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# Effect of certain fungicides on the incidence and severity of *Colletotrichum* leaf disease of immature rubber

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**Abstract**

Colletotrichum leaf disease (CLD) of rubber caused by *Colletotrichum acutatum* and *C. gloeosporioides* affect the young foliage of nursery and immature rubber plants (*Hevea brasiliensis*). Field studies were undertaken to assess the distribution of the two *Colletotrichum* species in the immature rubber plants. Propiconazole and SAAF (a combination product of mancozeb and carbendazim), which were selected based on the *in vitro* screening, were evaluated in the field during 2001, 2002, and 2003 seasons. On immature plants of the clone RRII 105, the incidence of *C. acutatum* and C. *gloeosporioides* was uniformly distributed. Propiconazole and SAAF completely inhibited the mycelial growth at 100ppm and spore germination at 250ppm. No variations in the sensitivity of fungicides were observed between the two *Colletotrichum spp.* Field studies showed that the fungicides SAAF (0.075%) and propiconazole (0.012%) were equally effective in checking the incidence and severity of CLD and was comparable to hexaconazole (0.02%), mancozeb(0.2%) and carbendazim(0.05%). The cost comparison studies clearly indicated that the fungicides SAAF (0.075%) and propiconazole (0.012%) are economic and can be used for the management of CLD in immature rubber.

*Key words* : *Hevea brasiliensis*, *Colletotrichum*, Propiconazole, SAAF, Leaf disease control

## Introduction

Colletotrichum leaf disease (CLD) causes considerable damage to the foliage of nursery and immature plants of rubber (*Hevea brasiliensis*). Two species of *Colletotrichum* (*C. acutatum* and *C. gloeosporioides* ) are known to cause the disease (Kumar *et al., 2002).* The severity of the disease depends mainly on the susceptibility of the clones to the pathogen and the prevailing weather conditions. Almost all the popular clones are susceptible to the disease with varying intensity of disease incidence. The clone RRII 105 which occupies 80 per cent of the rubber planted area in South India is the most susceptible clone (Manju *et al.,* 1999). *Colletotrichum* infects the new flushes of young rubber during the first four years of planting, causing severe deformation and defoliation of leaves. This results in growth retardation and prolongation of immaturity period of rubber plants. Reduction in crop production ranging from 7-45 per cent has been reported from Indonesia and 12 per cent from Sri Lanka (IRRDB, 1994) due to the incidence of this disease. Fortnightly application of mancozeb(0.2%), carbendazim(0.05%) or Bordeaux mixture(1%) is recommended for CLD control (Joseph *et al.*, 1994 ; Edathil *et al.*, 2000). Application of mancozeb (0.2%) and carbendazim (0.05%) either as combination or as alternate spraying was very effective in reducing the disease intensity (Joseph *et al.*, 2005). The present investigation was undertaken up to understand the distribution of two *Colletotrichum* species and to evaluate a triazole fungicide, propiconazole and a combination product of carbendazim and mancozeb (SAAF) to be integrated in the management of Colletotrichum leaf disease in immature rubber.

## Material and Methods

### *Incidence of different symptoms caused by Colletotrichum acutatum and C. gloeosporioides*

Observations on the occurrence of different symptoms on immature plants were studied at Kaliyar estate Thodupuzha. The symptoms viz. raised spot, papery

lesion and anthracnose were recorded from the first year plants of RRII 105. The study was carried out in an area of one hectare(400 plants). Upper two whorls were assessed for the incidence of different symptoms and the percent incidence was worked out.

***In vitro evaluation of fungicides***

The test fungi *Colletotrichum acutatum* and *C. gloeosporioides* were isolated from the infected *Hevea brasiliensis* leaves and the single spore isolates were maintained on potato dextrose agar (PDA) medium for the *in vitro* studies. *In vitro* efficacy of five fungicides viz. mancozeb, carbendazim, SAAF, hexaconazole and propiconazole were carried out. Concentrations ranging from 100 - 1000 ppm were selected for the evaluation.

***Spore germination inhibition***

Spore germination study was carried out by the hanging drop method in cavity slides. Spore suspension was prepared in sterile water using seven-day-old culture of *Colletotrichum*. Concentrations of 100 –1000ppm of each test fungicide were prepared with the spore suspension ($10^4$ spore/ml). The spore suspension was then transferred to cavity slides and placed in petriplates lined with moisture absorbent cotton at room temperature. Spore suspension in sterile water severed as control. Spore germination was recorded after 12 hours of incubation. The percent inhibition of spore germination with respect to control was calculated.

***Poisoned food technique***

Fungicides at desired quantity were mixed in the autoclaved PDA at 45±2°C to get the preferred concentration. The fungicide-amended medium was poured into petridishes and at the center; culture disc of 5mm diameter from the actively growing culture was placed. Control plates were maintained with out the addition of fungicides. Colony growth was measured when the diameter of growth in the control plates was 9cm. The percent inhibition of the mycelial growth in each treatment with respect to the growth in the control was calculated.

**Field evaluation**

Field experiments were carried out in two regions viz. at Thodupuza and Mundakayam, which are known to be the hot spot area for Colletotrichum leaf disease. Three field trials were carried out during the disease season of 2001, 2002 and 2003 using the RRII 105 plants planted in main field during those years (first year) at Kaliyar Estate, Thodupuzha, Malankara Estate, Thodupuzha and Manikal Estate, Mundakayam respectively. During 2001, SAAF(0.075%) and propiconazole(0.025%) were compared with hexaconazole and recommended fungicides viz. mancozeb and carbendazim, copper oxychloride and Bordeaux mixture. In 2002, SAAF (0.075%) and propiconazole at two concentrations (0.025% and 0.012%) were evaluated. SAAF (0.075%), propiconazole(0.012%) as such and alternated with mancozeb(0.2%) were evaluated during 2003 season.

The trials were laid out in randomised block design with four replications each comprising of 25 plants. An unsprayed control was maintained in each trial. Fungicides were applied from August to October at weekly intervals using a knap-sack sprayer. To estimate disease incidence the total number of leaves and the total number of diseased leaves in the upper two whorls were recorded. Disease incidence was calculated by dividing the number of infected leaves by the total number of leaves and expressed as a percentage (Samaradeewa *et al., 1985).* Disease severity was assessed on a 0-5 scale (Manju *et al.,* 1999*)* based on the intensity of spotting and deformity of the leaves and expressed as percentage disease intensity (PDI).

**Results and Discussion**

The observations on disease incidence by the two *Colletotrichum spp.* on the clone RRII 105 showed that the raised spot (73.6%) and papery lesion (71.5%) and anthracnose(12.4%) caused by the two species of *Colletotrichum acutatum* and *C. gloeosporioides* respectively distributed almost uniformly in the immature plants. The anthracnose symptom caused by *C. gloeosporioides* recorded an incidence of only 12.4 per cent. Kumar *et al.* (2002) found that the raised spots were produced by *Colletotrichum acutatum* and the anthracnose and papery lesions by *C. gloeosporioides.* This study showed that in the same plant itself a combined infection occurred. Jayasinghe *et al.* (1997) reported that the *C. acutatum* was more aggressive than *C. gloeosporioides*. In the present study, it was observed that the leaves with raised spots symptoms (*C. acutatum)* were more distorted than those with the other symptoms.

Hexaconazole at 100ppm, SAAF and propiconazole at 250ppm and carbendazim at 500ppm completely inhibited the spore germination. All these fungicides arrested the mycelial growth completely at 100ppm concentration. Mancozeb completely inhibited the spore germination and mycelial growth only at 1000ppm (Table 1a and 1b). Jayasinghe and Fernando (2000) observed that *C. acutatum and C. gloeosporioides* infecting rubber varied in their response to fungicides. However, in the present study not much variation in the sensitivity was observed between the two species at the tested concentrations. Benzamidazole fungicides like carbendazim are known to induce resistance in various

Table 1a. *In vitro* efficacy of fungicides – inhibition of spore germination

| Fungicide | Per cent spore germination inhibition | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *C. acutatum* | | | | *C. gloeosporioides* | | | |
| | 100 ppm | 250 ppm | 500 ppm | 1000 ppm | 100 ppm | 250 ppm | 500 ppm | 1000 ppm |
| Mancozeb | 8.0 | 30.0 | 80.0 | 100 | 5.0 | 25.0 | 80.0 | 100 |
| Carbendazim | 25.0 | 70.0 | 100 | 100 | 25.0 | 75.0 | 100 | 100 |
| SAAF | 90.0 | 100 | 100 | 100 | 90.0 | 100 | 100 | 100 |
| Hexaconazole | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Propiconazole | 95.0 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

Table 1b. *In vitro* efficacy of fungicides on growth of fungi – poisoned food technique

| Fungicide | Per cent inhibition of growth | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *C. acutatum* | | | | *C. gloeosporioides* | | | |
| | 100 ppm | 250 ppm | 500 ppm | 1000 ppm | 100 ppm | 250 ppm | 500 ppm | 1000 ppm |
| Mancozeb | 24.7 | 35.9 | 74.52 | 100 | 25.8 | 30.5 | 62.9 | 100 |
| Carbendazim | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| SAAF | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Hexaconazole | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Propiconazole | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |

groups of fungi (Nene and Thapliyal,1993). Carbendazim is recommended for the management of CLD in rubber plantations. Though it is very effective, the possibility of resistance build up in the fungal population (Georgopoulos, 1977) cannot be ruled out. This suggests the importance of screening fungicides with different modes of action for combined use.

In the field study during 2001, lowest disease incidence was recorded in the treatment SAAF(7.7%) followed by mancozeb(9.8%). Disease severity was minimum in the plots sprayed with carbendazim(3.6%) followed by SAAF(4.3%). Control plots recoded maximum disease incidence and severity (Table 2). The triazole fungicide propiconazole and SAAF were on par with hexaconazole and recommended fungicides, viz. mancozeb, carbendazim and Bordeaux mixture.

Results of the experiment during 2002 showed that all the fungicides evaluated were significantly superior to control in checking the incidence and severity of CLD (Table 3). The minimum disease incidence was recorded in the plots sprayed with hexaconazole(5.8%) followed by SAAF(8.9%). Lowest disease severity was recorded in the plots sprayed with SAAF(5.2%) followed by mancozeb(6.9%). The lower concentration of propiconazole(0.012%) was as effective as the higher concentration (0.025%) and on par with the other fungicides.

In the experiment conducted in 2003, the results showed that there was no significant difference in the disease incidence although it was the lowest (33.4%) in the SAAF applied plots (Table 4). Disease severity was also low in the SAAF applied plots (9.0%). All the fungicides treatments showed significantly lower severity compared to control. Lower concentration of propiconazole (0.012%) was on par and comparable to the other fungicides evaluated. Alternate use of

Table 2. Effect of fungicides on the incidence and severity of CLD at Kaliyar Estate, Thodupuzha (2001)

| Fungicide | Concentration Used(%) | Disease incidence (%) | Disease severity (%) |
|---|---|---|---|
| Mancozeb | 0.2 | 9.8 | 6.1 |
| Carbendazim | 0.05 | 10.2 | 3.6 |
| SAAF | 0.075 | 7.7 | 4.3 |
| Hexaconazole | 0.02 | 11.8 | 6.1 |
| Propiconazole | 0.025 | 10.4 | 7.1 |
| Copper oxychloride | 0.125 | 15 | 12.5 |
| Bordeaux mixture | 1.0 | 11.5 | 9.8 |
| Control | Unsprayed | 21.9 | 21.7 |
| CD (0.05) | | 5.2 | 4.2 |

Table 3. Effect of fungicides on the incidence and severity of CLD at Malankara Estate, Thodupuzha (2002)

| Fungicide | Concentration used(%) | Disease incidence (%) | Disease severity (%) |
|---|---|---|---|
| Mancozeb | 0.2 | 20.1 | 6.9 |
| Carbendazim | 0.05 | 16.2 | 7.6 |
| SAAF | 0.075 | 8.9 | 5.2 |
| Hexaconazole | 0.02 | 5.8 | 11 |
| Propiconazole | 0.025 | 24.3 | 8.7 |
| Propiconazole | 0.012 | 24.2 | 8.6 |
| Control | Unsprayed | 37.5 | 19.1 |
| CD (0.05) | | 10.5 | 6.6 |

**Table 4. Effect of fungicides on the incidence and severity of CLD at Manikal Estate, Mundakayam (2003)**

| Fungicide | Concentration used(%) | Disease incidence (%) | Disease severity (%) |
|---|---|---|---|
| Mancozeb | 0.2 | 35.2 | 10.3 |
| Carbendazim | 0.05 | 40.6 | 11.5 |
| SAAF | 0.075 | 33.4 | 9 |
| Hexaconazole | 0.02 | 42 | 14.3 |
| Mancozeb/Propiconazole[A] | 0.025 & 0.012 | 44.4 | 11.7 |
| Propiconazole | 0.012 | 36.4 | 13.5 |
| Control | Unsprayed | 54.7 | 21.8 |
| CD (0.05) | | NS | 6.2 |

[A] alternate spraying

mancozeb(0.2%) and propiconazole(0.012%) was found to give comparable protection as the other treatments.

The cost of different fungicides except hexaconazole(0.02%) and propiconazole (0.025%) were comparable (Table 5). Therefore, the fungicides SAAF(0.075%) and propiconazole(0.012%) can be economically used for the Colletotrichum leaf disease management.

**Table 5. Comparison of cost of different fungicides**

| Fungicides | Concentration used | Dosage (per litre) | Quantity required per hectare | Cost of fungicides per hectare* (Rs.) |
|---|---|---|---|---|
| Mancozeb | 0.20% | 2.66g | 53.2g | 13.8 |
| Carbendazim | 0.05% | 1g | 20g | 10 |
| SAAF | 0.08% | 1g | 20g | 13 |
| Hexaconazole | 0.02% | 4ml | 80ml | 41.6 |
| Propiconazole | 0.03% | 1ml | 20ml | 24 |
| Propiconazole | 0.01% | 0.5ml | 10ml | 12 |
| Copper oxychloride | 0.13% | 2.5g | 50g | 12 |
| Bordeaux mixture(1%) ($CuSO_4$+Lime) | 1% | 10g+10g | 200g+200g | 12.20[a]/32.8[b] |

* Cost as per rate in 2006; [a] Cost of $CuSO_4$ considered during 2005; [b] Cost of $CuSO_4$ considered during 2006

Spraying of mancozeb(0.2%) and carbendazim (0.05%) at fortnightly intervals is recommended for CLD control. However, Joseph *et al.(2005)* found that the weekly application of mancozeb (0.2%) or carbendazim(0.05%) either as combination or alternate spraying was more effective in checking the disease severity. Synergy between carbendazim and mancozeb in controlling leaf and fruit spot of pomegranate caused by *Colletotrichum* was observed by Jamadar *et al.* (1998) and Gaikwad (2000). In the present investigation, weekly spraying of a combination product of mancozeb and carbendazim (SAAF) was found to be superior in checking the disease incidence and severity in all the field experiments. Similarly, individual and alternate spraying of propiconazole (0.012%) with mancozeb(0.2%) was found to be effective. Effectiveness of SAAF (2g/l) against *Corynespora cassiicola* infecting rubber was reported by Manju *et al.*(2005). Propiconazole(125g/l) has been reported to effectively control secondary leaf fall (SLF) disease caused by *Colletotrichum* in Malaysia (Shamsuri *et al. 1997)*. The present study confirmed that the use of combination product of systemic and contact fungicides or their alternate application is effective for disease management. It may also slow down the resistance development in the pathogen (Delp, 1980 and Staub,1991).

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