Effect of selected leaf extracts on the mycelial radial growth of *Phytophthora* sp.causing corm rot of colocasia (*Colocasia esculenta* L.)

ABSTRACT

In the present study the pathogenicity of *Phytophthora* sp. causing corm rot of colocasia was proved by Koch's postulates. Sixteen infected corm samples were collected from different markets in two districts (West Godavari and East Godavari) of Andhra Pradesh and pot experiment was carried out in the Department of Plant Pathology, SHUATS, Prayagraj during *Rabi* season (2022-23). Out of those, the sample which collected from KovvurRythu Bazar, West Godavari district in Andhra Pradesh shows high pathogenicity. Five leaf extracts *viz.*, neem, mint, lantana, tulsi and periwinkle @ 5, 10 and 15% concentrations were evaluated by using poisoned food technique against *Phytophthora* sp. Among all the leaf extracts, neem leaf extract @ 5, 10 and 15% concentrations recorded minimum radial growth (26.43, 24.73 and 22.77 mm, respectively) and maximum per cent inhibition (33.27, 37.59 and 42.55%, respectively) of *Phytophthora* sp. at 120 hrs of incubation as compared to other leaf extracts, treated (Metalaxyl) and untreated checks. The present study is limited to one trial in Prayagraj conditions, therefore to substantiate the present results more such trials are required in future for further recommendation.

Key words: Colocasia, Corm rot, Koch's postulate, Leaf extracts, Pathogenicity, *Phytophthora* sp., Poisoned food technique.

1. INTRODUCTION

Taro [(*Colocasia esculenta* (L.) Schott], also known as old cocoyam, true cocoyam is a water-loving herbaceous plant. Taro is ranked the 14th most consumed tuber vegetable in the world, with production capacity of 12 million tonnes generated from approximately 2 million hectares of land with a corresponding average yield of 6.5 tonnes/ ha**(Abdulai *et al*., 2020)**.It is the most important edible species of the monocotyledonous family Araceae. The plant helps in achieving food security because it is a multipurpose crop in which both the corms and leaves are used in different forms as different food products **(Singh *et al*., 2012)**.

The leaves are rich in fiber and are sources of other important nutritional compounds such as vitamins A and C **(Ugwuja*et al*., 2022)**.Despite these numerous socioeconomic benefits of taro plants, the yield of this crop continues to decline yearly due to a numberof constraints. Taro is affected by at least 10 major diseases and pests in different parts of the world andamong themtaro blight disease and corm rot caused by a fungus-like oomycete *Phytophthora colocasiae*Racib is most important disease **(Nelson *et al*.,**

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2011). The disease was first reported and described by Raciborski on taro plants in Java in 1900. The pathogen is the most serious disease-causing organism that causes blight disease of taro worldwide and is the principal cause of huge yield losses up to 100% of both corm and leaf **(Adomako *et al.*, 2017)**.The disease has severely constrained taro production in American Samoa.American Samoa were devastated by an epidemic of taro leaf blight from 1993-1994 **(Misra *et al.*, 2008)**. Taro blight epidemics have the potential to reduce food availability and posing a serious threat to the rural dwellers and regional food security **(Abdulai *et al.*, 2020)**.*Phytophthora colocasiae*Raciborski is of prime importance because it can reduce corm yield by up to 50% and leaf yield by 95% in susceptible varieties. Corm rots usually develop after harvest and entire corms can decay in 7–10 days and causes heavy loss during storage **(Ugwuja*et al.*, 2022)**.

2. MATERIALS AND METHODS

2.1 Collection of Disease Samples

Sixteen infected corm samples were collected from different markets in two districts (West Godavari and East Godavari) of Andhra Pradesh and pot experiment was carried outin the Department of Plant Pathology, SHUATS, Prayagraj during *Rabi* season (2022-23).

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Table 1. Collection of disease samples from markets

S. No.	Name of the sample	Name of the market	Mandal	District	State
1	A1	Ganesh Chowk Rythu Bazar	Rajamahendravaram Rural Mandal	East Godavari	Andhra Pradesh
2	A2	AmalapuramRythu Bazar	Amalapuram Mandal	East Godavari	Andhra Pradesh
3	A3	GopalapuramRythu Bazar	Gopalapuram Mandal	West Godavari	Andhra Pradesh
4	A4	KovvuruRythu Bazar	Kovvur Mandal	West Godavari	Andhra Pradesh

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Example of table below

Figure 1. Collected colocasia corms from different markets in Andhra Pradesh

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There should be a flow.
Say something maybe like "some samples of infected or healthy corms" are shown in Fig. 1 below

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2.2 Isolation of the Pathogen

The infected corm showing characteristic symptoms was cut with healthy portion into small pieces (2-5mm),firstly surface sterilized with sterile distilled water and then sterilized with sodium hypochlorite (0.1%) for 15-30 seconds and again sterilized with sterile distilled water to remove thedisinfectant and blotted dry. The sterilized pieces were plated (4 pieces/dish) on carrot agar medium in ~~Petri~~ Petri-dishes under aseptic conditions. Incubated at 25 °C for five to ten days for obtaining sufficient quantity of inoculums. Pure cultures were obtained by ~~sub~~ sub-culturing.

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Figure 2. Pure culture of *Phytophthora* sp.

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2.3 Inoculation of Pathogen

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The Koch's postulate was verified by inoculating the pathogen thoroughly mixed with the soil. In treated check three rhizomes were sown in each pot and it was inoculated with 10 days old culture of the fungal isolate. In control, three rhizomes were sown in uninoculated pots. The symptoms were observed after 30 days of inoculation in treated pots, whereas uninoculated pots remained symptom-free.

2.4 Symptoms after Inoculation of Pathogen

Infection can occur on any part of the corm and develop rapidly after harvest.In the early stages of corm rot, the symptoms are subtle. When infected corm aresliced (plate 3A), tan colored, rubber-like and soft appearance can be observed. Later an expanding, brown discolored area with a diffuse, indistinct border

develops.Downy whitish growth on the surface of the leaflets and corms **(Nelson *et al.*, 2011)**. After the germination of ~~corm~~corm, the symptoms appeared on the leaf surface as small grey to brown discoloured water-soaked spots which later enlarged, became dark brown and coalesced, finally destroyed the entire leaf lamina**(Nath *et al.*, 2015)**.

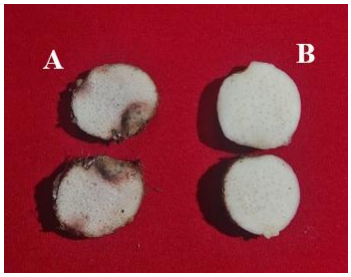

Figure 3. A) Infected and B) healthy corms of Colocasia

Figure 4. Phytophthora blight symptoms on leaves of Colocasia (after corm germination)

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(a)

(b)

Figure 5.(a and b) Colocasia plants in inoculated and uninoculated pots

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2.5 Pathogen ~~Reisolation~~sub-culturing

Pathogen ~~reisolation~~ sub-culturingwas done to fulfil the Koch's postulates. At the end of each test, corms were taken out from inoculated pots then washed with water. Corms were cut into small pieces and surface sterilized with sterile distilled water and with sodium hypochlorite then dried with sterile filter paperfor ~~reisolation~~sub-culturing. Sterilized pieces were plated on carrot agar poured petri plate and `incubated for four to seven days at 25°C in the dark.

2.6 *In vitro* Management of *Phytophthora* sp.

The experiment was conducted as a completely randomized design with seven treatments and three replications. The treatments were evaluated at three different concentrations @ 5%, 10% and 15% concentration of leaf extracts. The average diameter of the mycelial growth inhibition in each treatment was measured for five days post incubation (before the plates were completely covered with mycelia of the fungus). The per cent growth inhibition of the fungus in each treatment in comparison with control was calculated by the following formula as given by **Bliss (1934)**.

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

Where,

PGI = Per cent growth inhibition

C = Colony diameter (mm) in control

T = Colony diameter (mm) in treatment

Table 2. Detail of treatments

Treatments	Treatment details	Concentration (%)	References
T_0	Control	-	-
T_1	Neem leaf extract	5, 10 and 15 %	**Haider *et al.* (2020)**
T_2	Mint leaf extract	5, 10 and 15 %	**Mahaveer*et al.* (2022)**
T_3	Lantana leaf extract	5, 10 and 15 %	**Choga *et al.* (2021)**
T_4	Tulsi leaf extract	5, 10 and 15 %	**Mahaveer*et al.* (2022)**
T_5	Periwinkle leaf extract	5, 10 and 15 %	**Shah*et al.* (2022)**

T_6	Metalaxyl	0.025, 0.050 and 0.075 %	**Adomako*et al.* (2017)**

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2.7 Preparation of Leaf Extracts:

Fresh and healthy leaves were collected. The plant parts were washed thrice thoroughly under running tap water and once with 70% ethanol for 1minute. Then they are finally rinsed with sterile distilled water. Hundred grams of each plant part grinded by addition of 100ml of sterile distilled water with the help of mixer grinder. Then the mixture of each extract subjected/ passed through sterile double layered muslin cloth for twice and collected in conical flasks **(Chakrapani *et al.* 2020)**.

2.8 *In vitro* evaluation of leaf extracts against the pathogen:

The effect of selected botanicals against *Phytophthora* sp. were carried out by poisoned food technique, respectively.

2.8.1 Preparation of medium:

Calculated concentration of each leaf extracts (5, 10 and 15 ml) was thoroughly mixed with sterile molten carrot agar medium (95, 90 and 85 ml) in 250 ml conical flasks and sterilized at 1.1 kg /cm^2 (121.6 °C) pressure for 20 minutes in an autoclave.

2.8.2 Poison food technique:

Amended medium was poured into 90 mm sterile Petri plates and allowed to solidify. Mycelial disc of 5 mm from periphery of actively growing culture was cut out bysterile cork borer and one such disc was placed on the centre of each carrot agar poured platesThree replications and seven treatments were maintained. Control was maintained by growing the pathogen without any treatments. The plates were incubated at 25 ± 2°C. The data was recorded at 24, 72 and 120 hours after inoculation till full growth in control plate. Per cent inhibition of mycelial growth was calculated by using the formula given by**Bliss (1934)**.

3. Results and Discussion

The pathogen causing disease on the ~~colocasia~~Colocasia plants and corms was identified as *Phytophthora* sp. on the basis of morphological and cultural characters.

3.1 Morphological characteristics of pathogen

The morphological characteristics of pathogens are represented in plate 6. The mycelium was hyaline, coenocytic and inter or intra-cellular. The pathogen is a heterogeneous species that asexually produces sporangia, sporangiospores and zoospores. In contrast, as a result of sexual reproduction, it produces oospores **(Shah *et al.*, 2022)**. The sporangiophores are very slender and narrow at the tip.

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The shape of the sporangia varied from globose to ovoid,ellipsoid, elongated or pyriform **(Baysal-Gurel *et al*., 2022)**. The oogonium is spherical and yellowish and the amphigynous antheridium persists at the base of the oogonium for a considerable period after the oospores are formed **(Misra *et al*., 2008)**.

Flagellated zoospores are released from zoosporangia, which convert to cysts and germinate. The chlamydospores are globose or spherical in shape. The abundant production of zoosporangia, zoospores and cysts make *Phytophthora* sp. a devastating pathogen **(Baysal-Gurel *et al*., 2022)**.

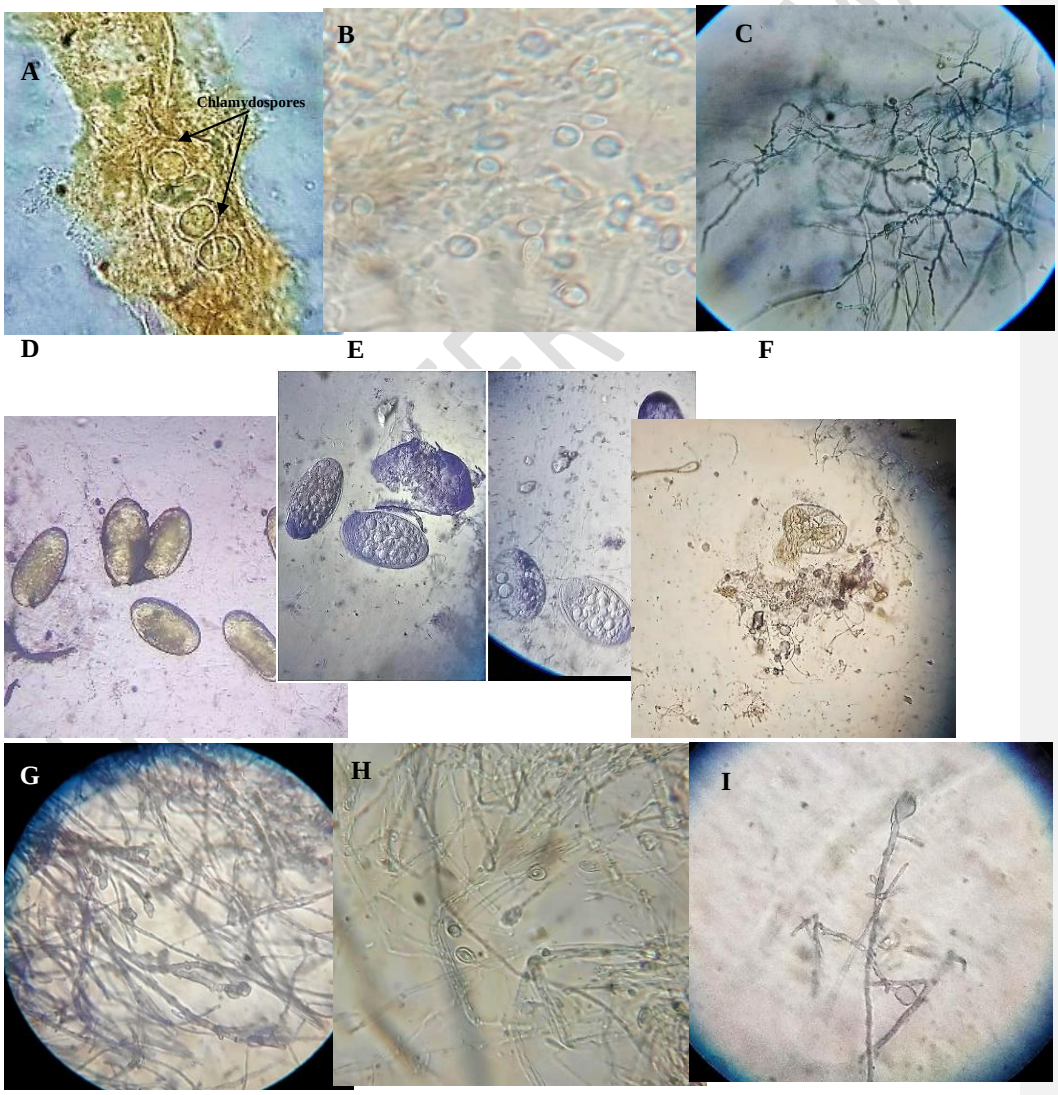

Figure 6. Microscopic view of *Phytophthora* sp. (A) thick walledchlamydospores in rotted corm tissue, (B) chlamydospores of *Phytophthora* sp. (40X), (C) chlamydospores with hyphal swelling (10X), (D) sporangia of *Phytophthora* sp. (40X), (E) zoosporangia (40X), (F) ruptured zoosporangia releasing zoospores (40X), (G) chlamydospore and sporangia with hyphal swelling, (H) internal nested sporangia (40X), (I) sporangium of *Phytophthora* sp. (40X)

Table 3. Efficacy of selected leaf extracts on the per cent inhibition (%) of *Phytophthora* sp. at 120 hrs of incubation as affected by treatments

Treatments	Treatment details	Per cent inhibition (%)*%) *		
		5%	10%	15%
T_0	Control	00.00	00.00	00.00
T_1	Neem leaf extract	33.27	37.59	42.55
T_2	Mint leaf extract	30.60	32.87	39.77
T_3	Lantana leaf extract	32.03	35.48	42.13
T_4	Tulsi leaf extract	31.44	35.06	40.19
T_5	Periwinkle leaf extract	27.49	30.69	34.22
T_6	Metalaxyl (0.025, 0.050, 0.075%)	59.12	65.85	75.18
	F test	S	S	S

*Mean of three replicates

(a)(b) **(c)**

Figure 7. Radial growth of *Phytophthora* sp. at 120 hrs of incubation (a) at 5% concentration, (b) at 10% concentration, (c) at 15% concentration of leaf extracts

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3.2 *In vitro* management of *Phytophthora* sp.

A total of 5 different botanicals were evaluated at various levels of concentrations by using poisoned food technique. Among all the 5 plant extracts evaluated under *in vitro* condition, two plant extracts showed the promising efficacy to suppress the test pathogen. Among these, neem leaf extract had shown 42.55% inhibition against the mycelial growth of fungus at all the three concentrations (5, 10 and 15 %) tested even after five days of incubation. While lantana leaf extract inhibited fungal

growthat all three concentrations (5, 10 and 15%), as the days progressed the potentiality of lantana leaf extract also slightly reducedfrom32.03% to 42.13%.The probable reasons for such findings may bebecause of phytoextracts of neem (*Azadirachta indica*) and lantana significantly inhibited the fungal growth bydisrupting the mitochondria, cell membrane and cell wallof pathogen.Secondary metabolites like azadirachtin, nimbin, polyphenols, terpenoids, saponins and flavonoids have potential to overcome fungal diseases without human health risks.Similar findings have been reported by**Haider *et al*. (2020)** and**Choga *et al*. (2021)**.

4. Conclusion

Sixteen infected corm samples were collected from two districts (West Godavari and East Godavari) of Andhra Pradesh. The samples collected from KovvurRythu Bazar, West Godavari district in Andhra Pradesh showed virulent when compared to other samples. The pathogenicity of *Phytophthora* sp. causing corm rot of colocasia was proved by Koch's postulate. Neem leaf extract @ 5, 10 and 15% conc. recorded minimum radial growth (mm) and maximum per cent inhibition (%) of *Phytophthora* sp. at 120 hrs of incubation as compared to other leaf extracts.

The present study is limited to one trial in Prayagraj conditions, therefore to substantiate the present result more such trials are required in future for further recommendation.

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