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SHORT COMMUNICATION

Ferret out a Natural Bio-Pesticide: *Ophicordyceps nutans* in Central India and Its Interaction Analysis with Tree Stink Bug

Jai Shankar Paul[1] · S. K. Jadhav[1,2] · Afaque Quraishi[1,2] · M. L. Naik[2]

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Abstract *Ophicordyceps* is a genus of fungi that grow on insects. In this paper we report for the first time the occurrence of *Ophicordyceps nutans* Pat. a species belonging to this entomopathogenic fungi group in Kanger Valley National Park in Bastar District of Chhattisgarh in Central India. The fruiting body or ascocarp of *O. nutans* was found in *Halyomorpha halys*, brown marmorated stink bug—an insect pest. The study highlighted the impact of *O. nutans* on the host insect and the damage it causes in trees and crops. The local people use this fungus in traditional medicine as an immune stimulator and also as a pest-control agent to protect crop and tree from stink bug. Therefore, it appears that *O. nutans* possesses great potential to be developed as natural medicine and bio-pesticide to save the agricultural crops and forest trees.

Keywords *Ophicordyceps nutans* · Entomoparasitic fungi · *Halyomorpha halys* · Bio-pesticide · Stink bug

Introduction

Ophicordyceps are well known parasitic fungi belonging to the ascomycetes group. *Ophicordyceps* sp. are the natural insecticides. They are entomoparasitic in nature. They grow up from different host insects and kill them (Hywel-Jones 1995; Sasaki et al. 2004; Friedrich et al. 2018; Luangsa-ard et al. 2018). *Ophicordyceps* sp. holds immense potential to be used as a biological pest control agent (Sasaki et al. 2008; Friedrich et al. 2018). There are several species of *Ophicordyceps* fungi (*Ophicordyceps sinensis*, *O. forquignonii, O. gracilis, O. militaris, O. coccinea*, etc.) having different medicinal properties (Sasaki et al. 2005). In China, *Ophicordyceps* sp. is traditionally used as medicine in a number of diseases (Hywel-Jones 1995). *Ophicordyceps nutans* Pat. is one of the fungal species, which is parasitic to stink bug insect, belongs to Order of Hemiptera (Karun and Sridhar, 2013). *O. nutans* are host-specific fungi that parasitize stink bug tree sap succulent insect *Halyomorpha halys* which damages several forest trees and agricultural crops. The fungi infect the insect and complete their life cycle in them. In the process, they extract precious nutrition from the insects that ultimately results in death of the insect. The fungal infection in *H. halys* is visible when the fruiting body is developed. In general, the infection spreads when the insects come in contact with each other. In addition, the fungal spores spread in the surrounding after death and degradation of the insect (Sasaki et al. 2008; Friedrich et al. 2018). *O. nutans* fungus has been reported from several Asian countries including Japan, China, New Guinea, Thailand, Taiwan, Nepal and Korea (Hywel-Jones 1995; Sasaki et al. 2004, 2012; Shrestha 2011; Luangsa-ard et al. 2018). In India, it is only found in the Western Ghat region (Karun and Sridhar 2013). However, there are very few studies on *O. nutans* with regards to its structure, behaviour, habitat mode of action, and interaction with host insect. Besides its entomopathogenic nature, the fungus is known to possess several medicinal and bio pesticide properties (Sridhar and Karun 2017; Wen et al. 2017) that warrants further investigation. Against this backdrop, we report for the first time the presence of *Ophicordyceps*

✉ S. K. Jadhav
jadhav9862@gmail.com

[1] School of Studies in Biotechnology, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India

[2] National Center for Natural Resources, Pt. Ravishankar Shukla University, Raipur, Chhattisgarh, India

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nutans Pat. in Kanger Valley National Park, Bastar District of Chhattisgarh in Central India.

Materials and Methods

Geographical Area Study and Sample Collection

As per previous records in India, *Ophicordyceps nutans* found only in the Western Ghat region (Karun and Sridhar 2013). In current investigation, *O. nutans* was found in the central region of India, at Kanger Valley National Park, Bastar District of Chhattisgarh State. The geographical location of *O. nutans* was found in 18.90′ 74.54″ N, 81.89′ 85.43″ E (Fig. 1) (Map courtesy google map).

Preservation and Microscopic Analysis

Collected fungi with its host were preserved in two ways, some samples were preserved in formalin solution and some of the samples were firstly dried in an oven at 60 °C, and then preserved in an airtight jar as a herbarium. Microscopic characters of fungal asci and ascospores were studied by preparing the lactophenol cotton blue slide mounts. The slide was observed under the microscope, and fungal microscopic characters and micrometery was studied (Sridhar and Karun 2017). Different morphological characteristics of fungi and bug were also studied like; size, length, width, colour, appearance, etc. and the mean values of data is mentioned.

Results and Discussion

Fungal Morphology

Ophicordyceps nutans was found on its host insect *Halyomorpha halys*. The fruit bodies of the fungi were generated between the thorax and the head region of the host. In some host insects, more than one fruit bodies of the fungi were found. Generally, the fungus infects the insect when it was alive and then fungal mycelium development takes place inside the host. When the fruiting bodies of the fungus are generated from the insect's thorax and abdomen, the host insect dies.

The stipe (stalk) of the fungus gets attached just behind the head and before the thorax region of *H. halys*. *O. nutans* is a host specific fungi, they come into their perfect stage only on their host (Karun and Sridhar 2013).The stipes were blackish or brown in colour and wiry like structure bent in apical portion, where the head was present (Fig. 2). Longest stipe was extended up to 125 mm (n = 15) in size and rest of the stipe was between 30 and 60 mm (n = 15) in size. At the apical portion of the stipe, a pod-like head was present. Size of the head was 8–13 mm long and 3–4 mm in diameter (n = 15) (Fig. 2). Pod shape head was reddish

Fig. 1 Geographical map of the study area

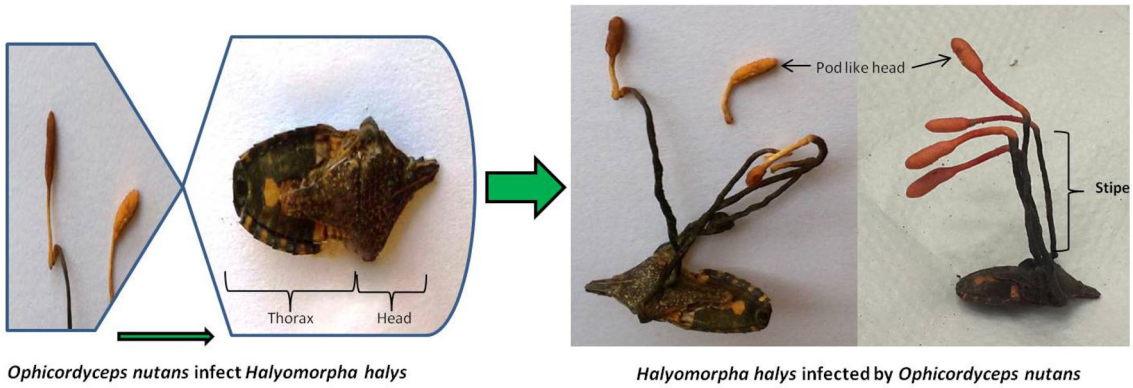

Fig. 2 *Ophiordyceps nutans* Pat. in host bug *Halyomorpha halys*

or saffron in colour and became yellowish on maturation. While studying the morphological characteristics, geographical linkage and the genetic variability of *O. nutans*, Friedrich et al. (2018) found a new strain of it. Several cylindrical compactly arranged 0.1 mm long filamentous asci were present inside an ascocarp or fruiting body. Each ascus having septate 2–5 µm oval shaped spore in the tip (Fig. 3). Each ascospore can infect the host and is capable of developing into a new fruiting body. Consistent with the present observations, similar morphological characteristics of *O. nutans* were observed by Karun and Sridhar (2013) and Sridhar and Karun (2017) in the Western Ghats region of Southern India.

Host Insect

The fruit bodies of *Ophicordyceps nutans* were found on the dead bodies of the host insect *Halyomorpha halys*. The insect is brown or black in colour. *H. halys* in order of Heteroptera is commonly known as stink bug. The average length of mature stink bug of this region (Kanger Valley National Park, Bastar District of Chhattisgarh) was between 18 and 26 mm and breadth of 12–16 mm (n = 30) (Fig. 2). The *H. halys* stink bug is a major insect pest that causes severe damage to several agricultural crops and forest trees (Sasaki et al. 2012; Karun and Sridhar 2013). We found it on and under the Sal tree (*Shorea robusta*). It damages the flower and fruits of the host tree in addition to many agricultural crops like soybeans, apple, green beans, pears, etc. (Sridhar and Karun 2017; McPherson 2017).

Habitat

In the present study the fungi were found in the forest of Kanger Valley National Park that contains fixed tropical dry deciduous forest. Fungi with its fruit body on its host insect were found after summer in August between the rainy seasons in a high humid climate. The annual rainfall of this region is 1300–1450 mm which is of tropical monsoon type. The annual average temperature of this region is a maximum of 35 °C and minimum of 23 °C. The lowest temperature of this region in the month of December–January goes down to 13 °C, and the highest temperature goes up to 40 °C in the month of May. Since the area is mostly forest-covered the humidity is generally high throughout the year. Average maximum and minimum relative humidity of this region is 89.3% and 49.7%, respectively. Cool and humid climate with high rainfall of

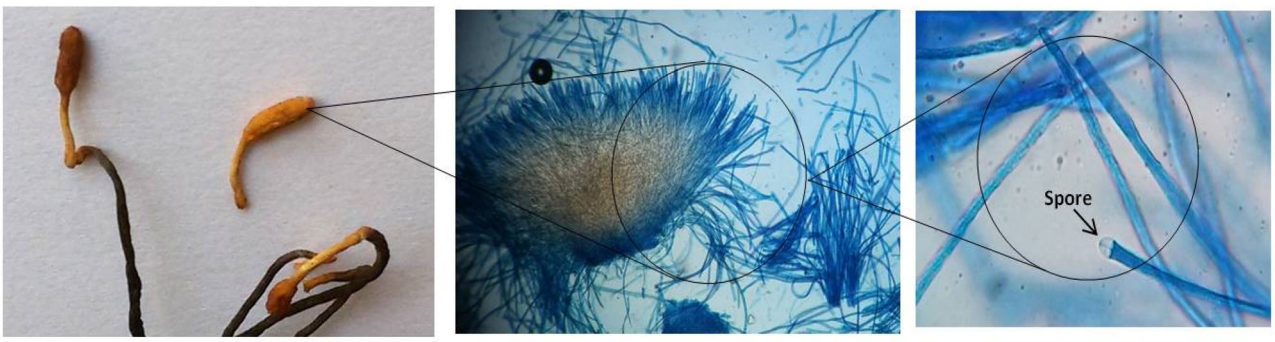

Fig. 3 Morphological characteristics (ascocarp and asci) of *Ophicordyceps nutans*

this region is suitable for this fungi and supports its growth (Karun and Sridhar 2013; Sridhar and Karun 2017; Friedrich et al. 2018).

Uses of *Ophicordyceps*

Ophicordyceps nutans is used as traditional medicine by the local people as an immune stimulator (Sridhar and Karun 2017). The fungi are also used as a biological control agent to protect the crop and trees against stink bug and several other insects (Sridhar and Karun 2017; Karun et al. 2017). However, the fungus is slow in killing the host which is a major disadvantage to develop it as a bio-pesticide. But the importance of the fungus lies is its medicinal properties. It contains cordycepin (3′-deoxyadenosine) which is a biologically active metabolite with potential use in the treatment of cancer as it induces apoptosis (Tuli et al. 2014; Sridhar and Karun 2017; Wen et al. 2017). Other fungal metabolites have anti-inflammatory and antioxidant activities for potential use in renal, hepatic and cardiovascular ailments. *O. nutans* also contains vitamin C and phenolic compound along with DPPH radical scavenging activity (Tuli et al. 2014; Karun et al. 2017). Other species of *Ophicordyceps* also contain varieties of bioactive metabolites including epicoccin, cordytropolone, cordypyridones and cordyanhydrides (Isaka et al. 2005; Lo et al. 2013; Tuli et al. 2014; Sridhar and Karun 2017).

Conclusion

The fungi of the genus *Ophicordyceps* have medicinal and entomopathogenic properties, which make them potential candidates for the production of natural medicine and biological pesticide. *Ophicordyceps nutans* efficiently kills the stink bugs which severely damage the forest trees and field crops. The entomopathogenic and medicinal properties of *O. nutans* can be exploited as a tool for future use as herbal medicine and biological pest control agent to save crops and trees naturally which, in turn, will reduce the harmful effect and use of chemical pesticide in the field.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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