease resistance to conventional fungicide and environmental contamination problems creates pressure on growers to adopt new control strategy in tomato production. The current global scenario firmly emphasizes the need to adopt eco-friendly agricultural practices for sustainable agriculture. Chemical management has made an adverse impact on the health-care of not only soil but also the beneficial soil microbial communities and the plants cultivated in these soils. This eventually has led to a high demand for organic produce by the present-day health conscious society. Therefore, biologically active plant derived pesticides are expected to play an increasingly significant role in crop protection strategies. Exploitation of naturally available chemicals from plants, which retards the reproduction and growth of plant pathogenic fungi, would be a more realistic and ecologically sound method for plant disease management.

Several reports mentioned that the many plant extracts play an important role in controlling different species of *Phytophthora* especially *P. cinnamomi* (Sivasithamparam 1981), *P. capsici* (Shashidhara *et al.*, 2008) [17], *P. nicotianae var. parasitica* (Sarpong, 2016) [16] and *P. cactorum* (Bhaik, 2017). The efficacy of cow-urine and cow-urine based extracts had also been reported against *Phyophthora* spp. by earlier workers i.e. Ashlesha *et al.* (2013) [3] reported the antifungal activity of cow-urine distillates of *Murraya koenigii* and *Melia azedarach* against *P. nicotianae* with 100 and 98 per cent mycelial inhibition, respectively at 10 per cent concentration and cow-urine (10%) alone inhibited the mycelial growth to 95.70 per cent. Krishan (2014) [9] had also observed 70 per cent mycelial growth inhibition of *P. nicotianae* var. *parasitica* by cow urine (10%).

Plants produce metabolites which have antifungal activities. Leaf extract of *Eucalyptus* sp. have fungitoxic effect due to presence of oleanolic and maslininc acid (Rastogi and Mehrotra, 1995) [14]. *Murraya* leaf water extract contains nicotinic acid as well as glucoside, koenigin which exhibit antimicrobial activity (Anonymous, 1962) [2]. *Melia azedarach* possesses both fungistatic and fungicidal activities due to presence of various organic molecules reported as vanillin, hydroxyl-3-methoxcinnamaldehyde and pinoresinol (Carpinella *et al*., 1999; Mishra *et al*., 2013) [5, 12]. The effectiveness of leaf extract of different botanicals in the present study can be corroborated to their fungitoxic effect due to presence of one or the other acid or organic molecules.

References

1. Alkhail AA. Antifungal activity of some extracts against some plant pathogenic fungi. Pakistan Journal of Biological Sciences. 2005; 8:413-17
2. Anonymous. The Wealth of India: A dictionary of India raw materials series and industrial products Publication and Information Directorate, CSIR, New Delhi. 1962; VI:446-48.
3. Ashlesha, Thakur S, Paul YS, Rameshwar, Payal. Antifungal activity of cow urine distillates of local botanicals against major pathogens of bell pepper. African Journal of Agricultural Research. 2013; 8:6171-77.
4. Bhaik A. Studies on Phytophthora fruit rot of apple and its management. M. Sc. Thesis, Department of Plant Pathology**.** Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, 2017, 39-44.
5. Carpinella MC, Herrero GG, Alonso RA, Palacios SM. Antifungal activity of *Melia azedarach* fruit extract. Fitoterapia. 1999; 70:296-98.
6. Falck R. Wachtumgesetze, wachstum Laktorehnund temperature wertde holzersterenden. Myceture. 1907; 32:38-39.
7. Gupta SK, Thind TS. Disease Problems in Vegetable Production. Scientific Publishers, Jodhpur. 2006, 576.
8. Jain SS, Sharma SL, Juneja SL. Studies on buckeye rot of tomato- new to Himachal Pradesh. Proceedings of Indian Science Congress Association. 1961; 50:351-52.
9. Krishan R. Organic management of Buckeye rot of tomato caused by *Phytophthora nicotianae* var. *parasitica* (Dastur) Waterhouse. Ph.D. Thesis. Department of Plant Pathology, CSK HPKV Palampur. 2014, 78.
10. Kuberan T, Balamurugan A, Vidlyapallavis R, Nepoleon P, Jayanthi R, Bealah T *et al. In vitro* evaluation of certain plant extracts against *Glomerella cingulata* causing Brown Blight Disease of tea. World Journal of Agricultural Sciences. 2012; 8:464-67.
11. Lyon GD, Beglinski T, Newton AC. Novel disease control compounds: the potential to 'immunise' plants against infection. Plant Pathology. 1995; 44:407-27.

12. Mishra G, Jawla S, Srivastva V. Melia azedarach: review. International Journal of Medical Chemistry and Analysis. 2013; 3:53-6.
13. Nene YL, Thapliyal PN. Fungicides in plant disease control. Oxford and IBH Publishing Company, New Delhi. 2011, 691.
14. Rastogi RP, Mehrotra BN. Compendium of Indian Medicinal Plants, CDRI, Lucknow, New Delhi**.** 1995; 4:613-15.
15. Rice MJ, Legg M, Powell KA. Natural products in Agriculture – A review from India. Pest Science. 1998; 52:184-88.
16. Sarpong MT. *In vitro* evaluation of the effect of selected plant extracts on the *Phytophthora* fungus causing disease in MD2 variety of pineapple in the central region of Ghana. EC Microbiology. 2016; 4:623-32.
17. Shashidhara S, Lokesh MS, Lingaraju S, Palakshappa MG. *In vitro* evaluation of microbial antagonists, botanicals and fungicides against *Phytophthora capsici* Leon. the causal agent of foot rot of black pepper. Karnataka Journal of Agricultural Sciences. 2008; 21:527-31.
18. Sivasithamparam K. Some effects of extracts from tree bark and saw dust on *Phytophthora cinnamomi*. Australasian Plant Pathology. 1981; 10:18-20.
19. Slusarenko AJ, Patel A, Portz D. Control of plant diseases by natural products: Allicin from garlic as a case study. European Journal of Plant Pathology. 2008; 121:313-22.
20. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1947; 159:850.