PAECILOMYCES LILACINUS ON EGG HATCHING AND LARVAL POPULATION OF *MELOIDOGYNE* SP.

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ABSTRACT

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Bio-control agent *Paecilomyces lilacinus* was tested against *Meloidogyne* sp. causing root knot disease in vegetables. The experiment was conducted in Microbiology & Bio-control Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh during the period from July, 2016 to October, 2017 to study *in-vitro* the effect of *P. lilacinus* as a bio-agent against *Meloidogyne* spp. Experiments were set up with four treatments viz., 1. Hatching of single egg mass in sterilized distilled water, 2. Hatching of 10 egg masses in sterilized distilled water, 3. Hatching of single egg mass in sterilized distilled water along with *P.lilacinus* and 4. Hatching of 10 egg masses in sterilized distilled water along with *P. lilacinus.* Hatching of egg masses in sterilized distilled water produced huge number of live and active of nematode. Hatching of eggs from egg masses in all samples from different areas greatly inhibited by the inoculation of *Paecilomyces lilacinus*. Eggs of *Meloidogyne* sp. were unable to hatch in the presence of *P. lilacinus*. Application of *P. lilacinus* decreased the nematode population over control which were less active, and some of them were dead. Moreover, some hatched larvae were directly attacked by mycelia of *P. lilacinus*, and larvae became inactive and died. Highest percent inhibition of *Meloidogyne* sp. population by *P. lilacinus* over control was 91.66%.

Key words: *Paecilomyces lilacinus*, *Meloidogyne* sp. Root knot, egg hatching, larval population.

INTRODUCTION

Root knot nematode, *Meloidogyne* spp. causes severe losse of vegetable crops all over the world. Among more than 80 species of the genus *Meloidogyne*, four important species viz., *M. incognita, M. Javanica, M. arenaria and M. hapla* were responsible for at least 90% of damages as root parasites (Castagnone-Sereno 2002). Solanaceous vegetables were highly susceptible to infestation by root-knot nematodes of the species *M. incognita* and *M. javanica* (Khan *et al.* 2006).

Number of good nematicides are available to control the problems of the root-knot nematodes associated with vegetables. But for killing the pests, nematicides exerts adverse effects on human beings, livestock and other living things, which come in contact directly or indirectly (Singh *et al.* 2018). In this condition, there is a need to apply bio-pesticides that are pest specific, nontoxic to human, less expensive, and safe for the environment (Suman and Dikshit 2010). Researchers all over the world are engaged in standardizing nematode management strategies by following non-chemical and eco-friendly approaches such as use of biological control agents to stabilize crop production (Sumathi *et al.* 2006). Several attempts have been made to use antagonistic fungi to control root-knot nematodes.

Among the various bio-control agents, the soil-inhabiting fungus *Paecilomyces lilacinus* is a unique strain with a wide range of activity against the most important plant parasitic nematodes. Tests on potted plants and field plots have shown the fungus to control a wide range of nematode species including the root-knot nematode, *Meloidogyne* spp. on a number of crops. Its effectiveness was comparable to several chemical nematicides tested (Jatala 1986). *P. lilacinus* is one of the most widely tested biological control agents used for management of plant-parasitic nematodes (Vasanthi & Kumaraswamy 1999). Siddiqui (2000) reported that *P. lilacinus* significantly reduced *Meloidogyne* spp. infection on vegetables. Several efforts for managing root knot nematode using chemicals are not satisfactory to control; cost of chemicals and residue problems has made the nematode management strategy unattractive for the growers and extension specialists. Thus, the present investigation was designed to evaluate the biological potential of *P. lilacinus* on egg hatching and larval population of *Meloidogyne* sp.

MATERIALS AND METHODS

The laboratory work was carried out in Microbiology & Bio-control Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. The experiment was conducted during July, 2016 to October 2017. The experiment was done following Completely Randomized Design (CRD).
P. lilacinus was collected from Microbiology & Bio-control Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. Previously, *P. lilacinus* was isolated from experimental formulation supplied by Haychem (Bangladesh) Limited on Potato Dextrose Agar (PDA) media. The isolated fungus was examined microscopically on the basis of their morphological traits and cultural characteristics of the fungi such as mycelium growth, colony texture, spores production and other characteristics (Abubakar *et al*. 2005; Ahmad and Jairajpuri 1993).

Collection and identification of egg masses of *Meloidogyne* sp.
Galled roots of tomato plants were collected by observing above ground symptoms from Bangladesh Agricultural University Farm and nearby areas. Five samples were selected by taking one sample from each area of collection. These were sample no. 1 (Noyarchor), sample no. 2 (Boyra), sample no. 3 (BAU farm), sample no. 4 (Bhabokhali), and sample no. 5 (Sutiakhali). Selected plants were uprooted by digging with spade and sickle. Caution was taken to get intact root system without leaving any root parts or gall produced by nematodes. Nematode eggs were separated from knotted tomato roots in the laboratory. Only well developed light brown and live egg masses were selected. Nematode identification was performed by using photographic microscope (Carl Zeiss Microscope GmbH, Model Stemi 508) at Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. *Meloidogyne* spp. was identified by observing their identifying characters such as vermiform (worm shaped), multi-cellular structure and stylet morphology (Eisenback and Hirschmann 1981).

Study of Interaction between *Paecilomyces lilacinus* and *Meloidogyne* spp.
The *In-vitro* experiment was conducted four different treatments viz. 1. Hatching of eggs from single egg mass in sterilized distilled water, 2. Hatching of eggs from 10 egg masses in sterilized distilled water, 3. Hatching of eggs from single egg mass in sterilized distilled water along with *Paecilomyces lilacinus*, 4. Hatching of eggs from 10 egg masses in sterilized distilled water along with *Paecilomyces lilacinus*. For

 e egg mass, small sized petridishes (4.5 cm diameter) were selected. For Hatching of 10 egg masses, petridishes of 9 cm diameter were taken. Egg masses collected from different places were placed in different petridishes and small amount of sterilized water was added to it so that the water kept the egg masses wet. Petridishes were incubated at 25 ºC ± 1 ºC. After 5 days, when the nematodes were hatched from the eggs, they were counted under stereomicroscope with the help of a counter.

RESULTS AND DISCUSSION

Paecilomyces lilacinus formeda dense mycelium which gives rise to conidiophores. These bear phialides from the ends of which spores were formed in long chains. Conidia were in divergent chains, globose to sub-globosein shape, and smooth walled to slightly roughened (Fig. 1). Egg-masses of *Meloidogyne* spp. were separated from the knotted roots and nematodes was characterized by their vermiform structures under the field of compound microscope (Fig. 2). Efficacy of *P. lilacinus* on hatching of egg was performed on single egg mass and ten egg masses, separately (Fig. 3). In case of single egg mass, hatching of *P. lilacinus* in sterilized distilled water, all hatched larvae of nematode were found live and active. Highest number of nematodes (116) was hatched from the single egg mass on sample no. 4 collected from Bhabokhali and second highest nematodes (90) were obtained from sample no. 2 collected from Boyra. Egg mass collected from sample no. 1 (Noyarchor), sample no. 3 (BAU farm) and sample no. 5 (Sutiakhali) yielded 76, 57, 54 larvae of *Meloidogyne* sp., respectively. Hatching of egg from egg masses of all areas greatly inhibited by the inoculation of *Paecilomyces lilacinus*. In the *Paecilomyces lilacinus* inoculated petridishes, lowest number of nematodes (7) was hatched from the egg masses of sample no. 1 (Noyarchor) and second lowest nematodes 8) from egg masses were obtained from sample no. 5 (Sutiakhali). In the presence of *Paecilomyces lilacinus*, single egg mass collected from sample no. 2 (Boyra), sample no. 3 (BAU farm) and sample no. 4 (Bhabokhali), hatched 9, 11, 10 nematodes, respectively. It can be mentioned that nematodes larvae hatched from egg mass with *Paecilomyces lilacinus* were less active and some of these were dead.

In case of experiment with 10 egg masses, similar types of results were observed. All hatched larvae from egg masses were found live and active. Highest number of larvae (1248) were hatched from the egg masses collected from sample no. 2 (Boyra) and second highest nematodes (1244) were hatched from

egg masses obtained from sample no. 4 (Bhabokhali). Hatching of egg masses of all areas greatly inhibited by the inoculation of *Paecilomyces lilacinus*. In the *Paecilomyces lilacinus* inoculated petridishes, lowest number of nematode (242) were hatched from the egg masses collected from sample no. 1 (Noyarchor). Number of larvae from sample no. 2 (Boyra), sample no. 3 (BAU farm) and sample no. 4 (Bhabokhali), sample no. 5 (Sutiakhali) were 278, 255, 261 and 283, respectively in the presence of *Paecilomyces lilacinus* with sterilized distilled water.

Inhibition of egg hatching of *Meloidogyne* sp. by *P. lilacinus* was observed in the laboratory (Table. 1). Highest percent inhibition of *Meloidogyne* spp. population by *P. lilacinus* over control was 91.66% in an area of egg masses collection. Application of *P. lilacinus* decreased the nematode population over control. Few larvae were hatched from egg masses of *Meloidogyne* sp. in the presence of *P. lilacinus* and these larvae were less active, and some of them were dead (Fig. 4). This finding is in agreement with Holland (2000) who stated that *P. lilacinus* reduced the number of viable eggs and juveniles of the second generation during the experimental period. Some juveniles of infected eggs showed various degrees of deformity and abnormal development and a number of juveniles that emerged from eggs were infected and showed mycelia growth over their body (Ganaie and Khan 2010 and Jatala 1986, Esphahani and Anaspour, 2006).

In the experiment, it was found that hatching of eggs of all areas greatly inhibited by the inoculation of *P. lilacinus*. Eggs of *Meloidogyne* sp. were unable to hatch in the presence of *P. lilacinus* (Fig. 4A&B). Some hatched larvae were directly attacked by mycelia of *P. lilacinus*, and larvae become inactive and finally died (Fig. 4C). The action of *P. lilacinus* against *Meloidogyne* sp. was interpreted in multitude investigations. Khan, *et al*. (2006) recorded the penetration of fungal hypha to the female cuticle of *Meloidogyne javanica* by transmission electron microscopy. Park *et al*. (2004) reported that *P. lilacinus* could produce leucino toxin and other nematicidal compounds. In the laboratory test, *P. lilacinus* infected the eggs of *Meloidogyne* spp. and destroys the embryos within 5 days because of simple penetration of the egg cuticle by individual hypha aided by mechanical and enzymatic activities (Jatala 1986). It was mentioned that *P. lilacinus* caused substantial deformation of eggs in *Meloidogyne* sp. which never matured or hatched (Jatala 1985). The serine protease produced by *P. lilacinus* might play a role in penetration of the fungus through the egg shell of the nematode (Bonants *et al*. 1995).

Although the isolates of *P. lilacinus* show some potentiality as a bio-control agent, the study must be continued to increase its efficacy. It is also important to know the fate of the bio-control agent after it has been applied to the soil (Esphahani and Anaspour 2006). Estimation of the persistence of *P. lilacinus* after application to the soil indicated that the levels fall after application and it disappears from the soil after a few months of inoculation. This suggests that *P. lilacinus* will only cause short-term disturbances to the soil biota and will not have any long-term effect as other bio-control agents do (Kerry & de Leij 1992, Lackey *et al.* 1994). Therefore, the fungus is a potential bio-control agent attacking the infective units of the root-knot nematodes (eggs and juveniles), checking the initiation of the disease and reducing the inoculum potential for successive crops. This effect of *P. lilacinus* is its most significant attribute.

P. lilacinus is not expected to cause any harm to plant roots. But, when egg masses and juveniles of *Meloidogyne* spp. were present, it attacked and destroyed them to a great extent and improved plant growth. It is clear that, eggs of *Meloidogyne* spp. unable to hatch into larvae in the presence of *P. lilacinus*. The entire contents of the egg are then used as a food resource by the fungus and completely destroy the embryo or larva (Khan *et al.* 1997). Therefore, it is expected that the presence of *P. lilacinus* before the nematode attack would offer greater protection to plants.

Table 1. Percent Inhibition of *Meloidogyne* sp. population by *Paecilomyces lilacinus* over control collected from different areas of Mymensingh

Sample No.	% Inhibition of nematode population by *Paecilomyces lilacinus* over control	
	Single egg mass	Ten egg masses
1	90.78	70.53
2	90	78.12
3	80.35	71.68
4	91.66	78.14
5	85.45	68.6

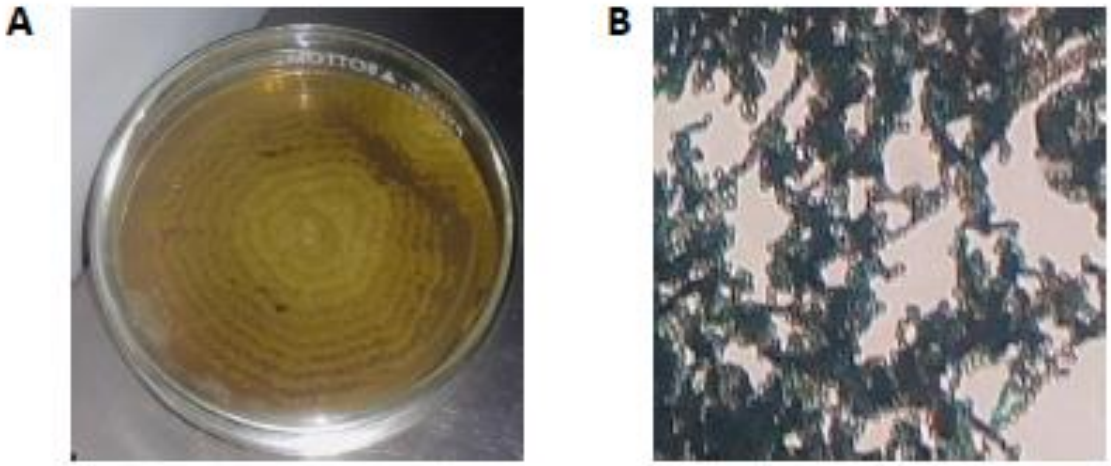


Fig. 1. Culturing *Paecilomyces lilacinus,* A. Pure culture of *P. lilacinus* and B. Microscopic view of *P. lilacinus.*

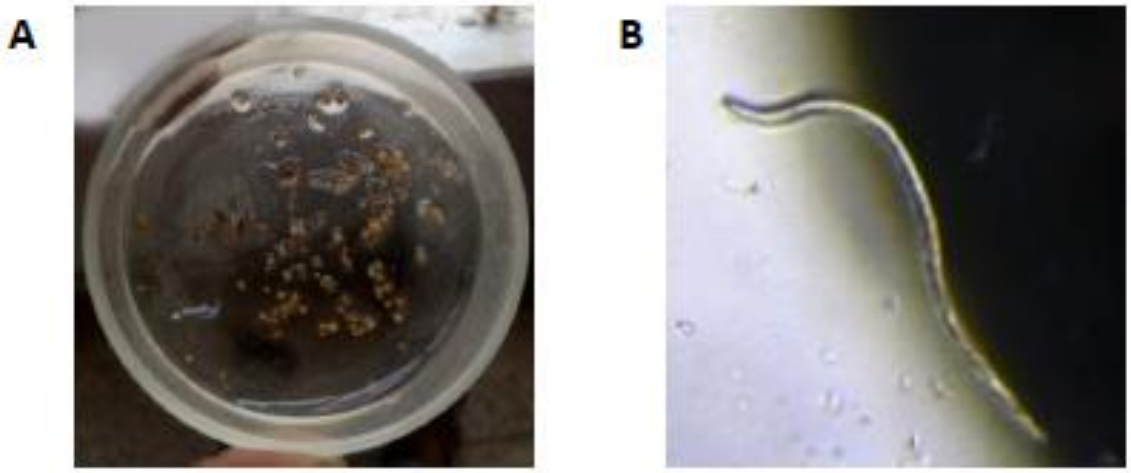
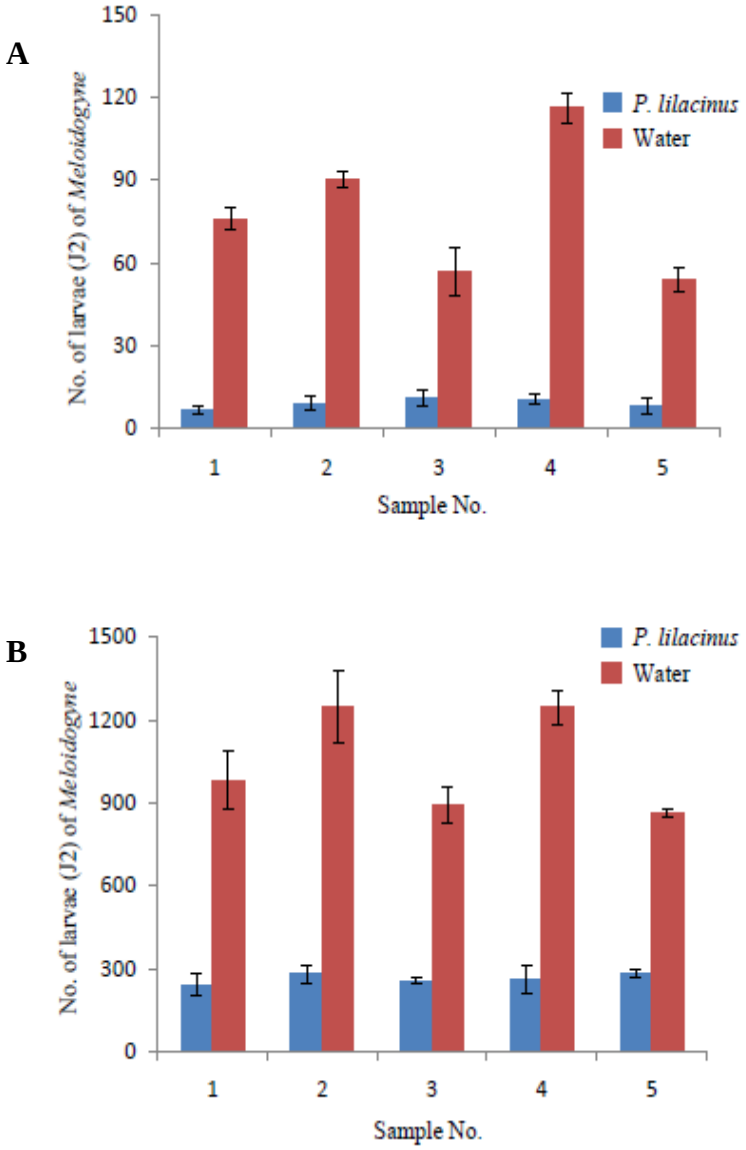


Fig. 2. Collection of *Meloidogyne* spp., A. Egg masses, B. Microscopic view of larva (J2) hatched from egg mass



A. Study was conducted on single egg mass and **B.** Study was conducted on 10 egg masses

Sample no. 1 (Noyarchor), sample no. 2 (Boyra), sample no. 3 (BAU farm) and sample no. 4 (Bhabokhali), and sample no. 5 (Sutiakhali).Each value represents the mean and standard deviation of 3 replicates.

Fig. 3. Effect of *Paecilomyces lilacinus* on egg hatching of *Meloidogyne* sp. in sterilized distilled water containing *Paecilomyces lilacinus* (blue bar) and in sterilized distilled water (red bar) collected from different areas of Mymensingh district.

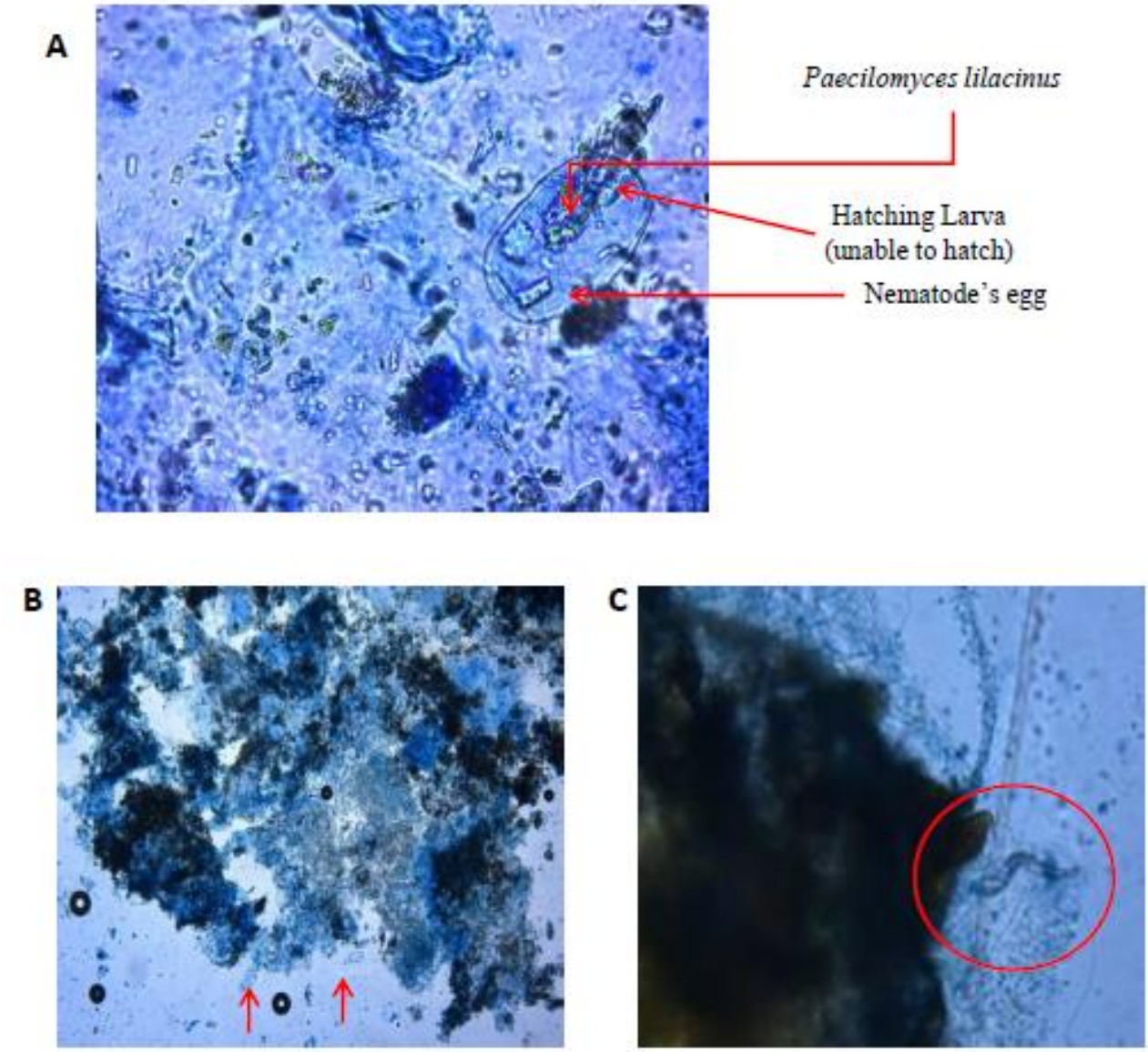


Fig. 4. Parasitizing the larva of *Meloidogyne* sp. by *P. lilacinus*. **A.** Larva of nematode was unable to hatch from egg due to the presence of *P. lilacinus,* **B.** Un-hatched eggs in a egg mass (red marked arrows) of *Meloidogyne* sp., **C.** Larva of hatched *Meloidogyne* sp. (within red cirle) is surrounded by mycelial mat of *P. lilacinus*

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