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Microscopic identification and antifungal activity of pNPP against *Peronophythora litchi*: the causal agent of downy blight disease of litchi (*Litchi chinensis* Sonn.)

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SUMMARY

Litchi is one of the most significant fruits on the planet. One of the major factors limiting the production of litchi fruits is the downy blight disease caused by *Peronophythora litchi*. The present study was conducted on fruit samples collected from five different areas of Dehradun in Uttarakhand to confirm the presence of the disease in the region. Furthermore, microscopic identification and characterization of *Peronophythora litchi* was done and antifungal activity of p-nitrophenyl phosphate (pNPP)) against *Peronophythora litchi* the causal agent of downy blight was evaluated.

Keywords: *Peronophythora litchi*, fruit rot, downy blight, pNPP

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INTRODUCTION

According to studies by Jiang et al. (2003), Sivakumar et al. (2007), and Kumar et al. (2008), litchi (Litchi chinensis Sonn.) is a highly significant crop within the Sapindaceae family. Litchi is a perennial tree that originated in southern China (Jiang et al., 2003) and thrives in both tropical and subtropical temperatures (Jiang et al., 2003, Sivakumar et al., 2007, Kumar et al., 2008).According to Jiang et al. (2003), this tree yields a pale, translucent fruit called an aril, which encases a lustrous brown seed. The aril is surrounded by a tough, inedible pinkish-red skin, or pericarp, that turns brown after harvest and dries out. As Singh and Kaur (2009) emphasize, litchi cultivation is highly dependent on specific climatic conditions, influencing key litchi-producing nations around the world.

India ranks second in litchi production, while the first place is bagged by China. Edible portion i.e. aril of the fruit is aromatic and has a good content of minerals and nutrients found to be effective against strokes and coronary heart diseases and provides several health benefits, and thus, can be used as a functional ingredient in food and pharmaceutical applications (Li et al., 2012). Insect pests and post-harvest losses stand out as key restrictions in litchi cultivation among the myriad factors influencing production and productivity. Fungal diseases occurring on litchi

are the most destructive pre-harvest infection and reasons for post-harvest losses. A fungus, *Peronophythora litchi* is widely found to contaminate leaves, panicles and fruit of litchi causing *Peronophythora* fruit rot or brown blight or downy blight disease of litchi which reduces its production and shelf life (Ann et al., 2011). The Infected part has white mildew which turns reddish-brown as the spores mature. *Peronophythora litchi* is a transitional species between *Peronospora* and *Phytophthora* of the family Peronosporaceae and Pythiaceae respectively (Ann and Ko, 1980; Ho et al., 1984; Zhang et al., 2007). It bears branched, determinate, differentiated sporangiophores that resemble the sporangiophores of the member of *Peronospora* and its mode of releasing sporangia from zoospore, the morphology of sex organs and ability to grow on artificial media resembles the characteristics of *Phytophthora* (Ann and Ko, 1980; Ho et al., 1984).This study focused on the microscopic identification and characterization of the causal agent of downy blight (*Peronophythora litchi*) in litchi fruits from five different areas of Dehradun, Uttarakhand.

MATERIALS AND METHODS

SAMPLE COLLECTION AND ISOLATION

Disease-affected litchi fruit materials (Fig. 1) were collected from five different places in Dehradun (Nanda Ki Chawki, Premnagar, Forest Research Institute, Rajpur Road and Clement Town).

The impacted fruit pericarp segments were first collected and placed in Petri plates with potato dextrose agar (PDA) and test tubes containing potato dextrose broth. These were then incubated for five days at a temperature of 28°C. The remaining samples were rigorously cleansed, dried in low light, then finely pulverized into a powder with liquid nitrogen and a mortar and pestle. The resulting powder, known as the plant extract, was scrupulously preserved individually at 20°C.

The powder obtained from each sample was also separately spread on petri plates containing PDA and on test tubes containing potato dextrose broth and incubated at 28°C for five days. Conidia of the fungus, *Peronophythora litchi,* was isolated from 4-5 days old culture grown on PDA media at 28 °C in the dark and was examined under the microscope after staining with Cotton Blue dye.

SCREENING OF ANTIFUNGAL ACTIVITY

The control of pNPP on spore germination of *Peronophythora litchi* was screened on PDA by petri plate assay. The antifungal potential of five different doses of pNPP (0.5, 1.0, 1.5, 2.0, and 2.5 mg/ml) was tested using the Agar Well Diffusion method. A sterile cork borer with a 6.0 mm diameter was used. PDA plates were inoculated with fungal strains that had been cultivated on PDA for 48 hours. A quantity of the inoculum (0.02 ml) was mixed with molten PDA and put into a petri dish using the pour plate technique. Following solidification, wells were created on the agar plate for the diffusion process using a cork borer. 0.05 ml of pNPP was injected into each of these wells. The plates were then incubated at 28°C for 24 to 48 hours to assess the extract's antifungal effectiveness.The zones of inhibition, which manifested as areas devoid of fungal growth around the extracts, were measured to assess antifungal

activity. The entire antifungal analysis was carried out under aseptic circumstances. The inhibition zones were measured in millimeters using an antibiotic zone scale, and the experiment was repeated three times to ensure accuracy.

RESULTS AND DISCUSSION

Peronophythora litchi, the causative agent of litchi downy blight, serves as an intermediate species between *Phytophthora* and *Peronospora* (Chen, 1961; Zhang et al., 2007). The fungus has branched, determinate sporangiophores similar to those of *Peronospora*. *Phytophthora* is distinguished by how zoospores are discharged from sporangia, the form of sexual organs, and the fungus's ability to live on artificial growth mediums (Ann and Ko, 1980; Ho et al., 1984).

Fungus showed colonization on Petri plates over 5 days (Figure 1). This ability to grow on artificial media resembles that of *Phytophthora.*Germinating oospores of *Peronophythora litchi* stained with cotton blue dye examined under the microscope resembled that of *Peronospora* or *Phytophthora.* The germ tube with many sporangia (Fig. 2) on the tip emerged from the antheridium, while that with many sporangia emerged. All sporangium were connected with the tube. Thus, the study confirmed the presence of the fungus *Peronophythora litchi* on litchi fruit causing downy blight disease of litchi inDehradun which limits the production of litchi and its post harvest shelf-life.

ANTIFUNGAL ACTIVITY PNPP AGAINST *PERONOPHYTHORA LITCHI*

In the present study, we have chosen p-nitrophenol as inhibiting substrate for In-Vitro control of the *Peronophythoralitchii*. All five different concentrations (0.5mg/ml, 1.0mg/ml, 1.5mg/ml, 2.0mg/ml, and 2.5mg/ml) of p-nitrophenol were active against *Peronophythoralitchii*(Fig. 3). Maximum zone of inhibition was shown by LC1 (16.4 mm in diameter), while minimum inhibition was displayed by LC 5 (Table 1).

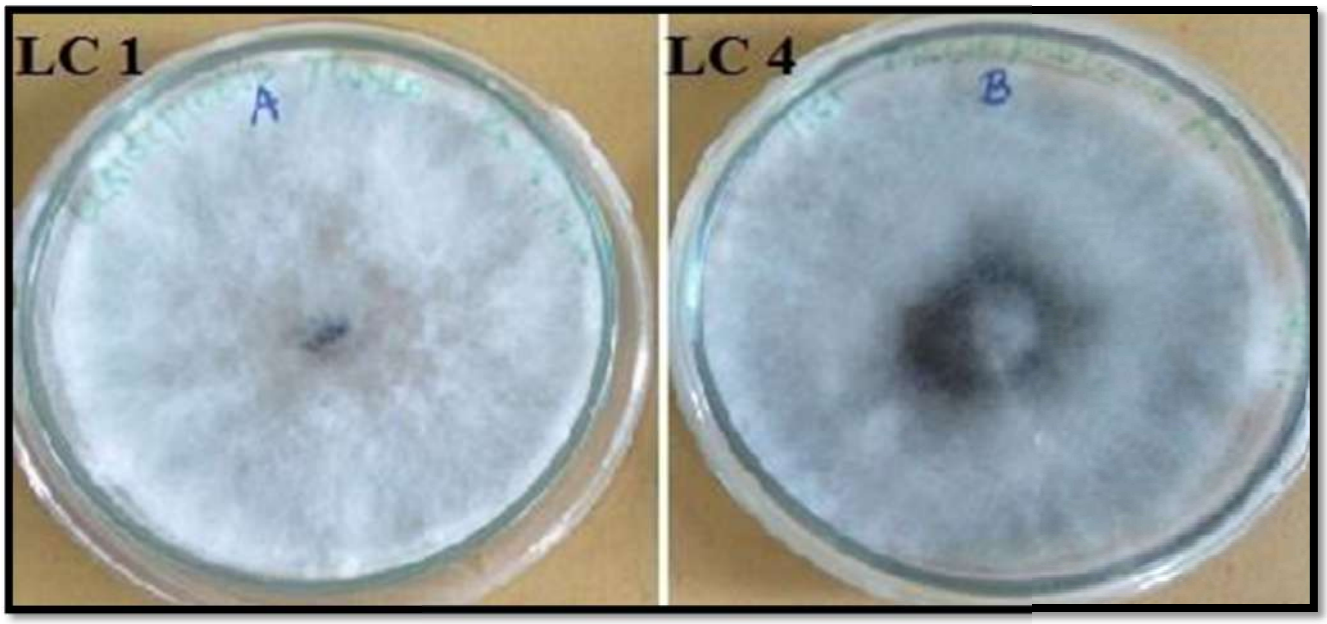
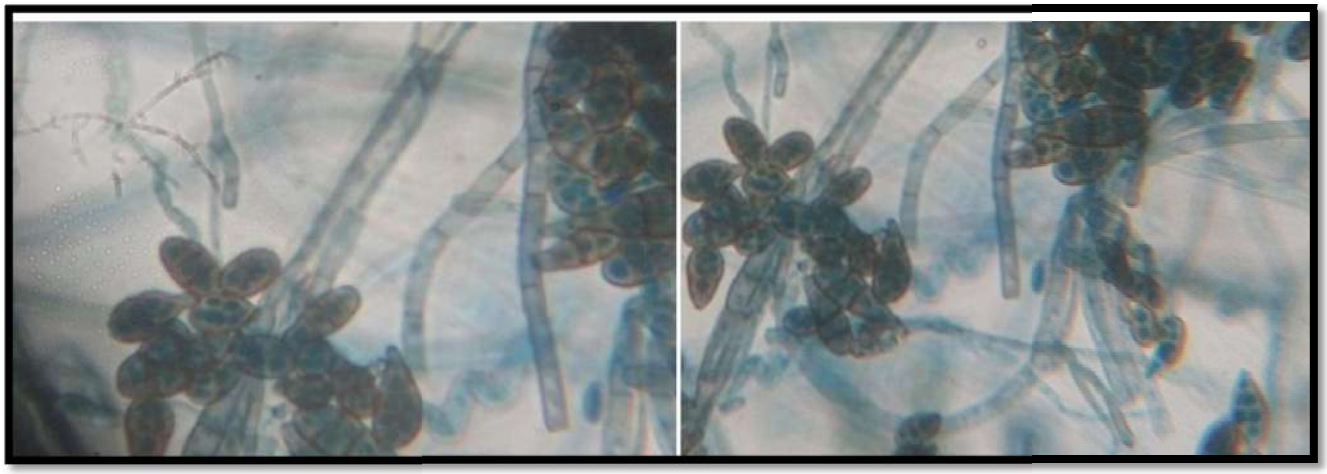


Figure 1: Colonization of isolates LC-1 and LC-2 on Potato Dextrose agar.



(A) (B)

Figure 2: Germinating oospore of *Peronophythora litchi* (A and B).

(A) LC1 **(B) LC2**

Figure 3: Antifungal activity pNPP against *Peronophythora litchi*

Table 1: Antifungal activity of pNPP against *Perenophythora litchi* (Zone of inhibition, mm in diameter).

pNPP mg/ml	Fungal isolates				
	LC1	**LC2**	**LC3**	**LC4**	**LC5**
5mg/ml	10.1	10.4	6.2	6.0	Nil
10mg/ml	12.0	10.5	5.1	9.0	Nil
15mg/ml	10.9	12.1	5.3	9.1	1.4
20mg/ml	15.9	12.2	6.5	10.1	1.1
25 mg/ml	16.4	13.1	10.0	11.0	10.4

Downy blight, caused by the activity of Peronophythora litchi, is a serious disease that affects litchi plants. This pathogenic agent attacks the fruits, panicles, and new shoots, causing panicle deterioration, withering, and the appearance of watery brown blotches on the fruits. These imperfections then produce downy, white sporangiophores. Furthermore, this disease causes significant losses during the post-harvest phase.The present experiment showed that p-nitrophenol phosphate exhibits antifungal effects against *Pereonphythora litchi.* So, it could be used as an antifungal drug (fungicides) to control the Downy blight caused by *Pereonphythora litchi.*

Further, the study can be continued to investigate the effect of direct application of p-nitrophenol phosphate on litchi tree during the infestation of Downy blight to reduce the occurrence of the same. It may enhance the yield, quality and shelf-life of the litchi.

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