Efficacy of phytoextracts and oils of certain medicinal plants against *Cercospora moricola* Cooke., incitant of mulberry (*Morus alba L.)* leaf spot

C. Raja Gopal Reddy* R.S.Nirmala and CH. Ramanamma**

ABSTRACT

Certain phytoextracts and plant oils were treated *in vitro* for their antifungal efficacy against the growth of *Cercospora moricola* Cooke, the incitant of leaf spot of Mulberry (*Morus alba* L.). Highest mycelial growth inhibition (72.59%) was recorded in *Eucalyptus globules* with 10% concentration. The next best plant extracts are, *Oscimum sanctum* (49.08%), *Phyllanthus emblica* (46.75%), *Aloe barbedensis* (45.75%), *Allium sativum* L. (41.08%) and *Azadirachta indica* (35.25%). Plant oils viz., *Madhuca indica* oil (3%) *Cymbopogon citratus* oil (0.05%) and neem oil (3%) also inhibited the mycelial growth of the fungus with 75.73%, 73.22% and 24.44% respectively, when compared to control. All the tested phytoextracts showed more or less inhibitory effect on mycelial growth on dry weight basis. Growth inhibition ranged from 49.24% to 60.12%. Significantly, the highest inhibition was recorded in *Eucalyptus globules* (10%) with 60.12%, followed by *Aloe barbedensis* (57.37%), *Oscimum sanctum* (56.40%), *Phyllanthus emblica* (54.26%), *Allium sativum* L.(51.68%) and *Azadirachta indica* (50.81%). In case of oils, neem oil (3%) showed highest per cent inhibition (40.44%), when compared to *Madhuca indica* oil (3%) with 40.23% and *Cymbopogon citratus* – (0.05%) with 30.62%.

Key Words: Mulberry, disease, *Cercospora moricola*, phytoextracts, plant oils, medicinal plants.

INTRODUCTION

Diseases are the major limiting factor in mulberry cultivation for production of quantitative and qualitative leaf production. Number of fungal pathogens cause variety of diseases to mulberry foliage, roots or stem right from its plantation. Out of them, foliar pathogens are of major significance, because of their direct accessibility to mulberry leaves as they are air-borne in nature. They cause 15-20% loss in leaf production, besides severe destruction of leaf lamina to about 20-25% which leads to leaf loss both by quantity and quality. Mulberry leaf is the sole feed of the silkworm. Feeding of the Silkworms with the diseased leaves leads to poor larval growth and are prone to different bacterial, viral and fungal diseases. The healthy and robust growth of the silkworms depends upon the quality mulberry leaf, which ultimately reflects in the qualitative and quantitative parameters of the cocoons. Because of the adverse environmental conditions and maintenance of the mulberry gardens round the year with so much of greenery in sericulture tracks of India, many diseases and insect pests of various agricultural crops are invaded mulberry plantations (Reddy *et al.*, 1999, 2000, 2002, 2003, 2007, 2007a, 2007b; Lakshmi Reddy *et al*., 2001; Suneetha Golla and Raja Gopal Reddy, 2008). So, there is every necessity in raising healthy mulberry plantation for production of quality leaf, duly following package of practices and protocols of pest management.

Cercospora moricola Cooke, the incitant of leaf spot disease causes serious damage to mulberry leaf yield and quality. It causes a direct leaf yield loss of about 5 % due to defoliation, which may, however, reach up to 35 per cent in most severe conditions. In addition, it also causes 20-25% destruction of leaf lamina (Sikdar and Krishnaswamy, 1980). The disease badly affects the nutritional value of leaves by reducing moisture, proteins, sugars and chlorophyll contents, and thus makes the leaves unsuitable for silkworm feeding (Sikdar *et al.*, 1979; Siddaramaiah and Hedge, 1990; Srikantaswamy *et al.*, 1996).

Cercospora moricola produces minute circular brownish spots on the leaves, which gradually increase in size, and turn dark brown surrounded by chlorotic holes. On severity, the spots coalesce resulting in larger spots, yellowing and defoliation probably because of the production of some toxins by the invading pathogen (Siddaramaiah *et al*., 1980). Among various fungicides tested, spraying of 0.2% solution of Carbendazim 50%WP twice at an interval of 15 days after disease appearance is

Table 1. Plant species screened in the experiment

Plant Species	Family
Azadirachta indica	*Meliaceae*
Allium cepa L.	*Liliaceae*
Allium sativum L.,	*Liliaceae*
Adathoda vasica	*Acanthaceae*
Aloe barbedensis	*Liliaceae*
Cassia tora L.	*Caesalpiniaceae.*
Catharanthus roseus	*Apocynaceae*
Eucalyptus globules	*Myrtaceae*
Lantana camara	*Verbenaceae*
Leucas aspera	*Labiatae*
Mentha viridis	*lameaceae*
Murraya Koenigii	*Rutaceae*
Oscimum sanctum	*Labiatae*
Parthenium hysterophorus L.	*Asteraceae*
Phyllanthus emblica	*Euphorbiacae*
Ricinus communis. L.	*Euphorbiacae*
Tagetes erecta	*Astreraceae*
Vitex negundo	*Vitaceae*
Zingiber officinale	*Zingiberaceae*

found most effective and easiest method of control (Siddramaiah *et al.*, 1978). Use of bio-degradable materials like fresh plant extracts has been taken up on top priority during the last three decades for plant disease control in view of the high cost of chemical pesticides and their hazardous nature (Mitra, 1984). Chemical control of mulberry disease is involved with so much of risk for having the residual toxicity to silkworm, when the chemical sprayed leaves are fed to them. So, there is a need to find out some bio-degradable natural products of non-toxic nature in containing the leaf spot of mulberry caused by *Cercospora moricola*.

MATERIALS AND METHODS

Isolation of *Cercospora moricola*

The leaf samples infected with *C. moricola* Cooke. were collected from the mulberry demonstration farm at Anantapur, A.P, in sterilized polythene bags separately for culturing and isolation. The diseased leaves having distinct leaf spot symptoms were collected for the identification of causal pathogen. Microscopic slides of free-hand sections of infected leaves were prepared and stained with Lactophenol-cotton blue and observed under microscope (400 X).

C. moricola infected areas of the mulberry leaf was cut into bits (0.5 cm) and surface sterilized with 0.1% mercuric chloride solution for one minute, washed in sterile distilled water thrice and plated in Potato Dextrose Agar Media. The plates were incubated at 28 ± 2° C. On the 4th day, the fungal growth around the bits were examined and aseptically transferred to PDA slants. The isolate was further purified by mono-hyphal tip method, identified and maintained on PDA medium.

Identification

Pathogenecity test was performed by atomizing the aqueous conidial suspension ($4.5x10^6$ conidia/ml) @ 8-10 ml/plant on to the 3-month-old potted mulberry plants of V1 variety. The control plants of were kept covered with polythene bags for 48 h to maintain sufficient moisture for spore germination and development of disease. After 15 days, the similar symptoms of leaf spot as seen on the original diseased plants were observed, thus fulfilling the Koch's postulates (Rangaswamy and Mahadevan, 1999). With the help of the illustrated manual and published papers (Ellis and Ellis, 1985; Biswas *et al.*, 1996; Hartman *et al.*, 1991; Singh and Bhalla, 2000) the fungus was identified as *Cercospora moricola* Cooke belongs to the order Moniliales of the class, Deuteromycetes.

The isolated fungus *Cercospora moricola* Cooke was maintained as stock culture in the slants of the PDA medium. Stock cultures of *Trichoderma viridae* and *Trichoderma harzianum* were produced from the Biological Pest Control Laboratory, Department of Agriculture, Anantapur (A.P) were also maintained in the PDA slants.

Maintainance of pure culture

The stock cultures of *Cercospora moricola* Cooke, were cultured in slants of potato dextrose agar medium which were maintained at 4ºC for further usage. They were sub cultured in fresh slants, every three months once. Two slants of the respective fungi were maintained. Out of them one is used for the regular culturing for utilizing it in the experimentation and other one is kept as stock culture.

Phytoextracts preparation

Plant products were prepared from different plant species (Table 1). Fresh leaves were collected and thoroughly washed in sterilized water before preparing their extract whereas bulbs and rhizomes were used in case of Garlic and Ginger, respectively. The extracts were prepared as per the method of Awuah (1989) as follows: the leaves of the selected plants were collected and cleaned with distilled water and dried under shade. Individual samples were ground in sterile distilled water (1.00 ml / g) with the help of mortar and pestle. Then this material was taken in a beaker and boiled at 80 °C for ten minutes in a hot water bath. The material was homogenized for five minutes and filtered through muslin cloth. The filtrate was centrifuged at 5000 RPM for fifteen minutes and the clear supernatant was collected. This was taken as 100% basic stock solution and further diluted to desired concentrations

(2.50, 5.00 and 10.00%) with distilled water before use. Then they were mixed with PDA medium and sterilized as per the method of poisoned food technique (McCallan, 1947).

Plant oils/products

A. indica (3%), *C. citratus* oil (0.05%), *M. indica* oil (3%), branded oils of neem, *C. citrates*, neem seed kernel extract (NSKE) (5%) and *M. indica* were used in the experiment. NSKE was prepared by the method of Pun *et al.* (2000). For preparation of neem seed kernel extract, 50 g of neem seed kernels were finely ground and soaked in 750 ml of water overnight. The slurry was filtered and the volume of the filtrate was made up to 1 litre by adding water. The prepared extract was directly used in the respective experiments.

***In vitro* evalution of phytoextracts and plant oils**

Poisoned food technique

The comparative toxicity of plant extracts on the growth of the fungus in *in vitro* was evaluated as per McCallan (1947). The plant extracts dissolved in sterile distilled water were added aseptically to sterilize potato Dextrose Agar medium in required concentrations and poured into Petri dishes. The medium without any plant extract in Petri dishes served as control. The plates were then inoculated with mycelial discs of 5mm diameter from the periphery of four- day -old culture of the test fungus, growing on Potato Dextrose Agar plates and incubated at room temperature (28° C). Five plates were maintained for each treatment. Radial growth of the mycelium in each plate was recorded as the average of two diameters measured at right angles to one another at 24 hours interval, till the control grow to the full plate. The per cent inhibition of growth was calculated according to the equation of Vincent (1947).

Dry weight method

The fungal isolates were cultured on 50ml aliquots of Czapek-Dox liquid medium with pH adjusted to 5.0 mixed with different plant products and fungicides in 250 ml Erlenmeyer flasks. After autoclaving at 10 lb. pressure for 20 minutes, each flask was seeded with a 5 mm mycelial disc of actively growing (4 days old) test fungus as per Garret (1936). The flasks were incubated for 15 days at room temperature (28±2 °C). Mycelial mat was harvested on pre-weighed Whatman's filter paper dried in an electric oven at 60°c for 48 hours and reweighed after cooling in desiccators. The per cent growth inhibition was calculated as per the formula of Bills (1934). Efficacy of fungicides and other plant products under *in vitro* was tried as per the method followed by Manica Tomar and Sunitha Chandel (2006) and Lalesh Kumari *et al.* (2006).

Statistical analyses

The data obtained from the laboratory and field experiments were analyzed by One way ANOVA using MSTAT computer software version 4.oo/E.M. (Freed, 1986). The least significant difference (LSD) test was used to separate group means when ANOVAs were significant.

RESULTS

Efficacy of plant products

All the plant extracts with 10% concentration were more or less inhibitory to mycelial growth. Mycelial growth

Table 2. *In vitro* efficacy of plant products on the inhibition (%) of mycelial growth (colony diameter) of *C.moricola*

Plants	Concentrations (in %)		
	2.5	5	10
Azadirachta indica	15.75 ± 2.22	23.06 ±2.58**	35.25 ±2.03**
Allium sativum L.,	24.00 ±2.16	23.25 ± 1.96**	41.08 ±2.47**
Adathoda vasica	16.75 ± 2.63	27.25 ±2.80**	40.00 ±2.18**
Aloe barbedensis	21.75 ± 1.71	34.25 ±4.03**	45.75 ±2.75**
Eucalyptus globules	12.25 ± 2.22	23.40 ±2.58**	72.59 ±2.17**
Oscimum sanctum	15.25 ± 2.22	38.65 ± 2.92**	49.08 ±2.34**
Parthenium hysterophorus L.	20.75 ± 1.26	26.25 ±2.20**	49.00 ±2.91**
Phyllanthus emblica	27.75 ± 6.24	27.50 ±2.85**	46.75 ±2.57**
Neem seed kernel extract	33.16 ± 3.24	39.45 ±2.87**	48.38 ±5.91**
Control	0.00	0.00	0.00

Values are represents of four replicates; ± values are SD, significant ** P = 0.001 when compared to the values of 2.5%

inhibition ranged from 35.25 to 72.59%. Significantly, the highest inhibition was recorded in plant extracts from *Eucalyptus globules* with 72.59% with 10% concentration. The next best plant extracts with 10% concentration are, *Oscimum sanctum* (49.08), *Parthenium hysterophorus L* (49.00), *Phyllanthus emblica* (46.75), *Aloe barbedensis* (45.75), *Allium sativum* L.(41.08), *Adathoda vasica* (40.00) and *Azadirachta indica* (35.25). In case of lower concentrations *i.e.*, at 2.5% and 5.0%, inhibition trend was shown accordingly with all the plant extracts as shown at 10% (Table 2).

***In vitro* efficacy of plant oils**

All the three Plant oils *viz*., *M. indica* oil (3 %) *C. citratus* oil - 0.05% and neem oil - 3% have inhibited the mycelial growth of the fungus with 75.73%,73.22% and 24.44%, when compared to control (Table 3).

Influence of plant products on the mycelial growth

All the plant extracts had shown more or less inhibitory effect on mycelial growth of the pathogen on dry weight basis. Normal growth of the test fungus was affected in presence of the plant extracts. The effectiveness of the extracts increased with an increase in concentration and maximum inhibition was recorded at 10%. In all most all cases, plant extracts with 10% concentration were most effective. Mycelial growth inhibition ranged from 49.24% to 60.12%. Significantly, the highest inhibition was recorded in plant extracts from *Eucalyptus globules* with 60.12% with 10% concentration. The next best plant extracts with 10% concentration are, *Aloe barbedensis* (57.37%), *O. sanctum* (56.40%), *P. emblica* (54.26%), *A. vasica* (53.32%), *A. sativum* (51.68%), *A. indica* (50.81%) and *P. hysterophorus* (49.24%). In case of the fungal mycelial growth on dry weight basis, neem oil-3% showed highest per cent of inhibition (40.44%), when compared to the performance of the plant oils like, *M. indica* oil (3%) with 40.23% and *C. citratus* (0.05%) with 36.32%. But all the oils showed significant inhibition (Table 4).

Table 3. *In vitro* efficacy of plant products, plant oils and antagonists against the growth of *C.moricola*

Treatments	Mycelial growth in diameter (cm)	Inhibition (%)
Neem oil (3%)	6.8 ± 0.14	24.44 ± 1.97**
C. citratus oil (0.05%)	2.41 ± 0.61	73.22 ± 1.13**
M. indica oil (3 %)	1.82 ± 0.24	75.73 ± 1.57**
T. viride	1.75 ± 0.54	80.55 ± 2.43**
T. harzianum	2.04 ± 0.13	60.91 ± 3.24**
Carbendazim (0.2%)	0.85 ± 0.11	90.55 ± 2.13
Control	9.00	0.00

Values are represents of four replicates; ± values are SD, significant ** P = 0.001

Table 4. Influence of plant products on the mycelial dry weight (50 mg / 50 ml culture broth) of *C. moricola*

Treatments	Dry weight	Inhibition (in %)
Azadirachta indica	106.83 ± 2.51	50.81 ± 1.97
Allium sativum	104.94 ± 2.17	51.68 ± 2.14
Adathoda vasica	101.36 ± 3.52	53.32 ± 2.67
Aloe barbedensis	92.57 ± 2.97	57.37 ± 2.54
Eucalyptus globules	86.60 ± 3.74	60.12 ± 2.17**
Oscimum sanctum	94.68 ± 2.64	56.40 ± 2.23
Parthenium hysterophorus	110.24 ± 2.59	49.24 ± 2.16
Phyllanthus emblica	99.32 ±2.36	54.26 ± 2.71
Neem oil	129.35 ± 2.96	40.44 ± 2.08**
Cymbopogon citratus oil	138.28 ± 3.25	36.32 ± 2.05**
Madhuca indica oil	129.80 ± 3.74	40.23 ± 2.55**
Carbendazim	9.12 ± 1.07	95.79 ± 0.56**
Control	217.18 ± 3.62	—

Values are represents of four replicates; ± values are SD, significant ** P = 0.001

DISCUSSION

The present results are correlating with the reports of the following researchers. Sesha Kiran *et al.* (2006) recorded the highest inhibition with *Prosopis julifera* (74%), followed by *Agave americana* (68%) and *Nerrium indicum* (54%). Antifungal properties of extracts of *Prosopis julifera* and *Cassia* species have also been reported by Ganesan (1993). Muthulakshmi (1990) has also reported on the efficacy of *Prosopis julifera* leaf extract against *Alternaria tenuis*. Suthinraj *et al.* (2003) have reported that some plant products for the control of *Macrophomia phaseolina* ,the incitant of Groundnut disease. Cold extracts of *A. sativum* with 10% concentration recorded maximum inhibition of fungal mycelium followed by *P. longifolia, A. indica, Lawsonia inermis.*

Antifungal property of *L. camara* and *O. sanctum* against *Drechslera sorokiniana* were reported (Varma *et al.*, 2002). Nishita Nailk *et al.* (2007) have reported that the plant extracts, *A. sativum* followed by *L. inermis* were found to be effective against *Fusarium solani*, the causative agent of the mulberry root rot. Manica Tomar and Sunita Chandel (2005) have worked on certain plant extracts against the *Fusarium* wilt, caused by *Fusarium oxysporum* f.sp. *gladioli* (L.Massey) and reported that *A.indica, A. sativum* and *O. sanctum* inhibited mycelial growth (by

60%). Deepa Khulbe *et al.* (2003) have also reported the Methanol extracts of leaves and roots of *Berberis aristata* with high degree of mycelial growth inhibition (50%-90%) against *Rhizoctonia solani*, *Macrophomina phaseolina*, *Alternaria brassicae* and *Fusarium oxysporium*.

These results are similar to the report of Vadivel and Ebenezar (2006) with cent per cent inhibition of mycelial growth of *A. solani* with (0.05 and 0.1%) *C. citratus* (0.05%), which was significantly superior to mancozeb (0.2%). Neem oil and *M. indica* oil (3%) were also partially effective in inhibiting the mycelial growth. Babu (1994) also got similar results. The effectiveness of the *C. citratus* (0.05%) and neem oil might be due to the presence of antifungal compounds like monoterpenes and azadiractin. The present findings are similar to the report of Babu (1994). The effectiveness of the *C. citratus* (0.05%) and neem oil might be due to the presence of antifungal compounds like monoterpenes and azadirachtin.

The present findings are in conformity with the results of Syed Zulfekher Ali (2007) on the mycelial growth on dry weight basis of *Capnodium* spp. *Prosopis julifera* showed highest per cent of inhibition (62.15%). Plant oils, neem oil (3%), *C. citratus* (0.05%), *M. indica* oil (3%) were also reported to be effective. The findings in this study are also similar to the report of Vadivel and Ebenezar (2006) on *Alternaria solani* and reported that the dried root extracts of *Acorus calamus* showed significant reduction in mycelial growth of the pathogen. At 10%, Acorus calamus dried root extract was superior in performance over Mancozeb in inhibiting mycelial growth. Mungkotnasa Wakul *et al.* (2002) also reported the efficacy of *Acorus calamus* dried root extract against the mycelial growth of *Alternaria* spp. *P. julifera* (10%) leaf extract and the bulb extract (5%) of garlic (*A. sativum*) were the next best treatments. Muthulakshmi (1990) reported the efficacy of *P. julifera* leaf extract against *A.tenuis*. Sevaral workers have reported the inhibitory effect of garlic bulb extract on the mycelial growth of *A. tenuis* (Bai,1992), *A. solani* (Babu,1994) and *A. brassicae* (Shivpuri *et al*., 1997).

In controlling the *Macrophomia phaseolina* ,the incitant of Groundnut disease, cold extracts of *Allium sativum* at 10% concentration recorded maximum inhibition of fungal mycelium followed by *Polyalthia longifolia*, *Azadirachta indica*, *Lawsonia inermis* and *Eucalyptus* sp. In case of mycelial dry weight, among the five selected plant products, cold and hot water extracts of *Allium sativum* recorded the maximum mycelial dry weight followed by *Polyalthia longifolia* (Suthinraj *et al.*, 2003).

Manica Tomar and Sharma (2005) have tested twenty different aqueous plant extracts against *Fusarium solani* and out of which, *A. sativum* showed a maximum inhibition (64.79%) of the mycelial growth, followed by *Aloe barbedensis* (62.89%). Out of the ten acetone based plant extracts tested at different concentrations against *Fusarium solani* under *in vitro* conditions, *O. sanctum* gave the overall maximum (81.97%) mycelial inhibition followed by *A. sativum* with a mean mycelial inhibition of 78.42%. Among the plant oils tested against *Colletotrichum capsici*, *Cymbopogon citratus* (0.1%) was found to be very effective and completely inhibited the mycelial growth. Neem oil (3%) and neem oil 60 EC (3%) recorded 42.84 % and 42.11% growth inhibition over control respectively. Based on the *in vitro* experimental results, it is to infer that some of the tested plant products and plant oils were found to be so effective in controlling the mycelial growth of *Cercospora moricola* Cooke, the incitant of leaf spot of Mulberry and a new line of research may be drawn with an in-depth study of testing the tried phytoextracts for their potentiality under *in vivo* condition.

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C. Raja Gopal Reddy* R.S.Nirmala and CH. Ramanamma**

Department of Biotechnology, School of Herbal Studies & Naturo Sciences, Dravidian University, Kuppam-517425, Andhra Pradesh,India.

**N. S. P. R. Govt. Degree College for Women, Hindupur – 515 201, Andhra Pradesh, India.

*Communication author E-mail: ctrgreddy@yahoo.co.in