# Comparative efficacy of plant products on the spore germination and disease incidence of coffee leaf rust pathogen

**Daivasikamani Subramani[1], Rajanaika[2], Kiran Kumar Kariyanakatte Chinnaswamy[1], Shyam Singh[1], Vinod Kumar Puthenveetil Kumar[1], Sudhakar Subray Bhat[1], Jayarama[1]**

[1]Division of Plant Pathology, Coffee Research Station, Central Coffee Research Institute, Chikmagalur, Karnataka 577117, India, Email: manicrs@gmail.com; [2]Department of Applied Botany, Kuvempu University, Shankaraghatta, Shimoga, Karnataka 577451, India

## ABSTRACT

Four plant products were evaluated in the laboratory for their effect on the spore germination of coffee leaf rust pathogen (*Hemileia vastatrix* Berk. & Br.) and in the field for its bioefficay on leaf rust disease incidence with *Coffea arabica* L. cultivar Cauvery as test material. The study was carried out at the Central Coffee Research Institute, Coffee Research Station, Chikmagalur, Karnataka for a period of two years. Leaf rust incidence was recorded at fortnightly intervals. Fresh leaves of *Adhatoda vasica, Azadirachta indica, Lantana camara* and *Ricinus communis* were used for extraction of plant based products. Efficacy of these plant extracts was evaluated in the laboratory by percent germination of urediniospores of the coffee leaf rust fungus and in the field the percentage of leaf rust incidence. The results indicated that inhibition of urediniospores to an extent of 73.86% with leaf extracts of *A. vasica*, 61.40% with *A. indica*, 69.91% with *L. camara* and 50.94% with *R. communis* was possible at 15% concentration of all the extracts. In the field, leaf extracts of *A. vasica* at 15% concentration decreased the disease incidence to 34.75% while that of *L. camara* by 25.12% and *A. indica* reduced the disease incidence to 19.60% while *R. communis* could reduce the rust incidence to an extent of only 8.35%. Though the management options using chemicals were encouraging, the leaf extracts of plants could be incorporated as a tool in the integrated disease management of leaf rust as an eco-friendly component.

**Keywords**: *Coffea arabica, Hemileia vastatrix,* leaf rust incidence, plant extracts, urediniospores.

## INTRODUCTION

Coffee belongs to the genus *Coffea* of the family, Rubiaceae [1]. The two economically important species of *Coffea* namely *Coffea arabica* L. (arabica coffee) and *Coffea canephora* Pierre ex Froehner (robusta coffee) are commercially cultivated throughout the coffee growing countries. Coffee is one of the most important commercial crops, cultivated in the Eastern and Western hilly tracts of India. During the year 2008-09 the total planted area of coffee in India was 3,94,352 ha. The average production of coffee was about 2.62 lakh MT with an average productivity of 765 kg $ha^{-1}$. Average productivity of arabica and robusta coffee was 624 and 874 kg $ha^{-1}$ respectively [2]. Among the cultivated coffee, arabica coffee is more susceptible to diseases compared to robusta coffee [3]. Coffee leaf rust (CLR) disease, incited by the Basidiomycetes fungus, *Hemileia vastatrix*

Berkeley & Broome, is the most devastating disease of coffee and is considered one of the major tropical diseases of crop plants [4]. This foliar disease was first found on cultivated coffee in India during 1869. The crop loss caused by the coffee leaf rust fungus, as estimated by various researchers from the coffee growing countries, varied between 30 and 80%, if no control measures are adopted [5,6]. In severely affected areas the pathogen may cause foliage loss up to 50% and berries up to 70% [7-9].

Plant protection against diseases continues to rely heavily upon fungicides. India is the third largest consumer of pesticides in the world and highest among the South Asian countries. In India, consumption of insecticides is 60%, fungicides 21%, herbicides 14% and others 5% [10]. The dependence on chemicals and indiscriminate use of pesticides is associated with problems such as environmental pollution, health hazards, destruction of biological communities, etc. Crop improvement and disease management have to be achieved with the use of bioresources such as antagonistic microbes, plant based products, etc. by replacing chemical pesticides and fertilizers. To combat the problems and for protecting crops from fungal pathogens, research on the development of pesticides of plant origin (botanicals or phytoextracts) and microbial antagonists (biopesticides or biological control agents) which are relatively safe for use in agriculture and measures for promoting rapid degradation of pesticides are being stepped up in the recent times.

Use of fungicides is a regular practice for the control of diseases of coffee in all the coffee growing countries of the world. Though, several fungicides have been tested for their efficacy against coffee leaf rust disease in India, the effect of plant based products (leaf extracts) has not been studied in detail. Given the current difficult economic situation and market demand for environment-friendly products, there are now good reasons to search for alternative control interventions to replace the more hazardous chemicals.

Therefore, there is a need to explore the possibility of using eco-friendly and environmentally safer formulations such as plant extracts which can fit into Integrated Disease Management (IDM) programme. Hence, studies were conducted to understand the effect of phytoextracts (leaf extracts from plants of *Adhatoda vasica*, *Azadirachta indica*, *Lantana camara* and *Ricinus communis*) in comparison with recommended fungicides (Bayleton and Bordeaux mixture) for the control of coffee leaf rust disease.

## MATERIALS AND METHODS

### Experimental site

Laboratory and field experiments were conducted during the years of 2006 and 2007 at the Central Coffee Research Institute (CCRI), Chikmagalur, Karnataka, India located at 13º 22' North Latitude and 75º 28' East Longitude at an altitude of 914 meters above MSL.

### Plant materials

The plant material used for laboratory (*in vitro*) and field (*in vivo*) studies was twenty years old *Coffea arabica* cv. Cauvery. The urediniospores of the coffee leaf rust fungus were collected from the infected leaves of Cauvery for *in vitro* studies. In the field experiment, to assess the bioefficacy of plant products, twenty years old Cauvery plants were used as test material. Four angiospermic flowering plants, viz., *Adhatoda vasica* Nees. (Acanthaceae), *Azadirachta indica* A. Juss. (Meliaceae), *Lantana camara* Linn. (Verbenaceae) and *Ricinus communis* Linn. (Euphorbiaceae) were used for extraction of plant based products from the leaves.

## Preparation of plant extracts

Plant extracts (cold water) were obtained from the leaves of *Adhatoda vasica, Azadirachta indica, Lantana camara* and *Ricinus communis* as described by Gerard et al. [11]. Fresh leaves were collected and washed with tap water and then in sterile water. It was then pulverized with sterile distilled water at the rate of 1 ml per gram of tissue (1:1 v/w) with a mixer grinder, filtered and centrifuged at 6000 rpm for 10 minutes. After centrifugation the supernatant was collected and stored in the refrigerator at 5ºC till further use and this formed the standard plant extract solution (100 per cent). All the above phytoextracts were tested at 15% concentration for *in vitro* and *in vivo* experiments.

## Laboratory bioassay of plant extracts on urediniospores germination

For the evaluation of antifungal effect of plant products on the germination of urediniospores of *H. vastatrix*, phyto extracts were obtained from the leaves of *A. vasica, A. indica, L. camara* and *R. communis* as described by Gerard et al. [11]. Water agar medium (2%) containing 15% phyto extracts were prepared. Fifteen ml of the medium was poured into 90 mm sterile Petri-plate (Corning make) and allowed to cool and solidify. Later, one ml of urediniospores suspension was pipetted out, poured and spread evenly on to the medium. The water agar medium without any phytoextract served as control. The different treatments were $T_1$- *A. vasica* leaf extract, $T_2$- *A. indica* leaf extract, $T_3$- *L. camara* leaf extract, $T_4$- *R. communis* leaf extract all at 15%, $T_5$- Bayleton 25 WP @ 0.02% a.i., $T_6$- Bordeaux mixture @ 0.5% and $T_7$- Untreated control (sterile distilled water). Five replicates were maintained and all the Petri plates were incubated overnight in the dark at room temperature (22±2ºC); observations were recorded after 18 h of incubation. The count on total number of urediniospores present and the total number of germinated urediniospores in each microscopic field were recorded using stereoscopic microscope (SMZ-800, Nikon, Japan). The values were expressed in percentage by using the formula: percentage of germinated urediniospores = number of urediniospores germinated × 100 / number of urediniospores in the microscopic field.

## Field evaluation of plant extracts on coffee leaf rust pathogen

The leaf extracts of four plants were evaluated in comparison with the two standard fungicides along with water sprayed control for their effectiveness in reducing the rust disease incidence in the field. The plant extracts were applied as foliar spray with high volume ASPEE - Rocking or Gator sprayer [12] on the most susceptible variety of arabica coffee-Cauvery during June (season I or pre-monsoon) and September (season II or post-monsoon). The different treatments were $T_1$- *A. vasica* leaf extract, $T_2$- *A. indica* leaf extract, $T_3$- *L. camara* leaf extract, $T_4$- *R. communis* leaf extract all at 15% concentration, $T_5$- Bayleton 25 WP @ 0.02% a.i., $T_6$- Bordeaux mixture @ 0.5% and $T_7$- Untreated control (water sprayed). Treatments were replicated five times with 15 plants per replication. Randomized Block Design (RBD) was used in the field experiment with optimum plot size [13]. Standard agronomic practices were followed in the experimental plot. The percentage of CLR disease incidence was assessed in the field by methods suggested by Muthappa [14] and Srinivasan [15]. Four secondary/tertiary branches at random in all the four directions and at top, middle and bottom of the plant were selected and marked in each treatment for uniformity. Pre-treatment count on rust incidence was recorded. After application of treatments, the coffee leaf rust incidence was scored once in fifteen days starting from fifteenth day till ninety days in both the seasons (season I and season II). The effect of plant extracts was compared with standard fungicides and also with water sprayed control. The total number of healthy and infected leaves from the marked branches was counted and the percent diseased leaves were worked out using the formula:

PDI = TNDL × 100 / TNHDL, where PDI = per cent disease incidence (percentage of infected leaves), TNDL = total number of diseased leaves in marked branch, and TNHDL = total number of healthy and diseased leaves in marked branch.

### Statistical analysis

Data recorded from laboratory and field experiments were statistically analysed using methods suitable for completely randomized or randomized block design. The percentage values were subjected to arc-sine or square root transformation. The treatment means were compared with LSD or Duncan's multiple range test (DMRT) for their significance [16].

## RESULTS AND DISCUSSION

With more than six billion people in the beginning of the third millennium, the governments are confronted with a herculean task of providing environmental and food security to the expanding population, particularly in the developing countries. This necessitates reorientation of strategies in agriculture to minimize the use of hazardous external inputs and so dependence increases on eco-friendly approaches to sustain food production without causing disruption to the fragile agro-ecosystem. In the recent years there is a shift in the control of plant diseases from the regular use of pesticides to an alternate and more eco-friendly biopesticide and plant based products. Use of plant based products in the cultivation of field crops and for controlling pest and diseases are well documented, but their effects on a perennial crop like coffee have not been studied in detail. Thus the present work is the first attempt to understand the effect of these botanicals on the control of *H. vastatrix*, the causal organism of leaf rust disease on coffee.

Mayee [17] stated that disease management in agricultural crops involves various strategies such as legislative, cultural, biological, biopesticides of plant origin, chemical, host resistant sources, breeding for resistance, etc. These methods may be practiced either individually or one or more methods may be followed as an integrated approach to tackle the disease problems. Vyas [18] stated that extensive use of systemic fungicides have led to several problems of toxicity, hazards to living beings, development of resistance in pathogen and non target effects of broad spectrum fungicides on associated soil micro flora.

From the present study, percentage spore germination in control treatment and percent inhibition of urediniospores of CLR in other treatments over control are presented in table 1. There was 56.34% urediniospores germination in control treatment. Inhibition percentage was highest (89.48%) in Bayleton 25 WP treatment at the dosage of 0.02% a.i. followed by 79.44% in Bordeaux mixture at the dose of 0.5%. Statistically all the treatments were significantly superior to control (P=0.05). Germination of urediniospores was inhibited to an extent of 73.86% in the leaf extracts of *A. vasica*, 61.40% in *A. indica*, 69.91% in *L. camara* and 50.94% in *R. communis* all at 15% test concentration. Statistically, there was no difference in the inhibition of spore germination between the leaf extracts of *A. vasica* and *L. camara*. Among the leaf extracts the property of inhibiting the urediniospores germination was more in *A. vasica* and *L. camara* followed by *A. indica* and *R. communis*. Field data recorded at fortnightly intervals on the effect of plant extracts were pooled. The per cent disease reduction over control based on the mean percentage of rust incidence of individual seasons of the year 2006 and 2007 and the cumulative mean data pertaining to season I and II of years 2006 and 2007 are presented in table 2.

During season I, the pooled mean data indicated a maximum disease reduction (63.83%) in Bayleton treatment at 0.02% a.i. when compared to control. Followed by Bayleton, there was 38.33% disease reduction in Bordeaux mixture treatment at 0.5% concentration. Among the plant products, leaf extract of *A. vasica* reduced the disease incidence to an extent of 34.75% while leaf

extract of *L. camara* recorded 25.12% disease reduction and *A. indica* leaf extract reduced the disease to 19.60%. The aqueous leaf extract of *R. communis* at 15% concentration could reduce the coffee rust disease to an extent of only 8.35%. The disease reduction during season II in different treatments were: Bayleton 54.68%, Bordeaux mixture 33.16%, *A. vasica* 28.63%, *L. camara* 21.50%, *A. indica* 17.24% and *R. communis* 8.42%.

Table 1. Effect of leaf extracts on germination of urediniospores of *H. vastatrix*.

| Treatment details | % germination | % inhibition over control |
|---|---|---|
| $T_1$ - *A. vasica* leaf extract @ 15% | 14.73 (22.12)$^{c}$ | 73.86 (57.26)$^{c}$ |
| $T_2$ - *A. indica* leaf extract @ 15% | 21.75 (27.28)$^{d}$ | 61.40 (49.57)$^{d}$ |
| $T_3$ - *L. camara* leaf extract @ 15% | 16.95 (23.70)$^{c}$ | 69.91 (56.70)$^{c}$ |
| $T_4$ - *R. communis* leaf extract @ 15% | 27.64 (31.30)$^{e}$ | 50.94 (46.54)$^{e}$ |
| $T_5$ - Bayleton 25 WP @ 0.02% a.i. | 5.93 (13.27)$^{a}$ | 89.48 (70.08)$^{a}$ |
| $T_6$ - Bordeaux mixture @ 0.5% | 11.58 (19.46)$^{b}$ | 79.44 (63.60)$^{b}$ |
| $T_7$ - Un-treated control (sterile distilled water) | 56.34 (48.50)$^{f}$ | - |
| S.Em. (±) | 1.32 | 2.13 |
| CD (P=0.05) | 2.69 | 4.72 |

*mean of five replications; figures in parentheses are *arc-sine* transformed values; in a column, means followed by same letter(s) are not significantly different as per LSD.

Table 2. Effect of leaf extracts on coffee leaf rust incidence in the field (cumulative mean of two years and four seasons).

| Treatment details | Per cent disease reduction over control* | | | | Pooled mean of years 2006 and 2007 | |
|---|---|---|---|---|---|---|
| | Year 2006 | | Year 2007 | | | |
| | Season I | Season II | Season I | Season II | Season I | Season II |
| $T_1$ - *A. vasica* leaf extract @ 15% | 33.25 (35.26)$^{bc}$ | 28.22 (31.86)$^{bc}$ | 36.24 (36.70)$^{b}$ | 29.04 (31.92)$^{bc}$ | 34.75 (35.53)$^{b}$ | 28.63 (31.92)$^{b}$ |
| $T_2$ - *A. indica* leaf extract @ 15% | 19.85 (25.84)$^{d}$ | 18.70 (25.10)$^{d}$ | 19.34 (25.71)$^{c}$ | 15.77 (22.78)$^{d}$ | 19.60 (25.87)$^{d}$ | 17.24 (24.87)$^{c}$ |
| $T_3$ - *L. camara* leaf extract @ 15% | 26.64 (29.86)$^{c}$ | 20.81 (26.86)$^{cd}$ | 23.60 (28.42)$^{c}$ | 22.18 (27.16)$^{cd}$ | 25.12 (28.17)$^{c}$ | 21.50 (26.96)$^{c}$ |
| $T_4$ - *R. communis* leaf extract @ 15% | 9.71 (15.32)$^{e}$ | 11.29 (19.34)$^{e}$ | 6.99 (14.28)$^{d}$ | 5.55 (13.17)$^{e}$ | 8.35 (14.72)$^{e}$ | 8.42 (15.14)$^{d}$ |
| $T_5$ - Bayleton 25 WP @ 0.02% a.i. | 63.95 (52.50)$^{a}$ | 53.80 (46.70)$^{a}$ | 63.71 (52.49)$^{a}$ | 55.56 (47.70)$^{a}$ | 63.83 (52.36)$^{a}$ | 54.68 (46.82)$^{a}$ |
| $T_6$ - Bordeaux mixture @ 0.5% | 35.76 (36.24)$^{b}$ | 31.99 (33.84)$^{b}$ | 40.90 (39.16)$^{b}$ | 34.33 (35.03)$^{b}$ | 38.33 (37.18)$^{b}$ | 33.16 (35.06)$^{b}$ |

*mean of five replications, figures in parentheses are *arc-sine* transformed values; in a column, means followed by same letter(s) are not significantly different at =0.05 as per DMRT.

The insecticidal properties of the neem tree, *A. indica* A. Juss (Meliaceae) and its products have been well described by Jacobson and Schmutterer et al. [19,20]. While studying the seed germination of infected tomatoes, Shekar and Darwish [21] described the antioxidative and antifungal activity of azardirachtin derivatives. Copping and Menn [22] has reported that many of the insect pest and fungal pathogen are controlled by the compounds derived from the neem tree. The most important active ingredient, azadirachtin, is a complex and highly oxygenated compound belonging to tetranor triterpenoid class and mostly concentrated in the seeds [23]. Dubey [24] reported that extracts from the plants of Dattura and neem did not inhibit the growth of *Rhizoctonia solani*. The antimicrobial activity of Zimmu (*Allium cepa* × *Allium sativum*) leaf extract against *R. solani* under *in vitro* conditions was described by Satya et al. [25]. Studies were conducted by Devi and Paul [26] by integrating plant extracts and biocontrol agents where ten plant species were selected and the plant extracts were evaluated for the management of pea wilt/root rot complex caused by *R. solani*. In horticultural crops, phytoextracts and fungicides were evaluated by Meena and Shah [27] against fruit rot disease caused by *Phomopsis citri* in Mandrin orange var. Nagpur Santra.

At present, fungicides are recommended for use as spray for the control of rust disease, especially when the incidence level builds up either by spraying prophylactic Bordeaux mixture or one of the systemic, curative fungicides. But there is always a risk of residues tainting the coffee. Coffee being an export oriented crop, use of fungicides and chemicals in plantations is subject to tight scrutiny in view of the strict minimum residue limits (MRL) of pesticide prescribed by different consuming countries. The coffee importing countries can use the MRL's as trade barriers at any time. Therefore, there is a need to explore the possibility of using eco-friendly and environmentally safer formulations such as plant extracts which can fit into integrated disease management programme.

As expected, the inhibition of urediniospores germination by the fungicides was much more than obtained using leaf extracts. Apart from growing field tolerant arabica coffee cultivars such as Chandragiri, Sln.5B, Sln.6 and Sln.9, use of leaf extracts of plants are the alternate tools available for integrated management of coffee leaf rust. The study using leaf extracts for the management of coffee leaf rust has shown some positive results. If the molecules inhibiting germination are identified, they could be synthesized in the laboratory and exploited commercially. Further studies are required to refine the method and time of application of the phytoextracts to improve their effect.

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