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# EMPIRICAL STUDY ON LEAFSPOT DISEASE IN MULBERRY PLANT USING BIO CHEMICAL METHODS

**P Nageswararao*** Department Of Botany, Andhra University, Visakapatnam, Andhra Pradesh. *Corresponding Author

**B Sujatha** Department Of Botany, Andhra University, Visakapatnam, Andhra Pradesh.

**ABSTRACT** Sericulture in India is as old as the ancient Indian culture. In the global scenario, India has emerged as one of the important sericulture practicing countries. The healthy and robust growth of the silkworms depends upon the quality mulberry leaf. Diseases are the major limiting factor in Mulberry cultivation, in which *Cercosporamoricola* Cooke, the incitant of leaf spot is one among them causing a major damage to the leaf production besides affects the silkworm's health. Four plant extracts and three plant oils tested against the pathogen, *Eucalyptus globules* at 10% (72.59%) and *Madhucaindica* oil (3%) evidenced a highest inhibition. Among the different fungal biocontrol agents tested against the pathogen, *Trichodermaviride* (80.55%) significantly recorded mycelial growth inhibition over control.

**KEYWORDS :** Sericulture,Cercospora, TrichodermaandGrowth.

## INTRODUCTION

Sericulture is an Agri-based enterprise. This culture is predominantly practiced by small and marginal farmers in the states of Karnataka, Andhra Pradesh, Tamil Nadu, West Bengal, Jammu and Kashmir and other states. 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 (Raja gopal Reddy *et al*, 1999). Diseases are one among the major limitations for this practice. Even though many fungi are reported causing leaf spot, *Cercosporamoricola*Cooke is widely occurring, found responsible for the disease and causes serious damage to mulberry leaf yield and quality and it also causes 20-25 destruction of leaf lamina (Sikdar and Krishnaswamy, 1980; Sukumar and Ramalingam, 1989). The infection on mulberry leaves caused by the pathogen diagnosed by 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).Production of organic mulberry leaf is slowly gaining importance and therefore, management of *Cercospora* leaf spot through eco-friendly methods is so vital. Use of biodegradable 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 *et al*, 1984).Keeping in view of the points explained above, present study has been taken up to manage the disease by plant products, plant oils and antagonistic fungi.

## MATERIALAND METHODS

### Isolation of *Cercosporamoricola*

Culture of *Cercosporamoricola* Cooke isolated from the infected leaves of mulberry plants were maintained on Potato Dextrose Agar (PDA) medium throughout the study.

### Poisoned food technique

The comparative toxicity of plant extracts, plant oils on the growth of the fungus *in vitro* evaluated as per McCallan (1947). The plant extracts dissolved in sterile distilled water were added aseptically to sterilize PDA in required concentrations and poured into Petri plates. 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 4 day old culture of the test fungus and incubated at room temperature (27±1 °C). 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.

### Dual culture technique

*In vitro* evaluation of bio-control agents was determined by dual culture technique (Sharvelle, 1961).

## RESULTS

**Table:1. In vitro effect of plant extracts on conidial germination of *Cercosporamoricola* Cooke**

| Plant Species | Conidial germination (%) | Inhibition in conidial germination (%) |
|---|---|---|
| Control (Distilled water) | 92.00 | 0.00 |
| *Azadirachtaindica* | 28.36 ± 1.94 | 70.08 ± 1.47 |
| *Allium cepa*L. | 74.26 ±1.26 | 26.57 ± 2.34 |
| *Allium sativum*L., | 24.00 ± 2.94 | 75.75 ± 3.4** |
| *Adathodavasica* | 13.78 ± 2.65 | 84.28 ± 2.04 |
| *Aloe barbedensis* | 24 ± 1.82 | 76.5 ± 2.64** |
| *Cassia tora* L. | 43.52±1.05 | 58.47 ± 1.78** |
| *Catharanthusroseus* | 65.49 ± 1.61 | 35.71 ± 1.93 |
| *Eucalyptus globules* | 14.17 ± 2.79 | 86.37 ± 1.83 |
| *Lantana camara* | 46 ± 2.16 | 55.25 ± 3.5** |
| *Leucasaspera* | 62.43 ± 1.69 | 17.52 ± 2.81 |
| *Menthaviridis* | 47.51 ± 2.71 | 51.68 ± 1.57 |
| *MurrayaKoenigii* | 45.5 ± 3.41 | 55.5 ± 3.69** |
| *Oscimum sanctum* | 18.23 ± 1.37 | 73.15 ± 1.48 |
| *Partheniumhysterophorus L.* | 25.96 ± 1.82 | 74.38 ± 2.68 |
| *Phyllanthusemblica* | 16.41 ± 2.83 | 73.64 ± 2.28 |
| *Ricinuscommunis. L.* | 74.92 ± 2.83 | 16.41 ± 1.51 |
| *Tageteserecta* | 54.75 ± 3.59 | 52 ± 4.69** |
| *Vitexnegundo* | 64 ± 3.16 | 43 ± 3.55** |
| *Zingiberofficinalis* | 85.52 ± 1.24 | 17.65 ± 1.62 |

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

**Table:2. In vivo effect of plant extracts on conidial germination of *Cercosporamoricola* Cooke**

| Plant Species | Inhibition in conidial germination (%) |
|---|---|
| Control (Distilled water) | 0.00 |
| *Azadirachtaindica* | 68.08 ± 1.27 |
| *Allium cepa*L. | 22.37 ± 2.74 |
| *Allium sativum L.,* | *71.71 ± 3.1*** |
| *Adathodavasica* | 81.23 ± 2.04 |
| Aloe barbedensis | 77.54 ± 2.94** |
| *Cassia tora* L. | 55.45 ± 1.48** |
| *Catharanthusroseus* | 36.71 ± 1.73 |
| *Eucalyptus globules* | 85.32 ± 1.63 |
| *Lantana camara* | 57.35 ± 2.5** |
| *Leucasaspera* | 19.72 ± 2.25 |
| *Menthaviridis* | 54.36 ± 2.71 |
| *MurrayaKoenigii* | 56.56 ± 2.41** |
| *Oscimum sanctum* | 74.76 ± 2.38 |
| *Partheniumhysterophorus L.* | 72.37 ± 2.86 |
| *Phyllanthusemblica* | 71.84 ± 2.17 |
| *Ricinuscommunis. L.* | 19.44 ± 1.27 |
| *Tageteserecta* | 56 ± 4.68** |
| *Vitexnegundo* | 46 ± 3.38** |
| *Zingiberofficinalis* | 21.72 ± 2.62 |

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

## DISCUSSION

### *In vitro* efficacy of plant products against conidial germination of *Capnodium* spp.

With regard to the inhibition in the fungal conidial germination *in vitro*, among the plant products tested, *Eucalyptus globules* showed the highest percent of inhibition in *Eucalyptus globules* (86.37%) followed by *Adathodavasica* with 84.28%, *Aloe barbedensis* (76.5), *Allium sativum* L.(75.75), *Phyllanthusemblica* (73.64), *Partheniumhysterophorus L* ( 74.38), *Oscimum sanctum* ( 73.15), .*Azadirachtaindica* (70.08), The other plant products like *Cassia tora*, *Lantana camara, Mentaviridis, MurryaKoenigii, Tagetuserecta* and *Vitexnegundo*also showed significant inhibition in spore germination when compared to the control (Table-1).

In case of the fungal conidial germination *in vivo*, among the plant products tested, *Eucalyptus globules* showed the highest percent of inhibition (86.32%) followed by *Adathodavasica* (81.23), *Aloe barbedensis* (77.54), *Oscimum sanctum* (74.76), *Partheniumhysterophorus L* (72.37), *Phyllanthusemblica* (71.84), *Allium sativum* L.(71.71) and *Azadirachtaindica* (68.08). The other plant products like *Cassia tora*, *Lantana camara, Menthaviridis, MurryaKoenigii, Tagetuserecta*and *Vitexnegundo*also showed significant inhibition in spore germination when compared to the control (Table-2).

The present findings are similar to the findings of the following. Santosh Kumar *et al* (2003) who have worked on the efficacy of the plant product ,*Lawsonia*with regard to the conidial germination and sporulation of *Colletotrichumfalcatum* Went, the incitant of root rot disease of sugarcane. *Lawsonia* at 40 per cent concentration has completely inhibited the spore germination and sporulation of the fungus and worked equally well with the fungicide, Carbendazim (0.1%) which was maintained as control.

The present findings are in conformity with Padma and Sukumar (2004) have workerd on the leaf extracts of *Brassica juncea, Allium cepa, Allium sativum, Tagetuspatula, Seasumumindicum, Leucasaspera, Oscimumadscendens, Azadirachtaindica, Catharanthus roseus,* and *Partheniumhysterophorus* and found that *Allium sativum* inhibited the conidial germination of Cercosporamoricola. They have also reported that *Calotropisgigantea* also inhibited the germination of pycnospores of *Lasiodiplodiatheobromae* pat.

Syed Zulfekher Ali (2007) has worked on the effect different plant extracts on the spore germination of the *Capnodium* Spp. Among the plant products tested ,*Prosopisjulifera* showed the highest percent of inhibition (75.75%) of fungal spore germination followed by Neem seed kernel extract with 72.5%. The other plant products like *Cassia occidentalis, Eucalyptus globulus, Lantana camera, Lawsoniainermis Prosopisjulifera, Tagetuspatula and Annona squamosa* also showed significant inhibition in spore germination when compared to the control.

Santosh Kumar *et al* (2003) have tested few natural products against the conidial germination and sporulation of *Colletototrichumfalcatum* Went. Among them garlic and *Lawsonia*with 40% concentration completely inhibited the spore germination and sporulation of the test fungus and were on par with the control fungicide.

The present findings are correlating with the report of Choudhury *et al* (2006) with the results of their screening of fifty botanicals. Among them Garlic bulb extract, *Hibiscus rosa-sinensis* L and *Leucas indica* (L) leaf extracts controlled the spore germination of *Alternariaporri*, the causal organism of the leaf blight of Niger, *Guizotiaabyssinica* (L.f).

### Efficacy of plant products on the inhibition (%) of mycelial growth of *Cercosporamoricola* Cooke

All the plant extracts were more or less inhibitory to mycelial growth and conidial germination of the pathogen. Normal growth and spore production 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 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),*Partheniumhysterophorus L* (49.00), *Phyllanthusemblica* (46.75),*Aloe barbedensis* (45.75), *Allium sativum* L.(41.08), *Adathodavasica* (40.00) and *Azadirachtaindica* (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%. Neem seed kernel extract has shown 33.16, 39.45 and 48.38% with 2.5. 5.0 and 10.0% concentrations respectively.

The present results are similar to the findings of Syed Zulfekher Ali (2007) on the mycelial inhibition of *Capnodium* spp. which ranged from 31 to 72%. Significantly, the highest inhibition was recorded in plant extracts from *Prosopisjulifera* with 74.50% with 10% concentration. The next best plant extracts were from Lantana camera (46.00%) to *Annona squamosa* (33.75%). Neem seed kernel extract has shown 27.75%, 37.75% and 49.25% with 2.5%, 5% and 10% respectively

The present results are correlating with the reports of the following researchers.

*Sesha*Kiran*et al* (2006) recorded the highest inhibition with*Prosopisjulifera* (74%), followed by *Agave americana* ( 68%) and *Neeriumindicum* (54%). Antifungal properties of extracts of *Prosopisjulifera*, *Cassia* species have also been reported by Ganesan (1993).

Muthulakshmi (1990) has also reported on the efficacy of *Prosopisjulifera* leaf extract against *Alternaria tenuis*. ManicaTomar and Sunita Chandel (2006) have worked on certain plant extracts against the *Fusarium* wilt, caused by *Fusariumoxysporum*f.sp. *gladioli* (L. Massey) Snyder and Hansen and reported that *Azadirachtaindica, Allium sativum L* and*Oscimum sanctum* inhibited mycelial growth (by 60%). Under field conditions, *Oscimum sanctum* and *Allium sativum* provided disease control (61-67%).

LaleshKumari*et al* (2006) have also reported garlic clove extract proved as most effective in checking the growth and conidial germination of *Alternariaalternata*, causative agent of *Alternaria* blight of Periwinkle (*Catharanthus roseus*). Garlic clove extract was reported to be effective against growth and/or conidial germination of *Alternaria* spp. pathogenic to various crops like pomegranate (Singh and Majumdar, 2001), potato (Choudhary *et al*, 2003), sunflower (Amaresh and Nargund, 2003) and tomato (Prasad and Naik, 2003).

Suthinraj*et al* (2003) have reported that some plant products for the control of Macrophomiaphaseolina ,the incitant of Groundnut disease. Cold extracts of Allium sativum with 10% concentration recorded maximum inhibition of fungal mycelium followed by *Polyalthialongifolia, Azadirachtaindica, Lawsoniainermis.* In case of hot water extracts, Allium sativum recorded the maximum inhibition of fungus followed by *Polyalthialongifolia.*

## CONCLUSION:

Foliar diseases create serious problems in qualitative and quantitative leaf production especially during rainy and winter seasons. Leaf spot disease caused by *Cercosporamoricola* Cooke is one of the most serious infestations, causing quantitative and qualitative leaf loss.

Occurrence of different fungi on leaf surface of mulberry was totally depending on the Biotic and Abiotic factors. In the integrated management of the *Cercospora*leaf spot fungus, growth and development of the pathogenic fungus is to be checked with the recommended plant products, plant oils and antagonistic fungi. Then only it will be possible to control leaf spot disease by way of developing certain effective protocols with the integration of the recommended plant products, oils and bio control agents under integrated pest management for the effective control of the leaf spot disease of Mulberry.

Basing on the experimental results, it is to infer that some of the tested plant products (6), plant oils (3) and antagonistic fungi (2) were found to be so effective in controlling the leaf spot disease caused by Cercosporamoricola Cooke to the level of certain percentage (%).

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