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Research Note

Incidence of entomophagous medicinal fungus, *Ophiocordyceps nutans* on stink bug, *Halyomorpha halys* (Stal) in the Western Ghats of India

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ABSTRACT: An entomophagous medicinal fungus *Ophiocordyceps nutans* growing on the sap sucking stink bug (*Halyomorpha halys*) was recorded for the first time from the forests of Western Ghats of India. This ascomycete emerges from the dead stink bugs in soil or humus during the southwest monsoon (June-August). In association with the host trees species (*Cassine glauca*), the stink bugs infected by *Hymenostilbe nutans* (anamorph of *O. nutans*). On completion of life cycle, the dead stink bugs accumulate in the base of host trees during summer (April-May). On the onset of southwest monsoon, *H. nutans* in dead stink bugs emerges as perfect state (*O. nutans*).

KEY WORDS: *Cassine glauca*, entomopathogen, *Halyomorpha halys*, *Hymenostilbe nutans*, medicinal fungi, *Ophiocordyceps nutans*, stink bugs.

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Cordyceps are well known medicinally valuable ascomycetous fungi (also called sac-fungi) encompass over 400 described species distributed in six continents in wide climatic zones and range of hosts (plants and insects) (Stensrud *et al*., 2005; Sung *et al*., 2007). They are either endoparasitic on arthropods or parasitic on other fungi (Zhang *et al*., 2012). As the genus *Cordyceps* is not monophyletic, it has been assigned to the genera like *Cordyceps*, *Elaphocordyceps*, *Metacordyceps* and *Ophiocordyceps* (Sung *et al*., 2007). The Chinese national flagship fungus, *Cordyceps sinensis* is used traditionally in Chinese medicine from ancient days, which is in high demand and its production went up to 200 tons/ annum (Cai and Sun, 2010; Shrestha *et al*., 2010; Cannon, 2011). Due to rarity, the price of *Cordyceps* elevated up to $75,000/kg during 2008 in USA (Mizuno, 1999; Holliday *et al*., 2004; Holliday and Cleaver, 2008). *Cordyceps* serve as nutraceutical product as they possess essential amino acids, vitamins, polysaccharides, proteins, sterols and minerals (Holliday *et al*., 2004; Hobbs, 1995; Holliday *et al*., 2005; Nie *et al*., 2013). Most of the *Cordyceps* are known to possess wiry to pliant or pigmented fibrous stromata (dark or partially dark) parasitize wood-inhabiting hosts buried in soil or embedded in decaying wood (Sung *et al*., 2007).

Ophiocordyceps nutans was first described from Japan by Patouillard (1887) and subsequently known from countries like China, Korea, Nepal, New Guinea, Taiwan and Costa Rica (Shrestha, 1985; Sung *et al*., 1993; Shimizu, 1994; Hywel-Jones, 1995; Liu *et al*., 1997; Chaverri, 2006; Sasaki *et al*., 2012). The teleomorph-anamorph connection has been linked by Hywel-Jones (1995) by demonstrating *Hymenostilbe nutans* as an anamorph of *O. nutans*. Literature search revealed no clear records of *O. nutans* from the Western Ghats of India although occurrence of the genus *Cordyceps* is mentioned by Bhagwat *et al*. (2005). The present study deals with new location of distribution of *O. nutans* on the stink bugs (*Halyomorpha halys*) and its anamorph, *Hymenostilbe nutans* on the host plant species (*Cassine glauca*) in the Western Ghat region of India.

Occurrence

During routine survey of macrofungi of the Western Ghats of Karnataka (reserve forests, shola forests, sacred groves and coffee agroforestry), fruit bodies of *Ophiocordyceps nutans* was recovered in southwest monsoon (June–August, 2012) from the dead stink bugs (*Halyomorpha halys*) accumulated or buried in soil/humus in the basins of *Cassine glauca* trees. Trees basins of *C. glauca* of evergreen forest and coffee agroforests near Virajpet and Makutta mixed jungle (75.8° N, 12.2 E) possess *O. nutans*. Average number of fruit bodies of fungus was 3.7 m^2 (n=10; range, 0-8) in agroforests of Virajpet, while only one fruit body was seen under the young tree of *C. glauca* in Makutta forest. The average air and soil temperature during sampling period (June–August, 2012) was 22 and 24°C, respectively.

Plant host

Cassine glauca (Rottb.) O. Kuntze [Family, Celastraceae; Syn.: *Mangifera glauca* Rottb., *Elaeodendron glaucum* (Rottb.) Pers. and *Celastrus glaucus* Vahl.; Sanskrit name, Bhutapala] is an economically valuable evergreen tree species distributed in India, Sri Lanka, Myanmar, Indo-China, Thailand, Indonesia, Malay and Philippines (Pullaiah, 2006). The pepper creeper *C. glauca* in mixed plantation helps hiding of the stink bugs *H. halys*. Association of these insects with *C. glauca* leads to distortion of bark (Fig. 1d). The bugs exist in large number during summer and on death accumulate on the litter strata of floor (Fig. 1e). On the onset of southwest monsoon, with sufficient moisture in leaf litter/soil/ humus, *Ophiocordyceps nutans* emerges from the dead stink bugs. Bark, inflorescence and fruits of *C. glauca* were also colonized by *Hymenostilbe nutans*, which is an imperfect state of *O. nutans*.

Insect host

Ophiocordyceps nutans is a host-specific pathogenic fungus parasitizes the pentatomid stink bugs (Hemiptera; Pentatomidae), the major insect pests of agricultural crops and forest trees (Sasaki *et al*., 2012). The range of parasitism of *O. nutans* encompasses up to 22 species of stink bugs detrimental to agriculture and forestry, thus serving as a possible potential selective biocontrol agent (Sung *et al*., 1993; Ito and Hirano, 1997; Li *et al*., 2006; Sasaki *et al*., 2008; Cai and Sun, 2010). Eleven hosts of stink bugs belonging to three families (Acanthosomatidae, Coreidae

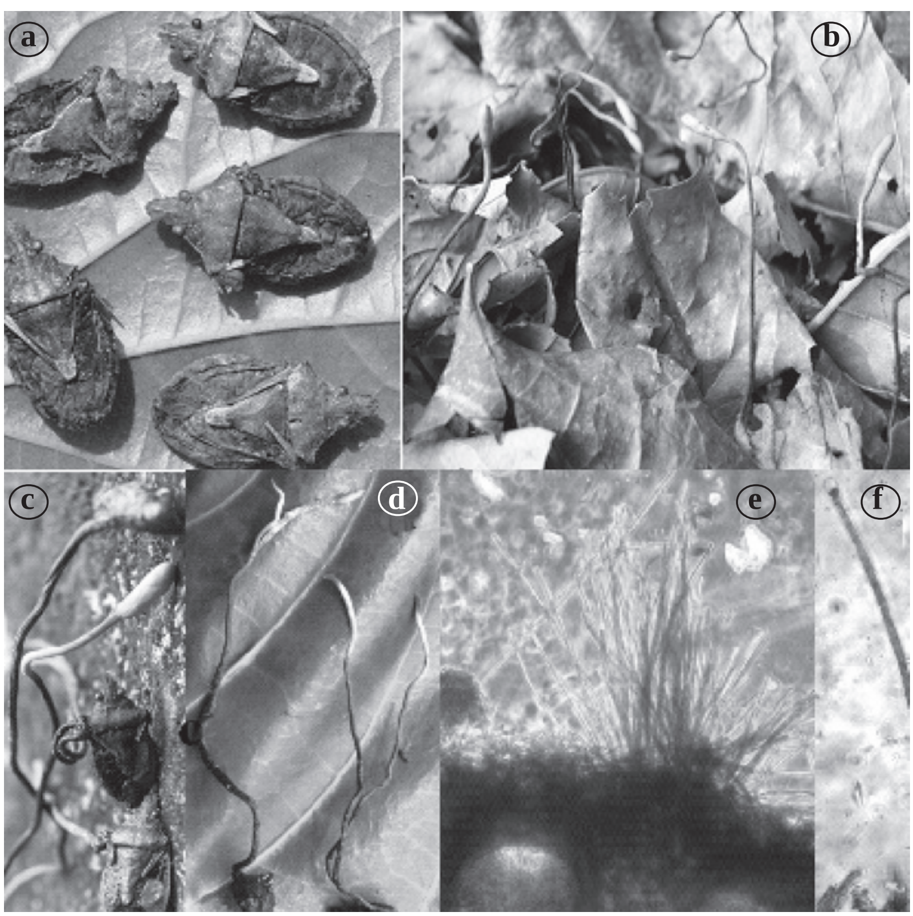

Fig. 1. ***(a) Halyomorpha halys*** **cadavers infected with** ***Ophiocordyceps nutans*****; (b) strong black wiry stipe (one or two) emerged out from thorax region; (c) stipe emerged out of abdomen (branched at tip) and (d) thorax (branched at base); (e) cross section of fruit body showing asci with mass of ascospores and (f) bulbous characteristic head portion of a typical lengthy ascus with ascospores.**

and Pentatomidae) have been identified by Sasaki *et al*. (2012). Based on the feeding behaviour, stink bugs are classified into two types: a) those suck juice mainly from grass and herbaceous plant stem (in grasslands); b) those live on trees and feed on fruits and cones (in forests) (Tomokuni *et al*., 1993). According to molecular studies, there are two types of *O. nutans* (Sasaki *et al*., 2012): a) type 1 has selective biocontrol of pests belonging to Coreidae; b) type 2 shows wide host specificity (hemipterans: Acanthosomatidae, Pentatomidae and Urostylidae). The fungus growing on the stink bugs in the present study belonging to type 2.

During intimate association of stink bugs *Halyomorpha halys* with host trees *Cassine glauca*, the adult bugs were infected by an imperfect fungus *Hymenostilbe nutans* and on death of insect it emerges as perfect state (*O. nutans*). Occurrence of *O. nutans* on nymps of *H. halys* is rare. Recently, association of *O. nutans* with *H. halys* was reported from Japan (Sasaki *et al*., 2012).

Fungi

From the fresh specimens collected from Virajpet agroforest, notes on stromatal details were taken followed by microscopic examinations. The identity of *Ophiocordyceps nutans* (Pat.) G. H. Sung, J. M. Sung, Hywel-Jones & Spatifora (Clavicipitaceae: Ascomycetes) was confirmed by monographs and descriptions (Hywel-Jones, 1995; Kobayasi, 1982; Sung *et al*., 2007). Specimens were fixed in formalin-acetic-alcohol and deposited in the herbarium (Department of Biosciences, Mangalore University, Mangalore, Karnataka: MUBSNC-KKRSMF-027).

The fruit bodies generally emerge from the thorax region (1 or 2 stipes) in 80% of bugs, sometimes one stipe emerges from thorax and another from the abdomen (occasionally from the abdomen alone). Stipe: black, strong, wiry and bend easily without breaking and coiled or wavy with orange to orange-yellow clavate head bearing ascocarp. Bulbous mature heads are common and needle-shaped bright red, yellow and orange immature tips were also seen. Occasionally two fertile heads branch out from single stipe or two separate fruit bodies emerge. Stroma: reddish-orange becoming yellowish-orange with age, clavate, finely punctate to warty, osteolate and measures 2.71 (1.7-3.8) ´ 0.29 (0.15-0.35) cm (n=10). Stipe: blackish-brown becoming blur-black with age, arising from thorax or abdomen and measures 10.2 (7.5-16.5) x 0.14 (0.1-0.17) cm (n=10). Perithecia: wholly embedded in stromal tissue, hyaline, sub-globose, ostioles seen on the surface and possess flesh pallid yellow. Asci: elongated, cylindrical, 8-spored, characteristically bulged, thick-walled at apex and measure 393.4 (362.94-428.69) x 4.73 (4.6-4.99) mm (n=10). Ascospores: hyaline, smooth, elongated-fusiform, parallel, multi-septate and finally separates, disintegrate into a large number of one-celled cylindrical or barrel-shaped spores with blunt ends and measure 11.46 (9.2-13.15) and 2.04 (1.97-2.63) mm (n=10).

Successful isolation of *O. nutans* was achieved by Sasaki *et al*. (2004) by surface sterilization of immature stipe and fungal structures from the abdominal tissues of insects as an alternative for isolation from the ascospores. Isolation rates from the abdominal tissues were high, possibly due to host-specific exclusive habitat of the fungus. Such isolation method was also successful for other *Cordyceps* spp. Imperfect state of *O. nutans*, the *Hymenostilbe nutans* Samson & H.C. Evans of *O. nutans* was obtained on damp incubation of pieces of bark, inflorescence and fruit integument of *Cassine glauca* on antibiotic-amended potato-dextrose agar medium along with *Aspergillus, Fusarium, Penicillium* and *Rhizopus*. Conidiogenous cells of *H. nutans* were pink (9.8-13.3 x 3.1-4.2 mm) (n=10) and conidia were light pink (3.9-6.7 x 2.5-3.5 mm) (n=10).

Cordyceps have a long history of medicinal use especially in traditional Chinese oriental medicine in treating respiratory, renal, liver and cardiovascular diseases (Holliday and Cleaver, 2008; Park *et al*., 2010; Nie *et al*., 2013). The entomopathogenicity of *Cordyceps* is disputed as parasitic or symbiontic as some studies revealed symbiotic than parasitic relationships (Holliday and Cleaver, 2008). It is likely *Cordyceps* attacks the host in its imperfect state (present in bark or leaf or inflorescence or fruit) without disease symptoms, subsequently on dead bugs on the floor during suitable environmental conditions in monsoon the perfect state of fungus emerges. This is the first report of the *Ophiodcordyceps* on pentatomid bugs. Further studies required to understand the infection of stink bugs by *O. nutans* and or *H. nutans* (e.g. degradation of cuticle and growth in hemocoel), duration required for its development and to produce fruit bodies. In spite of usefulness of *O. nutans* as host-specific biocontrol agent, being medicinally-valuable it is necessary to strike balance between control of hemipterans and accessibility of such specific hosts to the fungus for our benefits and future needs.

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