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Evaluation different botanicals against leaf blight disease of *colocasia* caused by *Phytophthora colocasiae* under *in vitro* and *in vivo* conditions

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Abstract
In order to find out the efficacy of different botanicals (phyto-extracts) against the leaf blight disease of *Colocasia* caused by *Phytophthora colocasiae* there were eight plants extract selected in both in vitro as well as in vivo condition namely, Nanma, *Sapindus mukorossi*, Manma, Neemraj, *Lantana camera*, *Eucalyptus tereticornis*, Yam bean and *Vitex negundo*. In an *in vitro* experiment among these botanicals the maximum per cent inhibition of tested pathogen (94.07%) was observed in *Sapindus mukorossi* seed extract which was significantly superior to rest of the all treatments. It was followed by Nanma (90.36%), Neemraj (72.58%). Among above mentioned plant-extracts from which under *in vivo* condition the minimum disease incidence was recorded in plot sprayed with *Sapindus mukorossi* (33.67%), it was followed by Nanma (37.00%) and Neemraj (39.67%). Maximum mean PDI (64.85%) was recorded in control.

Keywords: *Phytophthora colocasiae*, *colocasia* and botanicals

Introduction
Colocasia is a tuber crop belonging to Araceae family. It is grown throughout India due to its wide adaptability, large scale acceptability and high return unit area-1 (Gurung, 2001) [3]. In India, it is grown in Andhra Pradesh, Uttar Pradesh, Bihar, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra (*Konkan* region), Tamil Nadu and West Bengal. It grows well in lowland and upland areas. The corms, cormels and leaves of *colocasia* are eaten as fried and cooked vegetable. In the India, during the year 2012, the area under *colocasia* was 52000 ha with a production of 654000 tons with productivity of 13.0 tons ha^{-}1. The soil and climatic conditions of *Konkan* region of Maharashtra are favourable for extensive cultivation of *colocasia*.

The crop succumbs to a number of fungal, bacterial and viral diseases as well as some diseases of uncertain etiology. Major among them are, *Phytophthora* leaf blight (*Phytophthora colocasiae* Rae.), *Pythium* rot (*Pythium aphanidermatum*), *Phyllosticta* leaf spot (*Phyl1osticta colocasiophila* weedon) *Cladosporium* leaf spot (*Cladosporium colocasiae* Sawada), southern blight (*Sclerotium rolfsii* Sacc.), spongy black rot (*Botryodiplodia theobromae* Pat), lack rot (*Ceratocystis fimbriata* Ell. and HaIst.), storage rot of tubers (*Rhizopus stolonifer*), *Fusarium* dry Rot (*Fusarium solani* Mars.), bacterial soft rot (*Erwinia carotovora*), bacterial leaf spot (*Xanthomonas campestris* pv *dieffenbachiae*), Dasheen Mosaic (Dasheen Mosaic virus-DsMV), Alomae and Bobone (*Colocasia* bobone disease virus-CBDV) etc. Among all these diseases, leaf blight of taro caused by soil borne fungus *Phytophthora colocasiae* Racib., is the most destructive disease of worldwide occurrence. The pathogen infects all plant parts *viz*. leaves, petioles, corms and cormels leading to heavy yield losses to the tune of about 60 per cent in severe cases (Maheshwari *et al*., 2007) [6].

There is tremendous scope for popularizing this crop as an economically profitable crop among the farmers in the *Konkan* region. Area under this crop is increasing due to the efforts taken by AICRP on Tuber crops functioning at Central Experiment Station, Wakawali, of Dr. BSKKV., Dapoli. However, occurrence of *Phytophthora* blight and consequent yield loss may have a negative impact on acceptance of this crop by the farmers. In this situation, it was necessary to understand the time of occurrence of the disease, to find out a resistant variety if any among the available germplasm and to ascertain the possibility of use of phyto-extracts for the management of the disease under field conditions.

Materials and Methods

In vitro evaluation of botanicals against the pathogen

For this experiment, the extracts of 4 locally available plants with defined antifungal properties, yam been seed extract and 3 marketed formulations were used in this experiment to study their efficacy against the pathogen. The details are mentioned in table 1.

Table 1: Details of plant extracts tested against the pathogen

TTr. No	Name of marketed product(MP)/Scientific and local name of the plant (P)	Composition of marketed formulation	Plant part used	Conc. (%)
1.	Nanma (MP)	Neem and cassava leaf extract	-	10
2.	*Sapindus mukorossi*-soap nut (P)	--	Rind	20
3.	Manma (MP)	Cassava leaf extract	-	10
4.	Neemraj (MP)	Neem leaf extract	-	10
5.	*Lantana camera* –Ghaneri (P)	--	Leaves	20
6.	*Eucalyptus tereticornis* –Nilgiri (P)	--	Leaves	20
7.	*Pachyrhizus erosus*-Yam bean (P)	--	Seed	20
8.	*Vitex negundo* – Nirgudi (P)	--	Leaves	20
9.	Control (OMA)	--	-	-

Preparation of phyto-extracts

Aqueous phyto-extracts were obtained as per the method described by Bhatti (1988). A 100 gram sample (leaves/seeds) of each plant was washed with distilled, sterile water. Then each sample was then ground separately by using sterile pestle and mortal in 100 ml distilled sterile water. The extract of each sample thus obtained was filtered separately through a sterilized double layered muslin cloth to remove the bits of plant material in the filtrate. Then this extract was filtered through a filter paper (Whatman No-1). The filtered extracts were centrifuged at 4000 rpm for 5 minutes to get homogenous aqueous solution. After centrifuging, the supernatant of each extract was collected. This formed the standard plant extracts solution (100%). The marketed products were used by dissolving measured quantity of liquid formulation in water to get desired concentration.

Effect of botanicals against the pathogen

The effect of botanicals on mycelial growth was studied by 'Poisoned Food Technique' (Nene and Thapliyal, 1979) [7]. All glassware used in the study was sterilized before their use. All the plant extracts were tested at 20 per cent concentration and marketed products at 10 per cent concentration against the test pathogen using OMA as a basal medium. To obtain 20 per cent concentration of plant extracts, 20 ml quantity of each plant extract was dissolved separately, in 80 ml of lukewarm OMA in 250 ml conical flask, and then it was stirred well to obtain homogenized mixture. Similarly 10 ml quantity of liquid formulation of each marketed product was dissolved separately in 80 ml of lukewarm OMA in 250 ml conical flask. Twenty milliliter of such poisoned medium was then poured in each sterilized Petri plate and allowed to solidify. Mycelial discs of 5 mm diameter were cut from seven day old culture of test pathogen with the help of sterilized cork borer and transferred aseptically to the centre of each Petri plate already poured with poisoned medium. Petri plates with sole OMA served as control. The inoculated Petri plates were incubated as room temperature (27±2ºC) for further growth of the fungus.

Three replications were maintained for each treatment. The observation on colony diameter of the fungus were recorded when Petri plate in control treatment was fully covered with mycelial growth. Per cent inhibition of growth of the pathogen was calculated by the following formula (Horsfall, 1956) [4].

$$X = \frac{Y - Z}{Y} \times 100$$

Where,

X = Per cent inhibition

Y = Growth of fungus in control (mm)

Z = Growth of fungus in treatment (mm)

In vivo evaluation of botanicals against the pathogen

The field experiment was conducted on variety *Telia* during the *Kharif* season of 2015-16 at the AICRP on Tuber Crops, CES, Wakawali.

Plan of Layout

Season	:	*Kharif*, 2015
Plot size	:	3 X 1.35 m
Spacing	:	60 X 45 cm
Variety	:	*Telia*
Date of planting	:	17/06/2015
Design of experiment	:	Randomized Block Design (RBD)
Replications	:	Three
Treatments	:	9

Treatment Details

Tr. No	Name of marketed product(MP)/Scientific and local name of the plant (P)	Conc. (%)
T_1	Nanma (MP)	10
T_2	*Sapindus mukorossi*- soap nut (P)	20
T_3	Manma (MP)	10
T_4	Neemraj (MP)	10
T_5	*Lantana camera* –Ghaneri (P)	20
T_6	*Eucalyptus tereticornis* –Nilgiri (P)	20
T_7	*Pachyrhizus erosus*-Yam bean (P)	20
T_8	*Vitex negundo* – Nirgudi (P)	20
T_9	Control (water spary)	-

Schedule of spraying

The crop was observed carefully for initiation of disease. Three sprays of plant extracts were applied at an interval of 1 month starting from initiation of disease symptoms. The spray schedule was as under.

1. First spraying 01.08.2015
2. Second spraying 04.09.205
3. Third spraying 03.10.2015

Method of recording observations

Five plants per treatment per replication were randomly selected for recording disease incidence. Initial observations were recorded before first spray and final observations were recorded 1 month after the last spray.

Per cent disease intensity (PDI)

Per cent disease intensity was calculated by the following formula

$$PDI = \frac{\text{Sum of all numerical ratings}}{\text{Total number of leaves examined X Maximum rating}} X100$$

Per cent disease control (PDC)

The per cent disease control was calculated by using the formula given below:

$$PDC = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in control}} \times 100$$

Statistical analysis

The data obtained in all the experiments were statistically analyzed using methods suggested by Gomez and Gomez (1986) [2]. Completely Randomized Design (CRD) was used for radial growth and poisoned food technique and Randomized Block Design (RBD) was used for field experiments. The standard error (S. Em.) and critical difference (C.D.) at level P = 0.01 were worked out in lab experiment and at level P = 0.05 in field experiments. Results obtained in all the experiments were compared statistically.

Results and Discussion

The data obtained on the effect of different phyto-extracts on mycelial growth of the pathogen are presented in Table 2. The maximum per cent inhibition of the fungal mycelium (94.07%) was observed in T_2 (*Sapindus mukorossi*) rind extract which was significantly superior to rest of the treatments. It was followed by Nanma (90.36%), Neemraj (72.58%), *Lantana camera* (51.11%), Yam bean (52.58%), Manma (16.66%), *Eucalyptus tereticornis* (6.3%), and *Vitex negundo* (2.22%). The treatments T_4 and T_6, so also T_5 and T_7 were at par. Among the eight plant extracts, five (T_2, T_1, T_4, T_6, T_7 and T_5) were more effective as the inhibition of fungal mycelium in these extracts was more than 50 per cent. The performance of marketed formulation, Manma (T_3) and leaf extract of *Vitex negundo* was very poor. The efficacy of six plant extracts such as *Azadirachta indica*, *Ocimum bonilienum*, *Ocimum sanctum*, *Allium sativum* and *Allium cepa* was assayed by Shakywar *et al*., (2012) [9]. They found that, leaf extract of *Azadirachta indica* @10% recorded significantly maximum inhibition of mycelial growth (50.13%). In the present study also *Azadirachta indica* @10% inhibited the mycelial growth (72.58%). Similar results were also reported by Anandaraj and Leela (1996) who reported that mycelial growth of *P. capsici* was inhibited up to 75.5 per cent by *Azadirachta indica* extract (2.0%). Hence their results are comparative with the results of this study. As reported by Jagtap *et al*., (2012) [5], leaf extract of *Lantana camera* (5%) was effective against *P. colocasiae*. These results also concur with the results of current investigation as this plant extract recorded growth inhibition up to 51.11 per cent.

The results of the experiment on evaluation of the phyto-extracts against the disease under field conditions are presented in Table 3. All the phyto-extract treatments were significantly superior to control. The treatment T_2 was the most effective as it recorded minimum disease incidence (33.67%). It was followed by T_1 (37.00%) and T_4 (39.67%). But T_4 was statistically at par with T_7. Further the treatments T_3, T_5 and T_6 were at par. Maximum mean PDI (64.85%) was recorded in control. The plant extracts used in laboratory studies was also evaluated under field conditions. Among the plant extracts, rind extract of *Sapindus mukorossi* (20%) recorded PDC up to 48.09 per cent and it was significantly superior to rest of the treatments. This plant contains various alkaloids and saponins which might have restricted the fungal growth under field conditions. So also this plant contains certain chemicals which have high tenacity due to which they form a film on the lamina. Leaves of *colocasia* are glabrous and therefore the sprays of other plant extracts might have been easily washed off but the *Sapindus mukorossi* rind extract was retained for longer time due to natural tenacity and did not allow pathogen propagules to establish and penetrate in the host tissues. In present investigation it was observed that, the great difference between the initial and after spraying of phytoextract incidence of disease in *colocasia*. The incidence of leaf blight disease of *colocasia* was initially more but after spraying of phytoextracts the disease incidence was drastically reduced because of in *colocasia* crop the new leaves emerged gradually after old leaves drop off. Hence the newly emerged leaves were shows less incidence than old incidence due to the effect of phytoextract. Shakywar *et al*., (2014) [8] found that the leaf extract of *Azadirachta indica* @10% was effective against the pathogen under field conditions.

Table 2: *In vitro* efficacy of phyto-extracts against the pathogen

Tr. No.	Phyto - extracts	Conc. (%)	Mean (mm)	Inhibition (%)
T_1	Nanma	10	8.67	90.36
T_2	*Sapindus mukorossi*	20	5.33	94.07
T_3	Manma	10	75.00	16.66
T_4	Neemraj	10	24.67	72.58
T_5	*Lantana camera*	20	44.00	51.11
T_6	*Eucalyptus tereticornis*	20	24.67	72.58
T_7	Yam bean	20	42.67	52.58
T_8	*Vitex negundo*	20	88.00	2.22
T_9	Control	-	90.00	-
SEm ±	0.50			
CD at 1%	2.02			

Table 3: Per cent disease incidence (PDI) and per cent disease control (PDC) of *P. colocasiae* under field conditions

Tr. No.	Scientific Name	Conc. (%)	Mean PDI (%)		PDC (%)
			Before spraying	After spraying	
T_1	Nanma	10	56.67(48.83)*	37.00(37.46)	42.95
T_2	*Sapindus mukorossi*	20	55.33(48.07)	33.67(35.47)	48.09
T_3	Manma	10	53.33(47.87)	44.33(41.75)	31.64
T_4	Neemraj	10	54.67(47.68)	39.67(39.02)	38.83
T_5	*Lantana camera*	20	58.00(49.60)	45.33(42.32)	30.10
T_6	*Eucalyptus tereticornis*	20	55.00(47.87)	46.00(42.71)	29.07
T_7	Yam bean	20	59.00(50.19)	40.33(39.43)	37.81
T_8	*Vitex negundo*	20	56.33(48.64)	49.00(44.43)	24.44
T_9	Control	-	56.33(48.64)	64.85(53.65)	0.00
S. Em ±		0.54		0.68	
CD at 5%		1.62		2.04	

(*****Figures in parentheses are arc sine values)

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