

Bioefficacy of Some Plant Extracts to Control Black Rot Disease of Tea {*Camellia sinensis* (L.) O. Kuntze} Caused by *Corticium theae*

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The antifungal activity of aqueous leaf extracts of five weed species, viz., *Chromolaena odorata*, *Melastoma malabatricum*, *Clerodendrum viscosum*, *Polygonum sp* and *Ipomea carnea* was evaluated against black rot disease of tea causing pathogen *Corticium theae in vitro*. Linear growth reduction of the pathogen on PDA plates at 1%, 5% and 10% plant extract concentration was recorded in 3 days interval until the 15th day of inoculation. *Chromolaena odorata* leaf extract exhibited highest inhibition of the pathogenic fungus followed by *Ipomea carnea*, while *Polygonum sp* showed least inhibitory effect. The observations indicated the efficacy of aqueous plant extracts as potential inhibitors of *Corticium theae in vitro*. Application of plant extracts (which are easily available in Barak valley) for controlling plant diseases is non-pollutive, cost effective, non-hazardous and harmonious to ecological balance. Subsequently, field trial was conducted in Randomised Block Design. The plots were maintained in triplicate comprising five bushes in each replicate. Percent symptom and senility was found to be lowest in the plots sprayed with *Chromolaena odorata*. The present field evaluation proved the effectiveness of *Chromolaena odorata* in controlling the black rot disease of tea caused by *Corticium theae*. The same may be recommended to the tea plantations for controlling black rot disease of tea under the agroclimatic conditions of Barak valley (South Assam).

Key words: Antifungal activity, *Chromolaena odorata*, *Clerodendrum viscosum*, *Corticium theae*, *Ipomea carnea*, plant extracts, *Polygonum*.

Tea is a major plantation crop of India and is cultivated extensively throughout North east India. After water, tea is the most widely consumed beverage in the world. It has a cooling, slightly bitter, astringent flavour which many enjoy. Fungal pathogens cause a significant threat to tea bushes. To control this disease chemical fungicides are required to be used. However, the use of chemical

fungicides for tea plants has increasingly become unpopular due to an all-round awareness of its polluting effects, leading to tighter health and environmental regulations. This in turn has created a need for searching for alternative sourcing of fungicidal agent that can be developed for the treatment of fungal leaf disease of tea without having any detrimental effect on the finished product i.e. Black tea.

The traditional practice of using plant preparations to combat fungal disease has gained attention, and currently the focus is on the detection of new antifungal components from plants. Natural products isolated from plants appear to be one of the alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey,

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1999). Several authors have reported antifungal activity of plant extracts against the pathogens of rice, tomato, wheat, pea, and other important crops (Rana et al., 1999; Hu et al., 2001). However, such reports involving tea pathogens are very few (Chakarborty et al., 1991).

This paper presents a study on the efficacy of some plant extracts (weed species) against *Corticium theae*, the causal organism of black rot disease of tea under *in vitro* and field conditions.

MATERIALS AND METHODS

Infected leaves were collected from diseased plants showing characteristic symptoms of black rot, from Rosekandy Tea Estate, Cachar district, Assam. The infected plant parts were cut into pieces (2-3 mm), surface sterilized with 0.1 % sodium hypochlorite for 1 minute. The plant parts were washed three times with sterilized distilled water and then transferred aseptically on Potato Dextrose Agar (PDA) media containing petriplates. The inoculated plates were then incubated at room temperature ($27 \pm 2^{\circ}\text{C}$). After the development of the fungal colonies on the medium, stock cultures were prepared using PDA test tubes slants and stored in refrigerator at 4°C .

Plants used in the present study are *Chromolaena odorata* (Asteraceae), *Melastoma malabatricum* (Melastomaceae), *Polygonum sp* (Polygonaceae), *Ipomea carnea* (Convolvulaceae) and *Clerodendrum viscosum*.

Leaf Extract Preparation

Plant extracts were prepared from fresh leaves of the test plants, i.e. *Chromolaena odorata*, *Melastoma malabatricum*, *Polygonum sp*, *Ipomea carnea* and *Clerodendrum viscosum*. To obtain crude extracts the samples were washed thoroughly with tap water, surface sterilized with 1 % sodium hypochloride and repeatedly washed in sterilized water, and later cut into small pieces and crushed using a mortar and pestle. A 25 % w/v stock solution was prepared by soaking the crushed plant materials in sterilized distilled water for 24 h at room-temperature ($27 \pm 30^{\circ}\text{C}$), passing through muslin cloth and finally through Whatman filter paper no.1. The concentration of 1%, 5 % and 10% w/v was prepared by adding appropriate quantity of sterile water into the stock solution.

In vitro botanicals screening

Leaf extracts of different plants were tested at varied percentage for their efficacy against the black rot disease of tea using the poison food technique (Sinclair and Dhingra, 1985). For bioassay, 5 ml of each 1 %, 5 % and 10 % extract was mixed with 100 ml of molten PDA cooled to 45°C and sterilized in autoclave. The sterilized unamended medium served as control. For each treatment three replicates were maintained. These plates were inoculated with 4 mm disc of freshly grown culture of the pathogen and incubated at $27 \pm 2^{\circ}\text{C}$. Diameter of the fungal colony was measured by measuring the two opposite circumference of the colony growth at 72 h interval for 15 days.

Field Observations On The Disease Development/ Control

The experimental plants were examined for disease development through symptom and senility index. The tea bush plucking table was divided into four equal parts and values were assigned to each, proceeding from the infected part of the plucking table. Symptom expression in one-fourth of the plucking table was given the value 1; if half of the table was affected then the value 2 was given; if three quarter of the plucking table of the bush was affected value 3 was given, and if the symptoms are found throughout the plucking table or the plants are showing symptoms of total defoliation/ death, due to black rot disease the value 4 was given. A modified symptom and senility index described earlier by Dutta (1991) was used for calculating for each group of plants in a single treatment as a percentage figure.

$$\text{Symptom \& Senility index} = \frac{\text{Sum of the individual rating value} \times 100}{4 \times \text{no of plants assessed}}$$

RESULTS AND DISCUSSION

The result of the effect of plant extracts on the linear growth of the pathogenic fungus *Corticium theae* under *in vitro* condition are presented in table I. It can be seen that radial mycelial growth of *Corticium theae* was inhibited on PDA medium containing plant extracts as compared to the control. Three days after inoculation there was free growth in the control plates (53.4 mm). The plates consisting of PDA amended with plant extracts of the five plant species

separately, in respective treatments caused significant reduction in the radial growth of the pathogenic fungus. Maximum radial growth reduction by different plant extracts is observed in the following ascending order *Chromolaena odorata*, *Ipomea carnea*, *Clerodendrum viscosum*, *Melastoma malabatricum* and *Polygonum sp.*

All the plant extracts tested significantly ($p < 0.05$) inhibited the mycelial growth of the fungi in the amended PDA medium in vitro at all the concentrations tested. However the effectiveness of the plant extracts increased with the increase in concentration and this was found to be statistically significant ($p < 0.05$).

At all the concentrations, the extracts of *Chromolaena odorata* inhibited the growth of *Corticium theae* more than that of *Ipomea carnea*, *Melastoma malabatricum*, *Clerodendrum viscosum* and *Polygonum sp* respectively.

There was a gradual decrease in the disease symptom as per the symptom and senility index in the diseased plots sprayed with the aqueous leaf extract of *Chromolaena odorata* as compared with the plots sprayed only with water which served as control.

Considering the need for alternative biorational fungicides in tea plantations, it was

thought to be worthwhile to evaluate the antifungal effects of locally available plant/ weed extracts.

The difference in the toxicity of the different extracts may be due to the solubility of the active compound(s) in the solvents or due to the presence of inhibitors or the fungitoxic principle (Tewari and Nayak, 1991; Amadioha and Obi, 1998). However, the presence of the active component in plants is influenced by several factors such as method of extraction, age of the plant, time of harvesting the plant material and different extracting solvents (Nicolls, 1969).

The antifungal activity of the selected plants may be attributed to their constituents of biologically active compounds. Several studies have been conducted to understand the mechanism of action of antifungal activity of plant extracts and essential oils, but it is still unclear (Hadizadeh *et al.*, 2009). However, some researches attributed the antimicrobial activity to the phenolic compounds. The amphipathicity of these compounds can explain their interaction with biomembranes causing the inhibitory effect (Veldhuzen *et al.*, 2006). Omidbeygi *et al.*, (2007) suggested that extract components cross the cell membrane, interacting with enzymes and proteins of the membrane, producing a flux of protons

Table 1. Efficacy of plant extracts against *Corticium theae*

Treatment	Conc%	Radial growth of the fungus(mm)				
		3rd	6th	9th	12th	15th
<i>Ipomea carnea</i>	1510	23.7(±2.79)	27.58(±3.13)	35.37(±0.64)	42.38(±0.55)	45.77(±0.94)
		5.8(±0.11)	10.8(±1.15)	18.5(±0.86)	20.2(±1)	25.3(±0.4)
		5.6(±0.2)	6.15(±0.33)	9.38(±1.8)	10.98(±1.74)	11.88(±1.42)
<i>Chromolaena odorata</i>	1510	18.39(±0.6)	23(±1.21)	36.25(±0.58)	38.76(±0.28)	42.41(±0.93)
		6.39(±1.55)	6.4(±1.5)	6.8(±1.73)	7(±1.73)	7.89(±1.73)
		5.53(±1.64)	5.8(±1.87)	6.06(±1.67)	6.43(±1.54)	6.9(±1.62)
<i>Clerodendrum viscosum</i>	1510	46.5(±0.5)	49.66(±0.3)	52.5(±0.28)	55.5(±0.5)	59.33(±0.33)
		36.2(±1.79)	38.4(±0.44)	40.9(±0.51)	50.5(±3.88)	51.31(±3.78)
		18.5(±0.7)	18.66(±0.66)	24.33(±0.33)	27.66(±1.2)	28(±1)
<i>Polygonum sp</i>	1510	40.33(±0.5)	42.33(±0.72)	44.5(±0.5)	47.5(±0.18)	50.33(±0.95)
		29.6(±1.32)	49(±0.86)	54.1(±2.3)	61.7(±2.88)	64.7(±2)
		25.66(±0.1)	28.16(±0.33)	30.66(±0.6)	33(±0.57)	35.5(±0.76)
<i>Melastoma malabatricum</i>	1510	46.5(±2.5)	51.33(±2.12)	52.5(±1.8)	58.33(±0.6)	63(±1.3)
		42(±2.02)	44(±2.05)	45(±1.96)	48(±1.58)	52.27(±1.5)
		32(±0.5)	36.33(±1.09)	38.66(±0.72)	41.83(±0.88)	44.66(±0.92)
Control		53.4(±1.73)	58.5(±2.51)	61.2(±2.42)	63.5(±1.15)	79.25(±2.88)
CD at 5 %		10.43	10.55	12.99	13.43	13.66
CD at 1 %		14.47	14.63	18.02	18.63	18.94

*mean of three replications, the experiment is significant at 5 % level of significance

towards the cell exterior which induces changes in the cells and ultimately, their death. Sharma and Tripathi (2006) concluded that essential oils and plant extracts may act on the hyphae of the mycelium, provoking exit of components from the cytoplasm, the loss of rigidity and integrity of the hyphal cell wall, resulting in its collapse and death of the mycelium.

It is evident from the results of the present study that susceptibility of pathogen to plant extracts depends upon plant species (Bagwan, 2001), solvent used for extraction and extract concentration (Abou-Jawdah *et al.*, 2002) as well as the organism tested and phase of growth (Kumaran *et al.*, 2003). The variation in antifungal activity of the selected plants may be attributed to the difference in chemical nature and concentration of the active constituents of each plant that has been tested (Hadizadeh *et al.*, 2009).

Among the different plants whose extracts were found to be effective, *C. odorata* showed maximum potential in inhibiting the mycelial

growth of *Corticium theae in vitro*. The findings is in agreement with Ebele Martina Illondu, 2011 in which he evaluated the antifungal properties of *Chromolaena odorata*, *Carica papaya* and *Acalypha ciliata* on the growth of the isolates and observed that these plants' crude extracts possess some inhibitory components which cause significant reduction in mycelia growth of *Aspergillus niger*, *Fusarium solani* and *Botryodiplodia theobromae*. This agrees with the results of Amadioha (1998), Owolade & Osikaniu (1999) & Adejumo *et al.*, (2000) who reported the efficacy of extracts from *C. papaya*, *A. ciliata* and *C. odorata*, among other extracts in reducing the mycelia growth of *Erysiphe cichoracearum*, *Collectotrichum capsici* and *Protomyces phaseoli*, which compared favourably with the chemical pesticides Benlate & Ridomil. However, there are no previous reports on the antifungal activity of *C. odorata*, *I. carnea*, *C. viscosum*, *M. malabatricum* and *Polygonum sp* against *Corticium theae*.

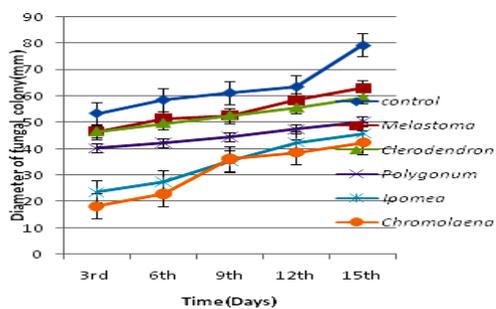


Fig. 1. Effect of some plant extracts (1 %) on the growth of *Corticium theae in vitro*

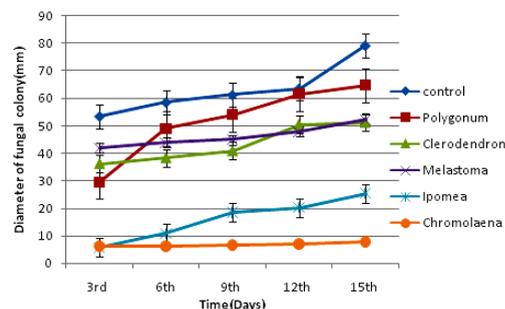


Fig. 2. Effect of some plant extracts (5 %) on the growth of *Corticium theae in vitro*

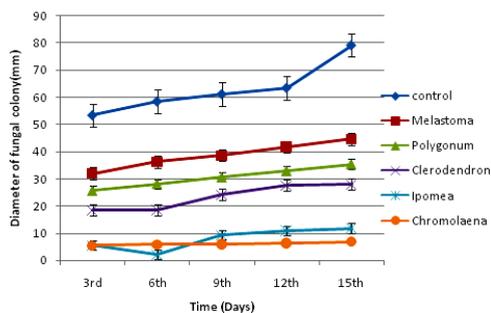


Fig. 3. Effect of some plant extracts (10 %) on the growth of *Corticium theae in vitro*

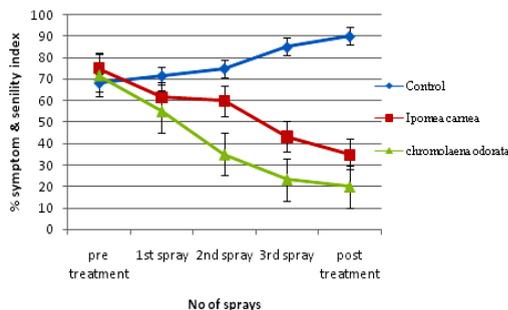


Fig. 4. Effect of foliar spray of plant extracts on the disease development in black rot infected tea bushes caused by *Corticium theae*

Various plant extracts have been examined by different investigators for their antifungal activity with the objective of exploring environmentally safe alternatives of plant disease control. Martinez et al., (2000) reported variable effects of *Sagassum filipendula* extracts on *Aspergillus* species including *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus parasiticus*. Saha et al., (2005) tested the antifungal activity of some plant extracts against the pathogens of tea i.e. *Pestalotiopsis theae*, *Curvularia eragrostidis*, *Colletotrichum camelliae* and *Botryodiplodia theobromae* and recorded that *Allium sativum* L., *Datura metel* L., *Dryopteris filix-mas* (L.) Schott, *Zingiber officinale* Rosc., *Smilax zeylanica* L., *Azadirachta indica*, A. Joss and *Cucurma longa* L. gave a 100 % inhibition of spore germination.

Ojo and Olufolaji (2005) reported that utilization of plant extracts may go a long way in providing better alternative to the over dependency on synthetic fungicides. The use of plant products in integrated pests and fungal disease management could reduce over reliance on one source of agricultural chemical to the farmers, as well as cut down the cost of production. The plants used in the present study are readily available and with easy method of extraction it can be exploited in the control of black rot disease of tea with ease.

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