

Original Research Article

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Isolation and Microscopic Study of Leaf Spot of *Chromolaena odorata* (Siam Weed) caused by *Phytophthora* spp. from Tripura

Durga Prasad Awasthi¹ and Sumen Kapali^{2*}

¹Department of Plant Pathology, College of Agriculture, Tripura, India

²Vocational Education Teacher (Agriculture), SIBIN Group, Kamalghat Higher Secondary School, India

*Corresponding author

ABSTRACT

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To explore bio-control options available for management of Siam weed, a survey was conducted during the year 2017-18 and 2018-19 in different districts of Tripura, India. During survey, symptoms of leaf blight were observed on leaves of Siam weed. Fungi isolated from infected sample on PDA media shows slow growth covering whole petri plate within 10 days. During incubation period fungal sporulation increases with increase in growth period. Identification of suspected causal agent was *Phytophthora* which was confirmed based on the morphology. Culture colonies of *Phytophthora* produce coenocytic hyphae, unbranched sporangiophores, ovoid or subglobose to globose hyphal swellings with an average diameter of $18.6 \pm 6.5 \mu\text{m}$. Pathogenicity tests were carried out for further confirmation. Based on morphological features, the fungus on Siam weed was identified as *Phytophthora* spp. Based on available reviews, this is the first incidence of report of *Phytophthora* spp. from Tripura under the family Asteraceae, causing Leaf blight on Siam weed.

Introduction

Siam weed (*Chromolaena odorata* & *C. squalida*), looks similar to Blue top or Billy goat weed (*Ageratum* spp.), mature plants have a growth habit similar to Lantana (*Lantana camara*).

Chromolaena odorata has become one of the worst terrestrial invasive plants in the humid tropics and northeastern India (Waterhouse, 1994) It contains very high nitrate levels and

may also cause skin complaints and asthma in allergy-prone people (Prasad *et al.*, 2005, Koutika *et al.*, 2010).

To explore bio-control options available for its management a survey was conducted during the year 2017-18 and 2018-19 in different districts of Tripura. The present study revealed incidence and occurrence of *Phytophthora* species causing leaf spot of Siam weed under Agro-climatic condition of Tripura.

Materials and Methods

During the year 2017-18 and 2018-19, a routine survey on plants of *Siam weed* was conducted in the different Districts of Tripura, India namely, Gomati, Siphahijala, Khowai, west, South, Dhalai, Unokoti, and North Tripura. *In vivo* observation of disease symptoms were carried out, time of occurrence of disease, plant part affected and development process was recorded at regular interval. Samples were brought into the laboratory and compared with standard Disease Atlas and Books. Pathogen concern is isolated aseptically in Potato Dextrose Agar (PDA) slants. Before isolation small bits from the diseased plant parts were cut and washed in running tap water followed by washing in distilled water. The washed samples were then dipped in 0.1% HgCl₂ (Mercuric Chloride) solution for 30 to 45 seconds for surface sterilization and then washed in sterile distilled water. The PDA slants were then incubated at 28 ± 1°C in B.O.D. incubator for 14 days. Further sub culturing was carried out by taking 6 mm discs. The isolated fungus was inoculated into healthy plants grown in pots for confirmation of Koch postulates. Leaves sample and isolated fungi were mounted in Canada balsam and stained with cotton blue. For taxonomic identification, the morphology of obtained isolates was examined at 10 X, 40 X and 100 X magnifications. Colony growth patterns were examined at incubating the pathogen for one week at 20 °C in the dark on agar media.

Results and Discussion

In vivo observation of affected leaves initially show small dark spots which enlarge rapidly and turn purplish brown at the margins and tips of the leaf (Fig. 1). As the disease progresses, the lesions (mostly along the leaf margin) continue to expand and frequently coalesce. The boundary between the healthy

and diseased tissues is usually indistinct and smooth. The morphology of *Phytophthora* spp. was examined at 10 X, 40 X and 100 X magnifications using a light microscope, the result revealed that the sexual morph of this fungus was absent. Culture colonies of *Phytophthora* isolates on the PDA medium (Fig. 2) shows creamy white, woolly slow growth fungi. At initial level Primary hyphae grows slowly often branching in a mono- or dichasium with the mother hypha ending in a short protruding tip largely submerged with limited felty aerial mycelium (Fig. 3 and 4). Initially, Sporangia were not observed on solid agar but are produced abundantly after 24 hours in the same agar plate, borne terminally on unbranched sporangiophores, non-caducous, and papillate (Fig. 5 and 6), ovoid or subglobose to globose and irregular hyphal swellings (Fig. 7 and 8) with an average diameter of 18.6 ± 6.5 µm produced abundantly. Chlamydospores were not observed. The morphological and microscopic study confirms the asexual morph of *Phytophthora* spp as causal organism of leaf spot of Siam weed. The pathogen is readily identified as an asexual morph of the genus *Phytophthora* which are according to the findings of Andre and Sendall, 2001; Goheen *et al.*, 2007; Latham *et al.*, 2015). In future course of study molecular sequence analyses need to be taken up for further confirmation.

In the pathogenicity test, inoculated leaves developed leaf spots symptoms 10 days after inoculation, where the control leaves remained healthy. The fungus present on the inoculated leaves was morphologically identical to that originally observed on disease plants.

Although no prominent report so far has been recorded about the incidence of *Phytophthora* in Siam weed from Tripura. The Siam weed belongs to the family Asteraceae it includes Aster, Dahlia, Marigold, Safflower,

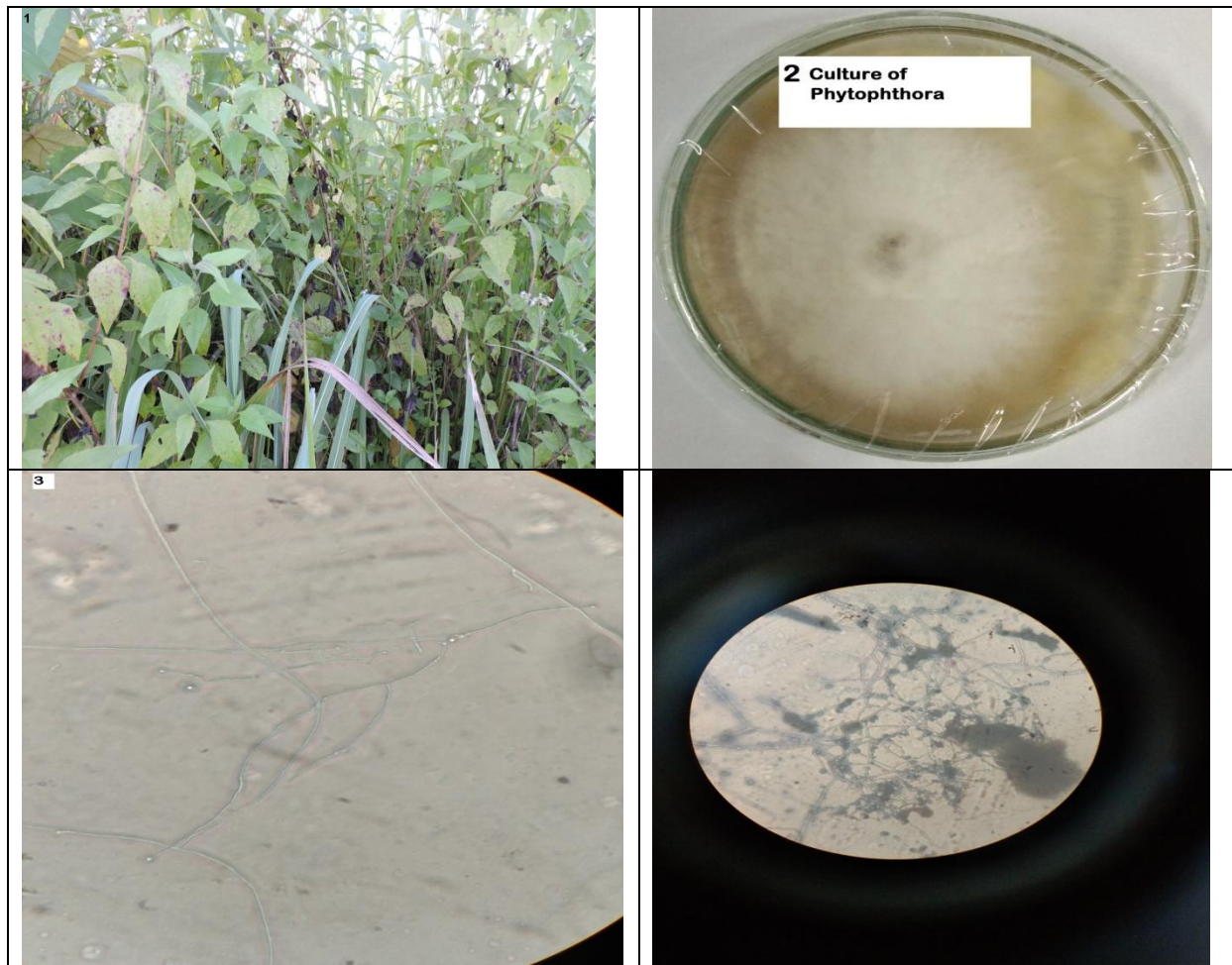
Sunflower, Stevia, Zinnia etc. Phytophthora Susceptible hosts includes the members of the Asteraceae, Ranunculaceae, Lamiaceae, Rhamnaceae, Phrymaceae, Rosaceae, and Verbenaceae plant families (Latham *et al.*,

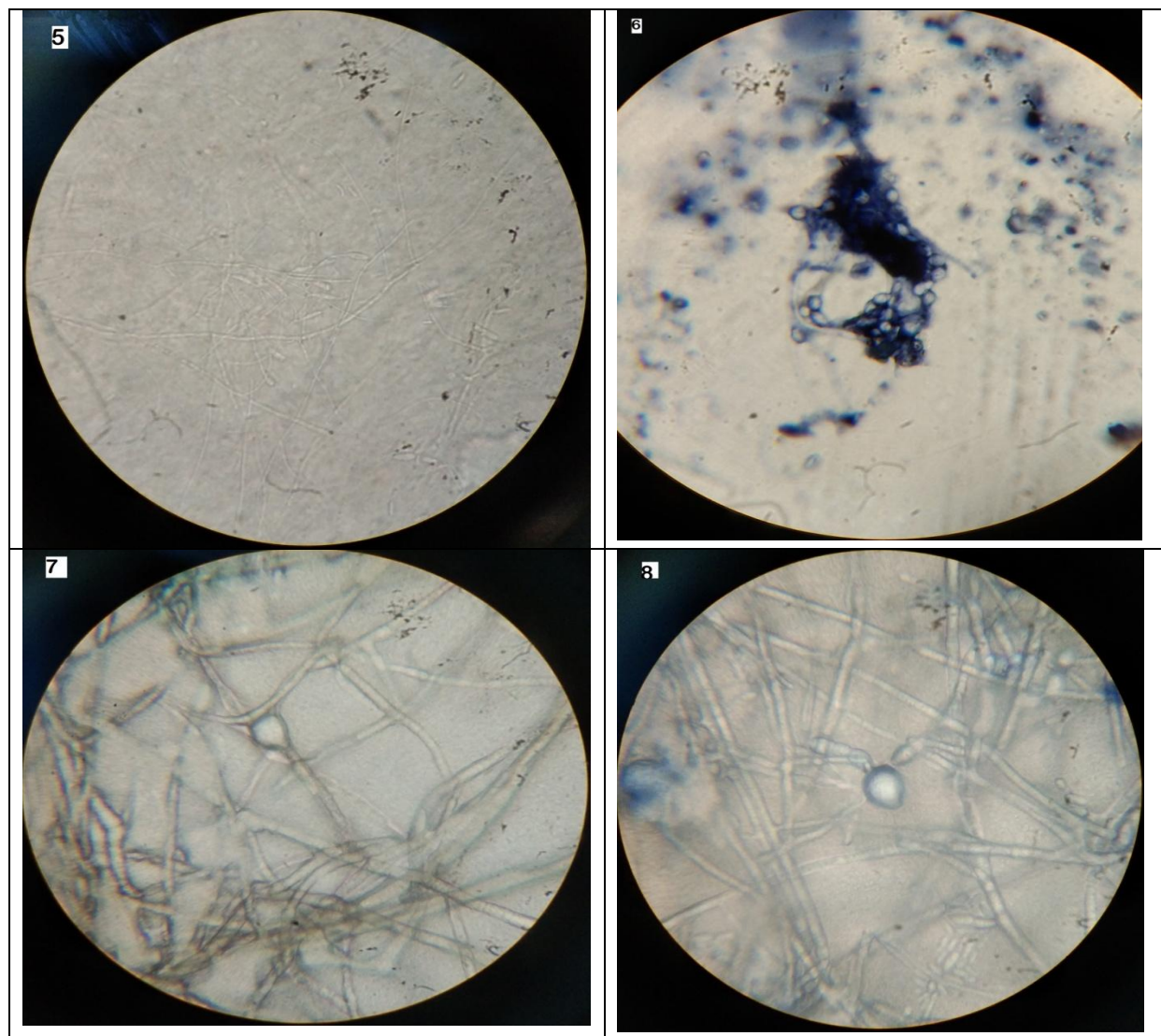
2015). *Chromolaena odorata* belongs to the Asteraceae (Compositae), a large, well-defined and highly evolved family (Toelken, 1983; Bremer, 1994; APG II, 2003) (Table 1).

Table.1 *In vivo* observation of disease symptoms, time of occurrence of disease, plant part affected and pathogen stages recorded

Sl.No.	Plant Part affected	Visibility of the symptoms	Time of Occurrence
1	Leaves	Usually visible to the naked eye	June-February
2	Stems (above ground)	Usually visible to the naked eye	

Fig.1 1. Phytophthora infected Siam Weed, 2. Culture of Phytophthora on PDA, 3. 4., Hyphae initially with slow growth branching with the hypha ending in a short protruding tip (10 X), 5., 6., Sporangia terminally on unbranched sporangiophores, non-caducous, and non-papillate (40 X), 7., 8., Sporangia ovoid or subglobose to globose or Non papillate (100 X)





Siam weed mainly of the tropics and subtropics, many of the countries in which it is a problem do not have the resources to implement comprehensive control programs using conventional methods. Consequently, biological control has become an important management tool (Boller *et al.*, 2006). Research into the potential of biological control for *C. odorata* through *Phytophthora* presumably used as a biological agent to keep it under control.

In conclusion, the pathogen produced purplish-brown, blackish water soaked lesions seen on margin of the leaves. Sporulation in *Phytophthora* cultures provides important

clues for identification. Chlamyospores were not observed. Sporangia are borne terminally on unbranched sporangiophores, non-caducous, papillate, ovoid, subglobose to globose with irregular hyphal swellings having average diameter of $18.6 \pm 6.5 \mu\text{m}$. This confirmed the fungi as asexual morph of *Phytophthora* spp. having high potential for exploring it as bio-control agent against Siam weed management.

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