

Management of Leaf Rust Disease of Medicinal Plant *Justicia gendarussa* Burm. F. With Antagonistic Fungi (*Trichoderma harzianum*)

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Abstract: In this study, antagonistic activity of *Trichoderma harzianum* against the causal agent of leaf rust of medicinal plant *Justicia gendarussa* Burm. f. were examined by dual culture technique. This is a common medicinal herb well known for its medicinal properties such as anti arthritic, anti inflammatory, anti oxidant and immunosuppressive activities. This was found to be severely infected by rust diseases caused by *Puccinia thwaitesii*. The disease was characterized by well defined concave or convex, discolored lesions surrounded by a wide yellow halo on the adaxial surface and corresponding lesions on the opposite abaxial surface. We isolated and tested *Trichoderma harzianum* for antagonistic activity. In vitro analysis showed that *Trichoderma harzianum* inhibited *Puccinia thwaitesii* by 80% inhibition of radial growth (PIRG). The present study stresses on control of diseases affecting medicinal plants by using biocontrol agents as an attempt for developing environmentally sound method towards disease management.

Key words: *Trichoderma harzianum* • *Puccinia thwaitesii* • *Justicia gendarussa* Burm. f. rust disease
• Antagonistic fungi.

INTRODUCTION

Justicia gendarussa (Burm. f.) belongs to plant family acanthaceae and is a quick growing, evergreen shrub found throughout India. The plant is used in traditional medicinal practices for chronic rheumatism, inflammations, bronchitis, vaginal discharges, dyspepsia, dysuria, eye diseases and fever [1]. It also shows signs of responses like antimicrobial, anti-inflammatory and anti cancerous effects [2]. Use of plant based remedies is also wide spread in many countries and numerous pharmaceuticals are resultant from plant compounds. Away from each other medicinal uses *Justicia gendarussa* Burm. f. dried leaves were used to repel insects from clothing and found to be most effective against malaria vector *Anopheles stephensi* [3].

One of the major problems in the cultivation of *Justicia gendarussa* Burm. f. is the rust disease caused by *Puccinia thwaitesii*, which lead to significant yield loss. *Puccinia thwaitesii* is one of the important plant

pathogens reported as causing rust diseases of *Justicia gendarussa* Burm. f. [4]. Disease protection by chemicals does not fit with the basic theory of usefulness of herbal drugs. Chemicals contaminate the soil and are also of serious concern from environmental point of view [5].

An environment friendly and sustainable answer for the problem is the introduction of biological control agents. Biological control is a nature friendly, ecological approach to overcome the problems caused by standard chemicals generally used in plant protection. Biological control of *Puccinia thwaitesii* has been considered as a potential control strategy in recent years and investigations for biological agents is increasing. Among the various antagonists used for the plant diseases, *Trichoderma harzianum* play a vital role in controlling the pathogen.

Trichoderma harzianum, a low cost biocontrol agent that it can establish on different pathosystems, has moderate effect on soil balance and does not harm beneficial organisms that contribute towards pathogen's

control [6]. *Trichoderma* species release antibiotics and other chemicals that are harmful to pathogens and inhibit growth (antibiosis). The mechanisms proposed to explain the biocontrol of plant pathogens by *Trichoderma* are presumptive. The suggested mechanisms for biocontrol are antibiosis, lysis, competition and mycoparasitism. These may act alone or in combination [7]. Therefore in the present study an attempt was made to test the viability of *Trichoderma harzianum* against *Justicia gendarussa* Burm. f. rust disease caused by *Puccinia thwaitesii*.

MATERIALS AND METHODS

Soil Sample Collection: Soil samples were collected from the farm area of Community Agro biodiversity Centre, MSSRF, Wayanad. The samples were taken from 15 cm depth from different localities of farm area. The samples were mixed, homogenized and stored in a sterile polythene bag, which were transported in to the Laboratory and stored at 4°C until use.

Isolation of *Trichoderma Harzianum* from Soil:

Trichoderma harzianum was isolated from soil by using soil suspension method [8]. One gram of collected soil sample was added to 99 ml of sterile distilled water. After one minute of occasional shaking 100 micro liter of soil suspension added to plate containing sterilized *Trichoderma* specific medium (g/liter distilled water : $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2; K_2HPO_4 , 0.9; KCl, 0.15; NH_4NO_3 , 1.0; Glucose, 3.0; Chloramphenicol, 0.25; Rose Bengal, 0.15; Agar, 20) containing biocidal ingredient [9]. The plate was then incubated at room temperature for five days. After incubation colonies were transferred to potato dextrose agar (PDA) plates and kept at 10 to 15 °C until required for further studies.

Identification of *Trichoderma Harzianum*: The cultures were sub cultured in potato dextrose agar medium and kept at room temperature for four days. The mycelium formed within three days in shades of green or yellow or less frequently white. For microscopic examination the mycelia stained with lacto phenol cotton blue and observed under microscope.

Isolation of *Puccinia thwaitesii*: Diseased leaves of *Justicia gendarussa* Burm. f. were identified and sampled from medicinal plant area of Community Agro biodiversity Centre of MSSRF, Wayanad. Leaf pieces were surface sterilized with 0.5% sodium hypochlorite solution, washed

with sterile distilled water and placed on PDA medium at room temperature for five days and daily observations were made. The isolates were then sub cultured to get pure culture and thereafter slanted in Potato Dextrose Agar (PDA) for further experimental purposes.

In vitro Antagonism of *Trichoderma Harzianum*:

Antagonistic potential of *Trichoderma harzianum* was evaluated against *Puccinia thwaitesii* by dual culture technique [10]. Mycelium discs of *Trichoderma harzianum* having five millimeter diameter was taken from seven days old culture and was paired against same sized mycelia discs of *Puccinia* at opposite end on PDA. The plates were incubated at 27°C for five days. The PDA plates inoculated only with phyto-pathogen served as a control. The growth of the pathogen in both control and test experiments were recorded. From the data, percent inhibition of radial growth (PIRG) was calculated using the formula given below:

$$\text{Percent inhibition of radial growth (PIRG)} = \frac{(R_1 - R_2)}{R_1} \times 100$$

R1 = Radial growth of pathogen in control plates.

R2 = Radial growth of pathogen in test plates.

RESULTS AND DISCUSSION

Disease management of medicinal plants needs to be focused on utilization of potential biocontrol agents. *Trichoderma harzianum* have been established to be antagonistic towards a number of pathogens. In the present work dual culture technique on PDA media was done to identify the antagonistic activity of *Trichoderma harzianum* against *Puccinia thwaitesii*.

The *Trichoderma harzianum* isolated from soil and sub cultured on potato dextrose agar medium *Trichoderma* colonies grew rapidly, white colonies developed to yellow-green color. Microscopic examinations showed that the conidiophores typically formed paired branches along the length of the main axis.

The symptoms of rust diseases of *Justicia gendarussa* noticed, were circular brown or reddish brown, granular, hypophyllous spots, which later developed into pustules. As the disease advanced, the red pustules became black spots on the leaves that were circular and small at first but often coalesced to form large patches [11]. Microscopic and morphological evidences showed that the isolated fungus was *Puccinia thwaitesii*.

Table 1: Antagonistic activity of against *Puccinia thwaitesii* by dual culture technique.

Antagonistic isolate	Radial diameter (cm)	PIRG (%)	Number of days
<i>Trichoderma harzianum</i>	1.2	85	5

In the dual culture technique hyphal contact between *Trichoderma harzianum* and *Puccinia thwaitesii* was observed. *Trichoderma harzianum* inhibited the growth of *Puccinia thwaitesii* by 85 inhibition percentage (Table 1).

Trichoderma harzianum is one of the most-promising bio control agents that can be used against many fungal plant pathogens. *T. harzianum* has multiple mechanisms of action, including co-parasitism via production of chitinases, β -1-3 glucanases and β -1-4 glucanases, antibiotics, competition, solubilisation of inorganic plant nutrients, induced resistance and inactivation of the pathogen's enzymes involved in the infection process [12]. Antibiosis is generally recognized principal mechanism of interference competition by which fungi may exclude other organism from resources potentially available to each other [13].

It is reported that rust disease caused by *Puccinia arachidis* was significantly reduced by *Trichoderma harzianum* on detached groundnut leaves. But no other reports assert the bio-control remedy of *Puccinia thwaitesii* by *Trichoderma harzianum*. So outcome of the total work might help the medicinal plant growers to reduce the leaf rust caused by *Puccinia thwaitesii* and thereby improving the quantity and quality of production.

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