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Evaluation of different Botanicals for the Management of Late Blight (*Phytophthora infestans*) of Potato in Karnataka

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ABSTRACT: Amongst all the vegetables, potato is one of the most important tuberous crops and is being constrained by many diseases like early blight, late blight, wart, black scurf, powdery scab etc. One of them which becomes endemic in a particular area and brings > 60 % yield loss is late blight of potato. It is caused by *Phytophthora infestans,* which can affect the crop both qualitatively as well as quantitatively if no early measures are adopted in a short period. Prolonged usage of fungicides has a long-lasting negative impact on bio-diversity with increase in cost of cultivation. With this back ground, totally 20 plant extracts (botanicals) were examined under *in-vitro* conditions, using three methods *viz.,* water extraction, methanol extraction and ethanol extraction methods. The results revealed that in water extraction method the clove extract was very effective in suppressing mycelial growth of *Phytophthora infestans*. While in methanol extraction, clove extract, black pepper, thyme, turmeric and pepper mint had shown a very good response to supress the pathogen at all the concentrations tested. But in ethanol extraction method, it was observed that clove and black pepper were the best ones with 100 *per cent* inhibition for ten days. Among all the methods, ethanol extraction method was found to be the most effective extractant whereas clove and thyme extracts had a good potential anti-fungal property.

Keywords: Botanical extracts, Late blight, Potato, *Phytophthora infestans.*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important vegetable tuber crops. It belongs to solanaceae family (Hijmans and Spooner 2001) with chromosome number 2n = 48 and believed to be originated from south America. It is commonly used as a vegetable or stock feed with industrial applications like starch extraction, alcohol preparation or other processed products. It is also known as a cash crop due to its highest cultivation to fulfil the huge demand of the world. Nutritionally, it is the cheapest source of carbohydrate, with doubled amount of protein in comparison with maize crop and has less fat content (Das, 2000). These tubers are enriched with vitamins like B_6, C and minerals like P, Ca, Mg, K, Fe, S and Cl. Botanically, it is an herbaceous cross-pollinated plant which bears compound leaves with white or purple flowers.

It is cultivated on larger areas in countries like China, Russia, Poland, USA and India being fifth largest producer covering an area of 1.97 lakh hectares with a total production of 344 lakh tonnes and average national yield per hectare is 21.10 tonnes per hectare. Uttar Pradesh, West Bengal, Bihar, Madhya Pradesh, Gujarat, Punjab, Assam, Haryana and Karnataka are the main potato growing states. In Karnataka, it is widely grown in districts of Dharwad, Belagavi, Kolar, Chikkaballapur and Hassan districts in an area of 41,000 hectares with the production of 3,61,000 tons. (Anon., 2015). Now a days, potato cultivation is constrained by many diseases like early blight, late blight, black scurf, powdery scab, Fusarium wilt, common scab, brown rot, soft rot, potato virus X, potato virus Y, crinkle mosaic. Amongst all, the late blight is one of the most devastating diseases which brings yield loss ranging from 50-100 % if not controlled at a right stage (Fry and Goodwin 1997).

Late blight is an important fungal disease caused by *Phytophthora infestans*, it damages all the aerial parts of plant and also affects tuber quality in storage. It was first discovered during Irish famine of Ireland (Large, 1940). In India it was discovered between 1870 and 1880 in Nilgiri hills (Butler, 1918) and in Hooghly district of West Bengal (Butler, 1903) and also was seen in Punjab, Haryana, Uttar Pradesh, Maharashtra, Bihar and Karnataka (Narayana Bhat *et al*., 2010). In South India, the disease is prevalent in the potato growing regions of Karnataka and Tamilnadu (Darwin and Thiru 2011). Initially they appear as brown to black spots on leaves and stem which requires temperature of > 10°C and relative humidity of 80%. Later these spots become blotches and white mold grows on leaves, tubers have dark patches and leads to rotting to cause a foul smell. Infected tubers are often invaded by soft rot bacteria which rapidly convert adjoining healthy potatoes into a rotten mass that must be discarded to

avoid further spread of inoculum (Schumann and Arcy 2000). Peerzada *et al*. (2013) reported the role of environmental factors such as temperature, topographical features and high relative humidity in the development of late blight epidemic.

The disease can be prevented through reduction in the spread of primary source of inoculum by burning of contaminated/infected heaps of tubers, covering dumps with plastic sheets (Cooke *et al*., 2011). Cultural methods can be adopted through crop rotation of legumes, early planting of seed tubers/ use of genetically resistant (Bhardwaj *et al*., 2013) and by changing date of planting (Sekhon and Sokhi 1999) or applying moderate rate of nitrogen fertilizer delays the growth of pathogen or through planting barrier crops (CPRI, 2004-2005). Systemic fungicides are prominently used for controlling fungus at a right time which also acts as a prominent strategy (Hermansen and Naerstad 2009). Even though farmers are adopting numerous strategies like timely application of fungicides and modified cultural practices, they are not being sufficient for the effective control of disease in turn they are having a negative impact on environment with residual ill-effects on human health and increased cost of cultivation. To reduce these negative effects of chemical fungicides, a natural method of plant derivatives *i.e.* botanicals can be used for checking the reactivity rate and their effectiveness to decrease the disease. This eco-friendly practice was first started in Europe and America in late 20th century through the usage of plant botanicals (Blaser *et al*., 1999). With this rationale, an investigation was carried out for identifying a better botanical formulation which has ample potential to decrease the disease severity in all stages of crop growth and further to be suggested to farmers.

MATERIAL AND METHODS

The experiment was carried out in the Department of Plant Pathology, College of Horticulture (GKVK), Bengaluru-560065, University of Horticultural Sciences, Bagalkot, Karnataka. There were 20 plant extracts (botanicals) (Table 1) with two replications and evaluated in a Factorial Complete Randomized Design (CRD) design under laboratory conditions. The plant materials were collected and shade dried to a powdered form for further usage in the experiment. Further infected plant samples brought from different parts of Karnataka, were surface sterilised and transferred to healthy tubers and were preserved in wax. Later pathogen growth was studied in suspended potato tubers, transferred to carrot juice agar media. The inoculated media plates were incubated at 14°C for 8 to 10 days in BOD chamber. The different botanicals were tested for the direct action on *Phytophthora infestans* under *in-vitro* conditions by poisoned food technique and the concentration of botanicals used in this study were 5, 10 and 15% by v/v. Observations were recorded by measuring the radial growth of the mycelium of the test pathogen at 1, 3, 5, 7, 9 and 10th day after the inoculation. Later *per cent* inhibition of growth of test fungus was determined.

Extraction of the botanicals: Three methods namely water extraction, methanol extraction and ethanol extraction methods were tried.

Water extraction method: Procedure: 30 grams of dried plant material was extracted in distilled water for 6 h at slow heat. Every 2 h it was filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected and the extract was then autoclaved at 121°C and 15 lbs. pressure for 15 min.

Methanol extraction method: Procedure: 30 grams of dried plant material was extracted with 90 ml of methanol kept on a rotary shaker for 24 h. Thereafter, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated. The left-over material in the container was utilized for the experimentation.

Ethanol extraction method: Procedure: 30 grams of dried plant material was extracted with 96% ethanol at the ratio of 1:10 w/v., then it was kept on a rotary shaker for 24 h. Thereafter, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated and the left-over material in the container was utilized for the experimentation.

Table 1: List of botanicals evaluated against *Phytophthora infestans* by poisoned food method.

Sr. No.	Common name	Scientific name	Concentration (%)		
1.	Garlic	*Allium sativum* L.	5	10	15
2.	Neem	*Azadirachta indica* A. Juss.	5	10	15
3.	Goldenrod	*Solidago canadensis* L.	5	10	15
4.	Ginger	*Ginger officinalis* L.	5	10	15
5.	Myrobalan	*Terminalia chebula* Retz.	5	10	15
6.	Turmeric	*Curcuma longa* L.	5	10	15
7.	Turmeric leaf oil	*Curcuma longa* L.	5	10	15
8.	Lemon grass	*Cymbopogan flexius* N.	5	10	15
9.	Thyme	*Thymus vulgaris* L.	5	10	15
10.	Pepper mint	*Mentha piperata* L.	5	10	15
11.	Dill	*Anethum graveolens* L.	5	10	15
12.	Hyssop	*Hyssopus officinalis* L.	5	10	15
13.	Lavender	*Lavandula stoechas* L.	5	10	15
14.	Rosemary	*Rosemarinus officinalis* L.	5	10	15
15.	Citronella	*Pelargonium citrosum* L.	5	10	15
16.	Fennel	*Foeniculum vulgare* Mill.	5	10	15
17.	Clove	*Syzygium aromaticum* L.	5	10	15
18.	Black pepper	*Piper nigrum* L.	5	10	15
19.	Datura	*Datura stromonium* L.	5	10	15
20.	Ocimum	*Ocimum sanctum* Linn.	5	10	15

RESULTS AND DISCUSSION

A total of 20 different botanicals were evaluated using three extraction methods to check the potentiality of each formulation and how it works effectively at various levels of concentrations by using poisoned food technique.

Water Extraction Method. In this method, among the 20 plant extracts evaluated under *in vitro* condition, two plant extracts showed the promising efficacy to suppress the test pathogen. Among these, clove extract had shown 100% inhibition against the mycelial growth of fungus at all the three concentrations (5, 10 and 15 %) tested even after ten days (Table 2 and Plates 1 and 2) of incubation. While the thyme extract at 5% concentration inhibited fungal growth, which varied from 95.24% to 87.10%. At 10% concentration, as the days progressed the potentiality of thyme extract also slightly reduced from 100% to 91.89%. At 15% concentration, it was observed that the zone of inhibition was between 100% to 99.32%. Remaining all the 18 botanical extracts tested had shown a decreasing trend of efficacy in suppressing the fungal growth at all the three concentrations. Among them, garlic extract had shown the zone of inhibition ranging between 78.13% to 68.30% in a span of ten days. On the contrary, among all the botanicals tested, hyssop extract at all the concentration *viz*., 5, 10 and 15 per cent the efficacy was least ranging between 29.07% to 46.57% at all the days of observation in suppressing the mycelial growth followed by dill (50.73%) and golden rod (32.46%) at 5 *per cent* concentration in a span of ten days. Similar kind of findings were also observed for garlic extract in order to control late blight by Paik (1989) due to the anti-fungal properties present in garlic cloves to inhibit the mycelial growth of *Phytophthora*. Pham *et al.* (2015) reported that water could extract the highest level of solid content and phenolic compounds from the plant products.

In thyme extract, the reactive capacity was slightly higher due to the presence of anti-fungal active principle and secondary metabolites in the botanicals, which are released only when extracted from water extraction method. In view of the above points, the clove extract was considered as the best plant extract against *Phytophthora infestans* growth at all the concentrations tested using the extract from water extraction method. Similar kind of findings were reported by Burt (2004); Halama and Van-Haluwin (2004); Rodino *et al*. (2013); Ngadze, (2014). Through their studies, it is reported that plant products in the form of botanicals can have a potential fungicidal property/fungistatic effects to control plant pathogens.

Table 2: Efficacy of botanicals extracted by water extraction method against *Phytophthora infestans* by poisoned food method.

	Botanicals	Concentration		
Days after inoculation		5%	10%	15%
First day	Clove	100	100	100
	Thyme	95.24	100	100
Third day	Clove	100	100	100
	Thyme	91.86	98.86	100
Fifth day	Clove	100	100	100
	Thyme	91.38	96.55	99.14
Seventh day	Clove	100	100	100
	Thyme	87.83	93.21	99.23
Ninth day	Clove	100	100	100
	Thyme	86.55	91.55	99.29
Tenth day	Clove	100	100	100
	Thyme	87.10	91.89	99.32

Methanol Extraction Method. In this method, methanol was used as a solvent to check the reactivity of different botanicals against *Phytophthora infestans* growth. It was observed that five formulations namely clove, black pepper, thyme, turmeric, peppermint and myrobalan were found effective after first day. The percent inhibition was ranged between 91.67 to 100% at 5% concentration, 95.24% to 100% at 10% conc, 98.81 to 100% inhibition at 15% of concentration. After ten days, clove extract had performed best in controlling pathogen efficiently with 100% zone of inhibition at all the three concentrations (Table 3 and Plates 1 and 2). Further, the thyme extract had shown 94.37%, 95.77%, 98.53% at 5, 10, 15 % concentration respectively after ten days of incubation. The formulations of hyssop, rosemary, lavender, fennel and citronella were the least performing items in all the three concentrations ranging between 50.51% to 56.25%. The similar results are reported by Pham *et al*. (2015) where, methanol was more effective for obtaining flavonoids and saponins than other solvents because the leaves had the highest amount of total phenolic compounds.

Ethanol Extraction Method. In this method the two extracts *viz*., clove and thyme had 100 per cent inhibition at all the concentrations tested. While, black pepper had 90 per cent inhibition (at 5 per cent conc.) and 100 per cent inhibition (at 15 per cent conc.). Similarly, myrobalan extract had 91.41 per cent inhibition (at 5 per cent conc.) and 100 per cent inhibition (at 15 per cent conc.). The plant extracts *viz*., citronella, rosemary, fennel and lavender were least inhibitory at all the concentrations tested. The results revealed that the ethanol extractants from clove, black pepper, thyme and myrobalan were very effective with 100 *per cent* inhibition at all the concentrations tested

(Table 4 and Plates 1-3). The similar trend was observed on fifth day, seventh day, ninth day and tenth day after incubation. This was because generally ethanol formulation has higher efficiency to inhibit *Phytophthora infestans* growth by releasing the antifungal compounds. And hence it can also refer that among all the extractants, ethanol was the best as they can produce phenolics and can possess high level of effectiveness being a polar solvent. Similar kind of observations were noted by Nashwa and Abo-Elyousr (2012). Among all the three extraction methods, clove, black pepper, thyme and myrobalan were very effective with 100 per cent inhibition at all concentration tested on first day.

Table 3: Efficacy of botanicals extracted by Methanol extraction method against *Phytophthora infestans* by poisoned food method.

Botanicals	Concentration																	
	5%						10%						15%					
Days after inoculation	First day	Third day	Fifth day	Seventh day	Ninth day	Tenth day	First day	Third day	Fifth day	Seventh day	Ninth day	Tenth day	First day	Third day	Fifth day	Seventh day	Ninth day	Tenth day
Clove	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Blackpepper	100	100	91.77	89.34	86.24	86.84	100	100	99.07	99.14	97.62	97.73	100	100	100	100	100	100
Thyme	100	97.78	94.45	93.65	94.12	94.37	100	100	95.45	95.24	95.59	95.77	100	100	98.18	98.41	98.53	98.53
Turmeric	98.68	96.67	92.73	92.06	91.18	91.55	100	97.78	94.55	94.44	94.12	94.37	100	100	100	100	100	100
Pepper mint	97.62	93.88	90.74	88.79	86.51	85.61	100	97.96	95.37	93.10	91.27	90.15	100	100	98.15	97.41	96.03	94.70
Myrobalan	91.67	88.78	87.04	85.34	83.33	81.06	95.24	91.84	90.74	89.66	88.89	88.64	98.81	96.94	95.37	94.83	93.65	93.94

Table 4: Efficacy of botanicals extracted by Ethanol extraction method against *Phytophthora infestans* by poisoned food method.

Botanicals	Concentration																	
	5%						10%						15%					
Days after inoculation	First day	Third day	Fifth day	Seventh day	Ninth day	Tenth day	First day	Third day	Fifth day	Seventh day	Ninth day	Tenth day	First day	Third day	Fifth day	Seventh day	Ninth day	Tenth day
Clove	100	100	98.91	99.04	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Thyme	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Blackpepper	97.44	100	92.39	90.38	89.29	90	100	100	98.91	99.04	99.11	99.17	100	100	100	100	100	100
Myrobalan	96.74	100	95	93.52	92.50	91.41	100	100	100	100	97.50	97.66	100	100	100	100	100	100
Turmeric	72.27	80.18	68.35	64.96	62.68	63.54	80.46	86.62	77.61	77.14	76.31	76.30	84.31	92.17	78.70	79.06	78.93	79.53

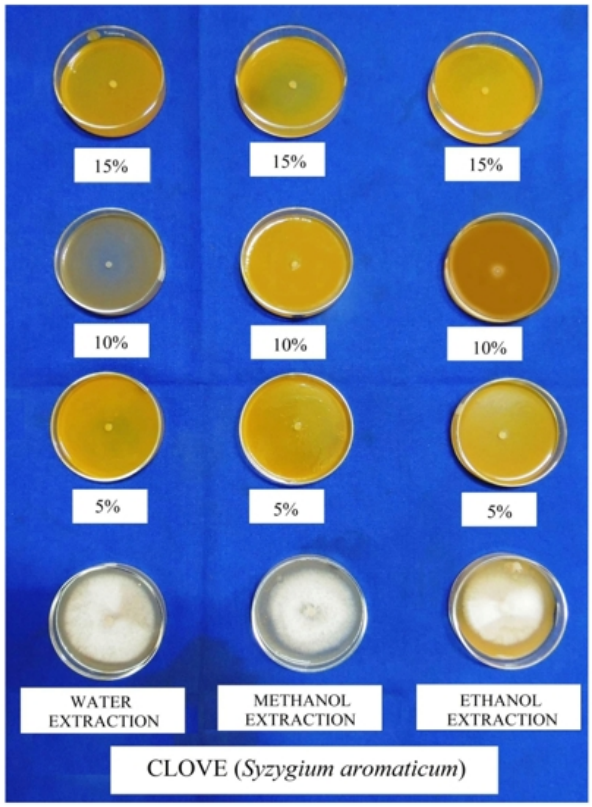

Plate 1 : Photograph showing the efficacy of different extraction methods of clove extract in suppressing the *Phytophthora infestans* on tenth day, after one week of inoculation

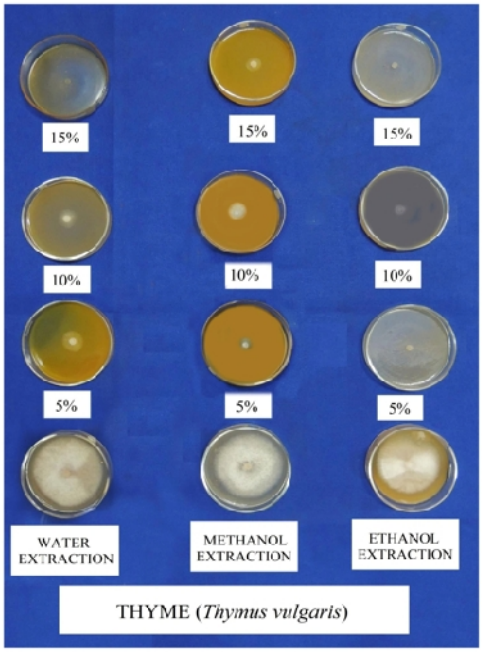

Plate 2 : Photograph showing the efficacy of different extraction methods of thyme extract in suppressing the *Phytophthora infestans* on tenth day, after one week of inoculation

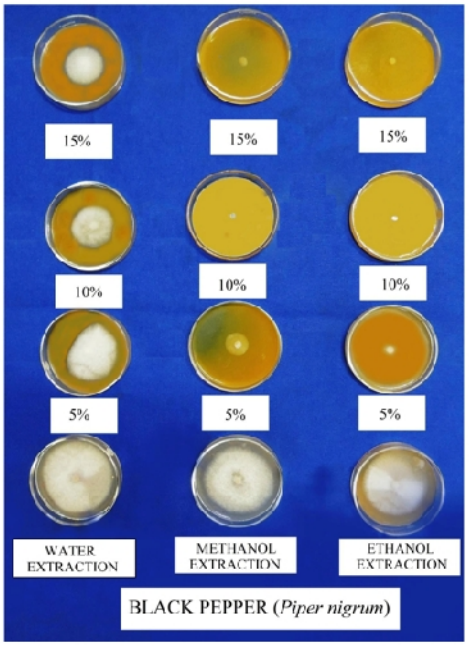

Plate 3 : Photograph showing the efficacy of different extraction methods of black pepper extract in suppressing the *Phytophthora infestans* on tenth day, after one week of inoculation

SUMMARY AND CONCLUSION

Late blight of potato is one of the serious fungal diseases which has become very difficult to control even with the continuous use of fungicides creating additional production costs and adverse environmental hazards besides the development of resistance against fungicides. In order to avoid these adverse effects, the importance must be given towards the usage of eco-friendly measures in the form of botanicals. Hence, 20 plant extracts were used using three extraction methods *viz*., water, ethanol and methanol extraction methods.

The results of the water extraction method and tested under *in vitro* conditions revealed that the extractants from clove was very effective (100 *per cent* inhibition) in suppressing the mycelial growth of *Phytophthora infestans* at all the concentrations *viz*., 5,10 and 15 *per cent* tested even after 10th day of incubation.

While in methanol extraction method, the clove extract has inhibited 100 per cent mycelial growth at all the concentrations tested followed by thyme extract with 94.37 per cent inhibition at 5 per cent concentration, 95.77 per cent at 10 per cent concentration and 98.53 per cent at 15 per cent concentration even after ten days of incubation. In ethanol extraction, clove and thyme extracts had 100 per cent inhibition at all the three concentrations tested even after ten days of incubation.

Among the different extraction methods, the ethanol extraction method was found to be effective, while among the plant products used clove and thyme extracts were very effective with 100 *per cent* inhibition against *Phytophthora infestans* growth under *in-vitro* conditions even after ten days of incubation. These products can be further studied under natural conditions to ascertain its field efficacy.

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Conflict of Interest. None.

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