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# Development of antifungal formulations and their evaluation against root rot disease of mulberry

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**ABSTRACT**

Mulberry (*Morus* sp.) cultivated throughout India for rearing of silkworm (*Bombyx mori* L.). Root rot caused by a group of fungi is a severe threat for mulberry (*Morus* spp.) leaf production, especially in southern states of India due to large scale mortality and enormous crop loss. Fungi such as *Fusarium solani*, *Fusarium oxysporum*, *Rhizoctonia bataticola* and *Botryodiplodia theobromae* are frequently isolated from the infected roots. Few control measures recommended could not sustain due to inconsistent results. In this perspective, studies were conducted to develop a broad spectrum formulation to contain the disease. Several plant products, synthetic fungicides and chemicals were screened for antifungal activities *in vitro* in solid and broth media using poisoned food technique. Five formulations were made using selected antifungal components and tested against root rot disease under artificial simulation. All the formulations significantly ($P<0.01$) reduced wilting and rotting compared with untreated control as well as plants treated existing control measure. Highest control of wilting (88.20%) and rotting (88.05%) was showed by formulation F-1, followed by F-2 and F-4 compared with untreated control. The highly effective formulation (F-1) was further tested in the hotspot areas of Karnataka showed revival of plants with range of 67-86 per cent. This eco-friendly formulation could be used for control of root rot disease of mulberry.

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## INTRODUCTION

Root rot is a serious disease of mulberry, occurs throughout the year in all kind of soils. The disease appears in isolated patches in the garden, spread gradually leads to complete destruction of the plants causing huge crop loss. The disease incidence is 10 to 16% in hotspot areas of Karnataka (Philip and Sharma, 1997) with 15% loss (Mallikarjuna *et al.*, 2010; Quadri *et al*., 2005). Maximum root rot incidence of 55 per cent and 24.33 per cent was reported in $V_1$ and $MR_2$,

respectively (Rajeswari and Angappan, 2018). Fungi such as *Fusarium solani* (Philip *et al.,* 1995*;* Sharma *et al.,* 2003*;* Manmohan and Govindaiah, 2012), *Fusarium oxysporum* (Beevi and Qadri, 2010 and Mallikarjuna *et al*., 2010), *Macrophomina phaseolina* (Chowdhary *et al*., 2011 and Muthuswami *et al.,* 2011) are reported to be associated with the disease. Though several chemicals (Sharma *et al*., 2003 and Choudhari *et al*., 2012), plant based products (Philip and Sharma, 1997) and biological control agents (Sridhar *et al*., 2000; Narayanan *et al*., 2015 and Pratheesh Kumar *et al*., 2017) are identified for management of the disease, only chemicals are used by the farmers. Growing concerns about health and environmental safety, the use of toxic, environmentally damaging chemicals are being discouraged. Along with this, development of resistance due to repeated use of chemicals, their cost and sensitive nature of silkworms are other impediments. This situation warrants alternative eco-friendly methods for control of root rot disease of mulberry. In view of these, experiments have been conducted to screen eco-friendly fungicides, chemicals and plant based materials, formulations were made combining effective components and tested under artificial simulation and effective formulation was further tested in the hotspot areas.

## MATERIAL AND METHODS

### *In vitro* evaluation of chemicals, fungicides and plant materials:

The pathogens *F. solani*, *F. oxysporum*, *M. phaseolia* and *B. theobromae* associated with root rot disease were isolated from roots of disease affected mulberry collected from the farmers' field. Various fungicides, chemicals and plant materials were tested through poisoned food technique (Aneja, 2017) both in Potato Dextrose Agar (PDA) and Potato Dextrose Broth (PDB) media (Himedia). The fungicides and chemicals were obtained from the market. The plant materials were obtained by ethanol extraction of the plant part mentioned in the Table 1. About 100 g plant materials were weighed and 100 ml ethanol solvent with a concentration of 96 per cent was added and extracted using mortar and pistil. The ethanol extract was evaporated using a rotary evaporator until about 25ml of extract was left in the container. This was further allowed to air dry in a vacuum desiccators until all the ethanol had evaporated. The solidified extracts were diluted in sterile distilled water to get desired concentration (500 ppm).

The chemicals, fungicides and plant material were tested individually against each pathogen in 500 ppm. The PDA was amended separately with 500 ppm of fungicides, chemicals and plant materials and poured in Petri-plates. After solidification, these Petri-plates were inoculated with 7 days old culture of the test fungi. Separate inoculations were done for each material against each test fungi. A control group was maintained for each fungi inoculated in the PDA without amending with the test materials. A whole set was then incubated at 28±2°C in a BOD incubator. The radial growth of mycelia was measured 7 days after inoculation.

Similarly, experiment was conducted by growing fungi in liquid PDB (Himedia) in polyurethane bottle. Each bottle was added with 100ml PDB media amended with 500 ppm test materials separately. Seven days old culture of test fungi were inoculated in bottle containing PDB media amended with test materials. The PDB inoculated only with test fungi were served as control. The whole set was incubated at 28±2°C for 15 days in a BOD incubator. After incubation the culture was observed for sporulation visually. The mycelia were filtered using Whatman filter paper. The filter paper containing mycelia was allowed to dry in an oven for 12 hrs at 60°C and determined the mycelia weight.

### Evaluation of various formulations against root rot disease under artificial simulation:

Five formulations (F-1 to F-5) were made with effective shortlisted materials. Each formulation included effective chemical, fungicide and plant material in different proportions. These combinations were tested on plants grown in earthen pots with artificial simulation of root rot disease. The root rot was simulated by challenge inoculating the plant with the culture all the fungi. Two weeks after inoculation when the plants showed the root rot symptoms; the plants were cut one foot above the collar region. Soil was removed up to 15-20 cm depth around the collar region 10 g of formulation was thoroughly mixed with 2 liter of water and poured on the cut stump completely drenching. Immediately the soil was put in place and pressed firmly. Two control groups one with the application of existing recommendation and other without application of any treatment was kept for comparison. Five replications were kept against each treatments and controls. The

data on wilting and rotting was collected one month after application of the treatment using the following formula:

$$\text{Wilting (\%)} = \frac{\text{Number of wilted leaves}}{\text{Total number of leaves}} \times 100$$

$$\text{Wilting (\%)} = \frac{\text{Weight of whole root} - \text{Weight of helthy root}}{\text{Weight of whole root}} \times 100$$

**Evaluation of Effective formulation in the hotspot area:**

The formulation-1 found effective was further evaluated in the selected farmers' field of Karnataka. The formulation was applied in the similar way as mentioned after cutting the infected plants one foot above the ground. After 30 days, the number of plants revived was counted and percentage of revival was calculated.

## RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

***In vitro* evaluation of plant extracts, fungicides and chemicals against root rot pathogens:**

All the treatments with plant extract significantly (P<0.05) reduced growth of all the test fungi. The fungus *M. phasiolina* failed to grow in the solid medium amended with *A. sativum, F. asafoitida* and a low growth was observed in presence of extract of *A. indica*. Growth of the fungus was also low in the liquid medium amended with these extracts. However maximum growth with high mycelia biomass was found in *P. pinnata* (0.471g). Similarly in case of *F. oxysporum* and *F. solani,* these fungi could not grow in the solid medium amended with extracts of *A.sativum* and *A. indica*. In presence of extracts of *P. pinnata, T. indica* and *B. nigra F. oxysporum* and *F. solani* showed low growth. Though these fungi grown in the liquid medium amended with these plant extracts, compared with that of other treatments the growth was very less. In case of *B. theobromae*, the fungus could not grow in the solid medium amended with *A. sativum* and the growth was very less (1.31 mm) in presence of extract of *A. indica* and *T. indica* (1.92 mm). The fungus showed least growth in the liquid medium amended with these plant extracts (Table 1).

All fungicides significantly (P<0.05) reduced radial growth of all tested fungi. Mancozeb and Benomyl completely suppressed growth of *M. phasiolina* in solid medium. The growth was also found less in media amended with Tricyclozole (2.00 mm) Captan (3.08 mm) Chlorothalonil (9.06 mm) and Copper oxychloride (9.25 mm). Growth was highest (40.83 mm in control). In liquid medium, the fungus showed comparatively less growth in presence of these fungicides. *F. oxysporum* and *F. solani* could not grow in the solid medium amended with Carbendazim as well as Benomyl and showed least growth in liquid medium amended with these fungicides. However in control the *F. oxysporum* (31.19 mm) and *F. solani* (34.37 mm) showed maximum growth in solid

**Table 1: Effect of various plant extracts on growth of root rot pathogens**

| Name of the plant | Radial growth of the fungus (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *M. phasiolina* | | *F.oxysporum* | | *F.solani* | | *B.theobromae* | |
| | Growth (mm) | Biomass (g) | Growth (mm) | Biomass (g) | Growth (mm) | Biomass (g) | Growth (mm) | Biomass (g) |
| *Allium sativum* (B) | 0.00 | 0.080 | 0.00 | 0.128 | 0.00 | 0.034 | 0.00 | 0.019 |
| *Guizotia abyssinica* (S) | 23.00 | 0.967 | 33.08 | 0.16 | 29.44 | 1.274 | 23.69 | 0.051 |
| *Ferula asafoetida* (R) | 0.00 | 0.261 | 2.22 | 0.379 | 2.33 | 0.569 | 34.75 | 0.25 |
| *Azadirachta indica* (S) | 0.47 | 0.153 | 0.00 | 0.076 | 0.00 | 0.060 | 1.31 | 0.020 |
| *Capsicum annuum* (F) | 41.28 | 0.260 | 43.78 | 0.485 | 44.28 | 0.137 | 13.67 | 0.932 |
| *Tamarindus indica* (F) | 37.11 | 0.263 | 18.67 | 0.126 | 13.78 | 0.222 | 1.92 | 0.229 |
| *Pongamia pinnata* (S) | 31.44 | 0.471 | 12.11 | 0.41 | 19.58 | 0.083 | 21.36 | 0.091 |
| *Calotropis procera* (L.) | 38.54 | 0.273 | 43.04 | 0.084 | 44.44 | 0.222 | 22.36 | 0.202 |
| *Lawsonia inermis* (L.) | 40.97 | 0.319 | 43.14 | 1.094 | 43.53 | 0.26 | 27.33 | 0.566 |
| *Brassica nigra* (S) | 25.83 | 0.269 | 14.31 | 0.340 | 26.11 | 0.784 | 10.86 | 0.707 |
| Control | 43.97 | 0.235 | 32.83 | 0.35 | 27.28 | 0.99 | 32.28 | 0.425 |
| C.D. (P<0.05) | 1.27 | | 0.94 | | 1.01 | | 1.34 | |

Plant parts used: B-Bulb, F-Fruit, L-Leaf, R- Resin, S-Seed

medium. *B. theobromae* could not grow in the solid medium amended with Mancozeb, Tricyclozole, Captan and Benomyl. Similarly, in the liquid medium amended with these fungicides, growth of the fungus was least (Table 2).

All chemicals significantly (P<0.05) influenced growth of the pathogens. TCCA was found highly effective by effectively suppressing all fungi in solid and liquid medium. The Phosphorus acid though completely suppress growth of *M. phaseolina*, *F. oxysporum* and *F. solani* in solid medium, it could not suppressed the growth of *B. theobromae* (Table 3).

**Evaluations of various formulations against root rot disease under artificial simulation and hot spot area:**

All the formulations and existing recommendation significantly (P<0.01) reduced the wilting and root rot of mulberry. The wilting was least (10.79%) in mulberry treated with formulation-1 followed by formulation-2 (23.99%) and formulation-4 (24.42%). However the wilting was highest in control (90.33%). These formulations controlled the wilting 88.05 per cent, 73.44 per cent and 72.96 per cent, respectively compared with untreated control. Likewise, the rotting was found least (8.73%) in formulation-1 treated plants followed by

**Table 2: Effect of various fungicides on growth root rot pathogens**

| Name of fungicides | Radial growth of the fungus (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *M. phasiolina* | | *F.oxysporum* | | *F.solani* | | *B.theobromae* | |
| | Growth (mm) | Biomass (g) | Growth (mm) | Biomass (g) | Growth (mm) | Biomass (g) | Growth (mm) | Biomass (g) |
| Mancozeb | 0.00 | 0.024 | 6.24 | 0.520 | 1.75 | 0.700 | 0.00 | 0.091 |
| Tricyclozole | 2.00 | 0.430 | 4.24 | 0.120 | 5.53 | 0.320 | 0.00 | 0.084 |
| Chlorothalonil | 9.06 | 0.170 | 12.48 | 0.710 | 13.03 | 0.520 | 8.56 | 0.171 |
| Metalaxyl | 26.08 | 0.520 | 22.19 | 0.340 | 9.15 | 0.430 | 9.22 | 0.115 |
| Thiophanate methyl | 36.03 | 0.140 | 14.30 | 0.350 | 7.08 | 0.500 | 3.25 | 0.154 |
| Carbendazim | 37.25 | 0.146 | 0.00 | 0.101 | 0.00 | 0.161 | 7.36 | 0.116 |
| Copper oxychloride | 9.25 | 0.145 | 10.40 | 0.265 | 17.97 | 0.179 | 11.25 | 0.153 |
| Fosetyl aluminum | 38.33 | 0.168 | 14.89 | 0.133 | 30.89 | 0.056 | 13.00 | 0.105 |
| Captan | 3.08 | 0.144 | 0.67 | 0.020 | 3.28 | 0.152 | 0.00 | 0.073 |
| Benomyl | 0.00 | 0.038 | 0.00 | 0.084 | 0.00 | 0.079 | 0.00 | 0.111 |
| Control | 40.83 | 0.350 | 31.19 | 0.950 | 34.37 | 0.990 | 34.44 | 0.269 |
| C.D. (P<0.05) | 2.15 | | 1.17 | | 1.04 | | 0.45 | |

**Table 3 : Effect of various chemicals on growth root rot pathogens**

| Name of the chemicals | Radial growth of the fungus (mm) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *M. phasiolina* | | *F.oxysporum* | | *F.solani* | | *B.theobromae* | |
| | Growth (mm) | Biomass (g) | Growth (mm) | Biomass (g) | Growth (mm) | Biomass (g) | Growth (mm) | Biomass (g) |
| Sulphur | 38.54 | 0.138 | 26.14 | 0.366 | 16.92 | 0.505 | 31.11 | 0.521 |
| Boric acid | 39.31 | 0.272 | 29.25 | 0.272 | 17.42 | 0.304 | 25.97 | 0.252 |
| Calcium hydroxide | 38.89 | 0.327 | 33.08 | 0.246 | 27.14 | 0.507 | 31.28 | 0.555 |
| Sodium bicarbonate | 33.83 | 0.327 | 32.83 | 0.370 | 29.94 | 0.306 | 32.11 | 0.390 |
| TCCA | 0.00 | 0.120 | 0.00 | 0.110 | 0.00 | 0.121 | 0.00 | 0.068 |
| Paraformaldehyde | 32.50 | 0.493 | 19.56 | 0.431 | 24.03 | 0.440 | 12.81 | 0.444 |
| Salicylic acid | 25.86 | 0.013 | 14.44 | 0.026 | 16.06 | 0.127 | 0.00 | 0.029 |
| Potassium bicarbonate | 34.25 | 0.153 | 34.53 | 0.152 | 27.75 | 0.159 | 29.00 | 0.152 |
| Ammonium bicarbonate | 15.00 | 0.960 | 23.03 | 0.027 | 32.03 | 0.316 | 13.69 | 0.969 |
| Sodium chloride | 38.64 | 0.023 | 30.86 | 0.070 | 30.42 | 0.043 | 0.00 | 0.069 |
| Phosphorus acid | 0.00 | 0.000 | 0.00 | 0.000 | 0.00 | 0.000 | 31.11 | 0.035 |
| Control | 41.94 | 0.235 | 35.33 | 0.350 | 32.28 | 0.990 | 34.61 | 0.269 |
| CD (P<0.05) | 1.82 | | 1.04 | | 8.79 | | 0.96 | |

**Table 4 : Effect of different formulations on wilting and rotting of mulberry**

| Formulations | Wilting (%) | | | SD | Rotting (%) | | | SD |
|---|---|---|---|---|---|---|---|---|
| F1 | 10.79 | (18.42)** | ± | 2.31 | 8.73 | ( 15.47) | ± | 2.05 |
| F2 | 23.99 | ( 26.79) | ± | 2.17 | 15.15 | ( 21.00) | ± | 2.02 |
| F3 | 25.81 | ( 27.82) | ± | 6.85 | 12.99 | ( 20.04) | ± | 3.34 |
| F4 | 24.42 | ( 27.03) | ± | 5.69 | 16.73 | ( 22.05) | ± | 1.58 |
| F5 | 29.45 | ( 29.24) | ± | 6.31 | 17.27 | ( 22.66) | ± | 6.27 |
| EC* | 36.29 | ( 32.31) | ± | 2.27 | 25.57 | ( 27.20) | ± | 3.19 |
| Control | 90.33 | (77.00) | | 5.23 | 74.04 | (59.38) | | 6.49 |
| C.D. (P<0.05) | 7.06 | | | | 10.55 | | | |

*EC- Existing control measure, ** Figures in parenthesis are arcsin transformed values

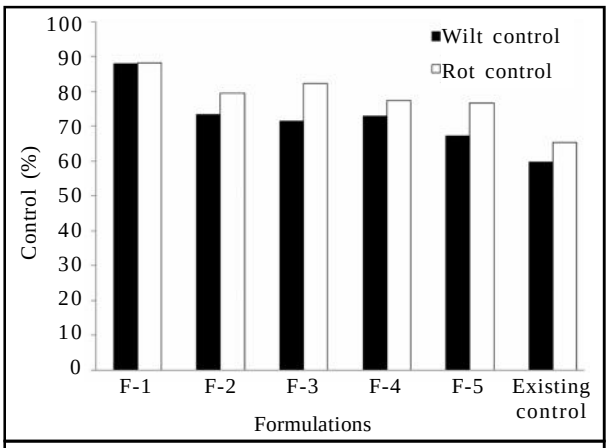


**Fig. 1 : Effect of various formulations on root rot control in mulberry**

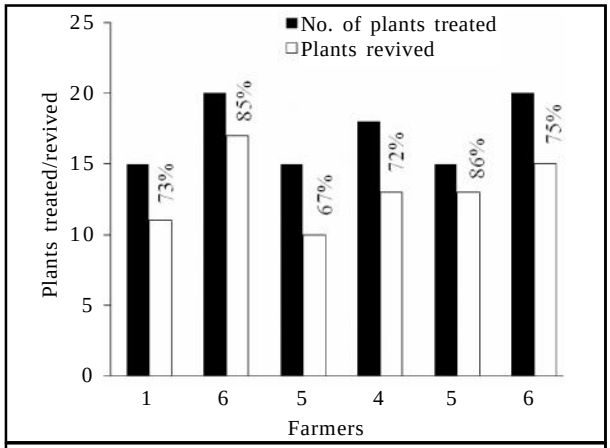


**Fig. 2 : Effect of formulation in revival of root rot infected mulberry**

formulation-3 treated plants (12.99%) and highest (74.04%) in untreated control. Compared to the control these treatments reduced the rotting 88.20 per cent and 82.45 per cent, respectively (Fig. 1). Among the five farmers field the formulation tested, the revival of the plants varied with 67 per cent to 87 per cent (Fig. 2).

Root rot is a widely distributed severe disease of mulberry leads to complete destruction of the plants resulting enormous loss. Though some control measures are recommended, the disease could not contain effectively with these. Also farmers are reluctant to apply chemicals for control of the disease due to environmental considerations. The formulation identified showed effective control of the disease both in the experimental field and in the hotspot area. The identified effective formulation contains 50 per cent plant components and 10.5 per cent sub lethal dose of chemicals (8% organic chemicals 2.5% inorganic chemicals) and rest 39.5 per cent organic and naturally found carrier material. In general, the formulation contains 89.5 per cent naturally found organic material and 8 per cent organic chemicals and only 2.5 per cent inorganic chemical is present. The chemical and plant component helps to kill the fungal pathogens associated with root rot disease and also offer resistance to the plant to sustain the damage caused by the pathogen. The identified formulation is environment friendly and could be used for control of root rot disease of mulberry.

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