ABSTRACT

*Itajahya rosea* was found growing in association with *Leucaena leucocephala* plants at Savitribai Phule Pune University campus in India. The species identity was confirmed by phylogenetic analysis based on ITS and LSU regions of rDNA, wherein, our fungus was placed along with *I. rosea* in phylogentic tree. It represents first record of *I. rosea* from India. Frequent visitation by *Drosophila* species on *I. rosea* fruiting body particularly on gleba was observed. The *Drosophila* got attracted to the detached gleba under the laboratory conditions and even sometimes, they prefer to sit over the gleba as compare to their food banana. It suggested that *I. rosea* gleba or pseudostipe produces some compounds for attraction and feeding behavior of *Drosophila* species. Therefore, we characterized the volatile attractants produced by gleba and pseudostipe of *I. rosea* by GC-MS analysis. Nineteen compounds were identified from gleba while nine compounds were recovered from the pseudostipe. Out of them, blends of three abundant odor producing volatile compounds were reported namely, Hexadecane, Pentadecane and Nonadecane, which are responsible for attraction of *Drosophila* toward the gleba. Three fatty acids namely 9,12-octadecadienoic acid (Z,Z), hexadecanoic acid and benzolic acid ethyl ester produced are served as an appetitive signal through olfactory response of *Drosophila*, so the flies were feed on the gleba. Two pheromones’ compounds, heneicosane and (++)-(5S,9S)-5,9-dimethylpentadecane, were also reported in pseudostipe and gleba, respectively, which play a role in *Drosophila* for breeding. Our study highlights an intriguing chemical ecology of fungus–*Drosophila* interaction.

1. Introduction

Phalloids (commonly known as “stinkhorns”) are currently classified in family Phallaceae, order Phallales E. Fisch., subclass Phallomycetidae [1]. First, *Itajahya* was reported by Moller in 1895 from Brazil. Later on, one more report of the phallolid from Bolivia [2]. Spegazzini listed a fungus from Argentina under the genus name *Alboffiella* [3], for which Fries and others were certain that it was synonymous to *Itajahya*. Therefore, *Itajahya* now is treated as a subgenus *Phallus* which include four species, with *I. galericulata* as type species [4,5]. These fungi exhibit very rare occurrence, thus, making the genus the least known members of the family *Phallaceae*. The main feature that distinguishes *Itajahya* from other taxa of this family is the presence of a structure termed the “calyptra” located at the apex of the gleba [6–8]. Based on the morphological characters like flat calyptra at apex, *Phallus roseus* was originally described from *Egypt* [9]. This is considered the taxonomic placement of *Ph. roseus*, a species assigned to the genera *Itajahya* or *Phallus* [4,5]. DNA sequencing and phylogenetic analyses of *Ph. rosea* and demonstrated that it does not cluster with other species of the genus *Phallus*, so it was, therefore, separated from *Phallus* and accepted as a member of the genus *Itajahya* [10]. *Itajahya rosea* is a rare species found not only in semidesertic habitats, known from Egypt, Morocco, Ghana, Israel, Pakistan, and southern France, but it also occurs in South America, from rich soils in Brazil and South Africa (Pretoria). There has not been any valid publication describing the occurrence and phylogenetic placement of *I. rosea* from India.

The stinkhorn fungi produce gelatinous, variously colored gleba with unpleasant smell similar to that of a rotten meat, which attract insects and play a role in basidiospore dispersal through these insects [11,12]. Seven different species of *Drosophila* were proved to breed in stinkhorn particularly reported on *Ph. impudicus* [13]. It has been an interesting aspect to find out what chemicals are produced by these fungi to attract the insects. Total 59
compounds were reported from *Ph. impudicus* at different developmental stages of basidiomata [14]. One drosophilid species reported to feed the spores of the *Ganoderma applanatum* have found to maintain germination capacity that have passed through the intestines, which suggests that this drosophilid acts as a spore dispersal agent [15]. Frequent visitation by *Drosophila* species on *I. rosea* fruiting body particularly on gleba was observed, and further the fungus also produces an unpleasant odor. However, chemical nature of these volatile attractants of *I. rosea* remains uncharacterized.

Therefore, the present paper deals with the correct identification, and phylogenetic placement of *I. rosea* from India and to investigate the identification of odorant compounds produced by both gleba and pseudosporocarp, and their function in *Drosophila* attraction.

2. **Materials and methods**

2.1. **Specimen collection and macroscopic analysis**

The eggs and basidiome of *I. rosea* were collected from Savitribai Phule Pune University Campus, India near root system of *Leucaena leucocephala*. The morphology of fresh basidiomata size, shape and colors was recorded. Microscopic characteristics of basidiomata were determined by vertical axis of the egg and basidiomata. Average spore size was done by measuring length and breadth of 25 spores.

2.2. **Molecular identification and phylogeny**

**DNA extraction:** The DNA was extracted from Basidioma with some modification [16]. The amplification of complete ITS and partial LSU regions was performed using primer ITS1/ITS4 and LROR/LR7 [17,18]. The PCR reaction mixture consisted of DNA template 2 μl (10–20 ng), 5 μl Taq DNA polymerase buffer (10×) (Sigma-Aldrich, Mumbai, India), dNTP 1 μl (200 μM) (Sigma-Aldrich, Mumbai, India), 1 μl of 10 pmol primers and the final volume made up by MiliQ H2O (Sterile Ultra-Pure Water, Sigma-Aldrich, Mumbai, India). Amplification was done by using Mastercycler (Eppendorf, Hamburg, Germany) as follows: first step was 95°C for 5 min, 1 min at 95°C with repetition of 30 cycles, annealing temperature was for 30 s at 55°C (for ITS rDNA) and 30 s at 52°C (for D1D2 region of LSU rDNA), extension was for 1 min at 72°C (for ITS) and 2 min at 72°C (for LSU), and a final extension step having 7 min at 72°C. The PCR products were purified with FavorPrep PCR Purification Kit (Favorgen Biotech Corp., Macomb, MI) and sequenced by using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Bedford, MA). The generated sequences were then submitted in the NCBI GenBank. The DNA sequences were subjected to BLASTn search and identified according to the closely related isolates that were available in the database (www.ncbi.nlm.nih.gov/geo). The 23 and 26 sequences of ITS and LSU, respectively, representing different allied species were aligned by using MUSCLE (see Table 1). The phylogenetic tree was constructed based on the neighbor-joining method with *Trappea darkeri* as an outgroup species by using MEGA7 software package [19–21].

2.3. **In vitro behavioral studies of Drosophila melanogaster**

An experiment was performed to check whether fruit flies reared in lab get attracted to the mucoid gleba of *I. rosea* and whether they prefer this mucoid gleba over a fermented Banana (*Musa paradisiaca*), for which a clean transparent plastic box was taken ensuring that the fly should have sufficient amount of air to respire and space to spread out for at least 24 h. An equal amount of mucoid substrate from *I. rosea* gleba and smashed 2-day fermented banana were weighed in two sterile petri dishes and kept at two opposite sides of the clean transparent plastic box. Approximately, 400–500 wild-type strain of *Drosophila melanogaster* (+;+;+) flies were anesthetized using ether and were collected in a vial from a bottle. This vial of flies was gently emptied at the center of the box and the lid was closed tightly. The box was kept at room temperature and was observed initially for an hour, making sure that all flies are conscious again and then after 0, 12, and 24 h, the feeding behaviors and preferences were assessed.

To analyze whether the behavior of flies is based on visual or olfactory, an experiment was performed in a three chambered box, wherein, the three chambers were created using two cardboards with a hole so as to allow the passage for flies. Approximately, 250 anesthetized flies were kept in the middle chamber, while gleba and laboratory prepared feed for *Drosophila* (the laboratory prepared feed contains yeast and some volatile compounds e.g., orthophosphoric acid, 10% methyl benzoate, and propionic acid in concentrated form) were placed inside chambers having hole passage. Schematic of the setting is shown in Figure 1(a–c). When the flies become conscious, the visual block prevents the preference of food based on vision and allows flies to decide the direction of food based on smell only.

As *Drosophila* is a human model organism, generally is cultured in a vial with feed following circadian rhythm. All the above experiments were
performed under same conditions i.e., following the day and the night cycle.

2.4. GC-MS analysis

Sample extraction: To study the odorant chemicals which attract the *Drosophila* toward the fruitbody, the fresh detached gleba and pseudostipe were extracted separately by using Hexane solvent. The extracts were filtered through Nylon syringe filter 0.45 μm size. The filtered solution was subjected to GC-MS analysis. GC-MS system consisted of an Agilent 6890N gas chromatograph and having detector (Agilent VR 5973 Network MSD, Santa Clara, CA). The column consisted of 30 m DB-5 column (J & W Scientific, Folsom, CA). Injection of samples was completed via injector (Split 1:40 at 250 °C, 2.0 μL; carrier gas: helium 1.1 mL/min (60 kPa) at 110 °C; pressure rise: 6 kPa/min). Full scan mass spectra were obtained from 35 to 350 m/z at a rate of 4.5 scans/s and with a 5.00 min solvent delay. Chromatographic peaks and mass spectra were then identified by using the National Institute of Standards and Technology (NIST) reference libraries. The percentage abundance of compounds was calculated by percentage of peak area of each compound by peak area of total compounds. The GC-MS analysis was conducted in duplicate for gleba and pseudostipe.

2.4. Statistical analysis

Data of Gleba feed after 12 and 24 h of feeding were analyzed by *T*-test, results were considered significantly different at *p* < 0.05. Data of Gleba versus Banana and Gleba versus laboratory feed after 12 and 24 h were analyzed by a one-way ANOVA with the Tukey post-test to evaluate the differences between treatments. The post-hoc test of the least significant difference (LSD) was used to assess the differences between treatments. Results were considered significantly different at *p* < 0.01.

Table 1. The ITS and LSU sequences and their GenBank accession numbers used for phylogenetic analysis.

| Sr. No. | Species              | GenBank Accession Number (ITS) | GenBank Accession Number (LSU) |
|---------|----------------------|-------------------------------|-------------------------------|
| 1       | Itahajaya rosea      | MN135577                      | MN134400                      |
| 2       | Itahajaya rosea      | KF481955                      | JF968441                      |
| 3       | Itahajaya galericulata | MF506816                   | KRO71850                      |
| 4       | Itahajaya galericulata | –                         | KRO71851                      |
| 5       | Anthurus archeri     | –                             | DQ218624                      |
| 6       | Asaero rubra         | –                             | DQ218625                      |
| 7       | Clathrus chrysomelinus | –                         | DQ218626                      |
| 8       | Laternae triscapa    | –                             | DQ218640                      |
| 9       | Abrachium florumorme | –                             | JF968440                      |
| 10      | Illeidictyon gracies | –                             | DQ218636                      |
| 11      | Illeidictyon cibarium | –                         | DQ218633                      |
| 12      | Protubera canescens  | –                             | DQ218645                      |
| 13      | Protubera parvivora  | –                             | DQ218648                      |
| 14      | Protubera jamaicensis| –                             | DQ218647                      |
| 15      | Protubera marajuca   | –                             | DQ218518                      |
| 16      | Kobayasina nipponica | –                             | DQ218638                      |
| 17      | Protubera borealis   | –                             | DQ218649                      |
| 18      | Protubera sabulonensis | –                       | DQ218649                      |
| 19      | Dicytophara duplicate | –                         | DQ218481                      |
| 20      | Dicytophara multicolor | –                        | DQ218628                      |
| 21      | Dicytophara indusiate | –                         | DQ218627                      |
| 22      | Phallus costatus     | –                             | DQ218513                      |
| 23      | Phallus haitangensis | KU705384                      | –                             |
| 24      | Phallus haitangensis | KR155668                      | –                             |
| 25      | Phallus serrata      | KF052623                      | –                             |
| 26      | Phallus serrata      | KF052622                      | –                             |
| 27      | Phallus mengsongensis| KF052625                     | –                             |
| 28      | Phallus mengsongensis| KF052627                     | –                             |
| 29      | Phallus indusatus    | MH862183                      | –                             |
| 30      | Phallus indusatus    | MF372140                      | –                             |
| 31      | Phallus aureolatus   | MF372135                      | –                             |
| 32      | Phallus hadriani     | KF481956                      | DQ218514                      |
| 33      | Phallus hadriani     | KU516100                      | –                             |
| 34      | Phallus hadriani     | MG678525                      | –                             |
| 35      | Dicytophara rubrovolvata | MF939504               | –                             |
| 36      | Phallus ultraduplicatus | KJ391385                | –                             |
| 37      | Phallus impudicus    | MH424916                      | –                             |
| 38      | Phallus impudicus    | KU516099                      | –                             |
| 39      | Phallus campanulatus | MF372139                      | –                             |
| 40      | Phallus ravenelli    | DQ218515                      | –                             |
| 41      | Phallus rugulosus    | MF372142                      | –                             |
| 42      | Phallus rugulosus    | MK182315                      | –                             |
| 43      | Phallobata alba      | –                             | DQ218642                      |
| 44      | Trappea darkeri      | MK752546                      | DQ218651                      |
3. Results

3.1. Taxonomical analysis of *Itajahya rosea*

3.1.1. Taxonomy

*Itajahya rosea* (Delile) E. Fisch., *Ber. dt. bot. Ges.* 47(5): 294. 1929 (Figure 2(A–D))

**Synonym:** *Phallus roseus* Delile 1813

**Description:** Egg are subglobose or pyriform, 5–6 cm high by 3–4 cm wide, white to yellowish brown, with developed rhizomorph Exoperdium ochre to dun, presence of three layers outer most layer thick 2–2.5 mm, white to beige, middle layer 5–6 mm, mucilagenous, inner layer less than 1 mm, whitish covering over green gleba. Basidioma without indusium, 4–5 cm tall and width 4.2 cm. Receptacle cylindrical with granulose to rugulose surface, receptacle surface is non-merrellid, receptacle surface wig-like, Pseudostipe pink, spongy, hollow, cylindrical, 3–4.5 cm tall and 1.5–2 cm wide, formed by pseudoparenchymatous cells, tappering toward the base, receptaculum conic with reticulate surface. Gleba mucilagenous, deliquiscent, dark green, gleba odor fetid. Calyptra at the apex white-beige discoid, 1.5–2 cm in diameter forming sterile platform above the receptacle. Basidiospores 3.5 × 1.8 μm average, cylindrical hyaline, slightly green, thin walled, smooth (Figure 2(E–F)).

**Edibility:** Not known.

**Habitat:** This fungus appears in sandy soils July–October, and is found in cluster associated with the roots of *Leucaena leucocephala* (Fabaceae) in India.

**Distribution:** Africa, Southern Yemen, North America, Southern France, Israel, India.

**Material examined:** India. Savitribai Phule Pune University Campus, MH, India. August 2018, M. Borde, Y. Kshirsagar, R. Jadhav & A. Baghela.

3.1.2. Phylogenetic analysis of *Itajahya rosea*

The ITS and LSU sequences of *I. rosea* were deposite in the NCBI GenBank with the accession numbers ITS sequence: MN135577, and LSU sequence: MN134400. The phylogenetic analysis of ITS sequences by the neighbor-joining method confirmed the identity of our fungus, *I. rosea* formed a sister clade with *I. galericulata* with bootstrap support (100%) (Figure 3). Phylogenetic analysis of LSU sequences by the neighbor-joining method confirmed the identity of our fungus, *I. rosea* formed sister clade with *I. galericulata* species with bootstrap support (99%) (Figure 4).

![Figure 1](image_url). Diagrammatic representation of behavioral experiment set ups. (A) initial trial test for attraction toward Gleba as food substrate; (B) to check the preference of food as Gleba and Banana; (C) experiment showing card board bifurcations with a passage hole separating the two food substrate (Gleba and laboratory prepared feed) with anesthetized flies in the center.
3.2. Behavioral studies on Drosophila

The *Drosophila* were found to be attracted toward gleba portion of *I. rosea* fruiting body (Figure 2(G)). Under laboratory conditions, the *Drosophila* were found to get significantly attracted toward the gleba of *I. rosea* (Figure 5(A)). Initially for few hours it was not clear whether the flies would choose to feed on gleba or not because a random distribution pattern of flies around the box was observed as flies take time to acclimatize to the new environment though few were feeding on gleba; however, within few hours, it became clear that most of the flies were getting attracted to the gleba preferring over the banana. It was observed that significant number of flies were in fungal substrate (gleba) zone and feeding on the gleba after 24 h of the inclusion in the box (Figure 5(B)).

The three chamber experiment showed that flies were significantly attracted more toward laboratory prepared feed as compared to gleba after 12 and 24 h, as it contains yeast and some volatile compounds (e.g., orthophosphoric acid, 10% methyl benzoate, and propionic acid) in concentrated form which exhibits anti-microbial properties and helps in attracting flies. During this experiment, flies could not see the type of feed kept in two chambers, while they could smell them and flew to their favorite food chamber (Figure 5(C)). Therefore, it confirms that flies response was an olfactory response rather than a visual response.

3.3. GC-MS analysis

GC-MS analysis from pseudostipe of *I. rosea* was identified for the first time showed that, total twenty two compounds were detected from both the gleba and pseudostipe. Nineteen compounds from gleba and nine compounds from pseudostipe and their molecular formula, retention time and percent Abundance were mention in (Table 2). Nine compounds with total abundance (78.11%) were found in pseudostipe of *I. rosea* namely, (1) pentadecane, (2) hexadecane, (3) nonadecane, (4) eicosane, (5) eicosane, (6) heneicosane, (7) 1,2-benzenedicarboxylic acid *bis*(2-methylpropyl) ester, (8) 4-(5'-nitro-2'-thienyl)-3-iodo-2-butanone, (9) phenol, 2,4-*bis*(1,1-dimethylethyl)- (Figure 6). The most abundant compounds were 1,2-benzenedicarboxylic acid *bis*(2-methylpropyl) ester, Heneicosane, Nonadecane, Eicosane, and Pentadecane. Nineteen compounds identified from Gleba with total abundance (84.76%) included (1) hexadecane, (2) pentadecane, (3) hexadecane, (4) hexadecane, (5) (−)-(55,95)-5,9-dimethylpentadecane, (6) (cis)-1-butyl-2-undecyclop propane, (7) nonadecane, (8) eicosane, (9)
benzene1,2-dimethoxy-, (10) 9,12-octadecadienoic acid (Z,Z)-, (11) benzoic acid ethyl ester (Figure 7(a)), (12) hexadecanoic acid, (13) 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, (14) anthraergostatetraenol hexahydrobenzoate, (15) N,N’-dicarbazoylhydrazine, (16) (S)-(−)-4-benzoyloxy-5-oxopentyl pivalat, (17) ethyl 5-methyl-4-(2-phenylimidazol-1-yl)butyl-1,2,4-triazine-6-carboxylate, (18) phenol,2,4-bis(1,1-dimethylethyl)−, (19) 2-(4-methoxyphenyl)-5,6-diphenyl-7H-pyrrolo[2,3-d]pyrimidin-4-ylidene)-malononitrile (Figure 7(b)). Three fatty acids were reported most abundant, 9,12-octadecadienoic acid (Z,Z)-, benzoic acid ethyl ester, and hexadecanoic acid followed by anthraergostatetraenol hexahydrobenzoate and mixture of volatiles such as Eicosane, Nonadecane, Pentadecane. Seven compounds were found to be common in gleba and pseudostipe.

4. Discussion

Four species of Itajahya have been discovered till date, however, only I. galericulata has been reported from India. The present study represents the first record of I. rosea from India. The most relevant distinguishing characteristic features in between I. rosea and I. galericulata have been the presence of a calyptra at the apex of the receptacle and the pinkish pseudostipe. The species is morphologically very close to I. galericulata. The peridium of the immature basidiome is an important feature separating the species: presence of three-layered in I. rosea and presence of four-layered peridium in I. galericulata. Itajahya galericulata has been documented in dry sandy environments of New Mexico and Arizona in Brazil either in clay banks of forest streams or among roots of dead trees [6,22]. In contrast, in
South Africa, it was found to be associated with litter of \( J. \) mimosifolia [23]. \( I. \) rosea as a rare species characteristic of desert and semiarid regions, as reported from Yemen and Northeastern Brazil [8,24,25]. Similarly, the Australian \( I. \) hornseyi is also reported from a sandy soil habitat [26]. The \( L. \) leucocephala is originated in Southern Mexico and Northern Central America where the climate is temperate and tropical zone. The \( L. \) leucocephala plant is invasive species may show association with \( I. \) rosea from its origin and there is possibility of dissemination of this association from Mexico to India via Africa and could be through seed-borne. The present molecular study showed that two gene regions namely, ITS and LSU have shown clearly that \( I. \) rosea was separated from other species. Our results confirm that \( I. \) rosea from India is phylogenetically closed to the \( I. \) galericulata, however, still branches out as a separate species. It also resulted that the inclusion of \( I. \) rosea in the \( I. \) hornseyi genus in Phallales by phylogenetic studies.

As in the case of many herbivores orientation toward host odors can be assumed to be of importance to fungivorous insects colonizing new patches [27]. Fungal odors have been shown to attract insects living on fungi or on substrates decayed by fungi [28–34]. For example, stinkhorn mushrooms attract flies and some other insects by emitting a foul-smelling odor and these insects disperse their spores [11,12,15].

In our study, we have shown for the first time that \( D. \) species gets attracted to the gleba of \( I. \) rosea. Further, we have also demonstrated that the laboratory reared \( D. \) could also get attracted to the isolated crude gleba material under the laboratory conditions. The laboratory-based experiments have also shown that the laboratory reared \( D. \) preferred the gleba over banana as a substrate for feeding. This behavioral study confirmed that there were some volatile compounds being produced by \( I. \) rosea, therefore, for the first time, we identified those compounds by GC-MS analysis.

The GC-MS analysis of gleba and pseudostipe from \( I. \) were studied, total 19 compounds were reported from gleba and some of their potential role and functions are discussed here. Blends of odorant compounds that include docosane, heneicosane, hexadecane, pentadecane, nonadecane, and...
1,2-dimethoxybenzene produced by *I. rosea* play a role in attraction of *Drosophila*. Some of these alkanes are previously reported in *P. impudicus* [35]. Heneicosane is reported to be an attractant of mosquitoes in the genus *Aedes* [36]. A single odorant in gleba may be less effective to attract but blend of odorants is effective for attraction toward food source [37,38]. Other odorant such as 1,2-dimethoxybenzene is reported to key compound produced by flower for pollinator attraction [39,40]. Thus, the Blends of odorant compounds produced by *I. rosea* showed an adaptive mechanisms for attraction of *Drosophila*.

Fatty acids, such as 12-octadecadienoic acid (Z,Z)- unsaturated fatty acid also known as Linoleic acid, are abundantly present in gleba, Benzoic acid ethylester is an ester formed by condensation of benzoic acid and ethanol and third one hexadecenoic acid which activates olfactory receptors of *Drosophila* and also has fruit odor quality [41,42]. These fatty acids serve as an appetitive signal for *Drosophila* and they are detected through sweet-sensing neurons by Phospholipase C signaling in gustatory system, which promote the feeding [43]. It has also been hypothesized that the *Drosophila* feed on gleba containing spores, which if not digested might come out through feces [44–46]. Thereby helps in dispersal; however, there is need to perform more experiments to claim the spore dispersal through feces.

The (+)-(5S,9S)-5,9-dimethylpentadecane is probably one of the sex pheromone produced in gleba of *I. rosea*, the same pheromone was also reported from female coffee leaf minor, *Leucoptera coffeella* one of the pest of coffee trees in Brazil [47]. The presence of such pheromones support the breeding of *Drosophila* after visit on *I. rosea*. One steroidal terpenoid compound abundantly produced from gleba named, anthraergostatetraenol hexahydrobenzoate is also responsible
for feeding, similar compound reported from other fungi \[48,49\]. Similarly, various woodland Drosophila species were reported as a food generalist, facultative fungal breeders and also a number of mycophagous organisms are attracted to \textit{P. impudicus} for the program of oviposition.

Table 2. List of compounds identified in \textit{Itajahya rosea} from pseudostipe and gleba by using GC-MS analysis.

| S. no. | Name of compound | Molecular formula | Pseudostipe (RT) | Abundance (%) | Gleba (RT) | Abundance (%) |
|--------|------------------|-------------------|------------------|---------------|------------|---------------|
| 1      | (S)-(–)-4-Benzoyloxy-5-oxopentyl pivalate | C$_{17}$H$_{22}$O$_5$ | – | – | 12.063 | 0.061 |
| 2      | Benzene, 1,2-dimethoxy- | C$_{8}$H$_{10}$O$_2$ | – | – | 13.275 | 0.152 |
| 3      | Benzoic acid, ethyl ester | C$_{6}$H$_{5}$CO$_2$H | – | – | 13.879 | 16.701 |
| 4      | N,N-Dicarbazoylhydrazine | C$_{9}$H$_{9}$N$_4$O$_2$ | – | – | 18.001 | 0.236 |
| 5      | Phenol, 2,4-bis(1,1-dimethyl)- | C$_{8}$H$_{8}$O | 22.869 | 4.182 | 22.871 | 0.312 |
| 6      | Hexadecane | C$_{16}$H$_{34}$ | – | – | 24.883 | 0.149 |
| 7      | Pentadecane | C$_{15}$H$_{32}$ | 27.123 | 6.134 | 25.991 | 1.203 |
| 8      | Hexadecane, 2,6,10-trimethyl- | C$_{32}$H$_{64}$ | 27.251 | 5.353 | 27.253 | 0.732 |
| 9      | Hexadecane, 2,6,10,14-tetramethyl- | C$_{36}$H$_{72}$ | – | – | 29.446 | 2.864 |
| 10     | Docosane | C$_{32}$H$_{64}$ | 29.447 | 5.455 | 30.714 | 1.982 |
| 11     | 1,2-Benzenedicarboxylic acid, bis[2-methylpropyl] ester | C$_{17}$H$_{36}$ | – | – | – | – |
| 12     | (±)-(33.95)-5,9-Dimethylpentadecane | C$_{20}$H$_{42}$ | – | – | 31.138 | 0.597 |
| 13     | Hexadecanoic acid | C$_{16}$H$_{32}$O$_2$ | – | – | 32.564 | 2.450 |
| 14     | 9,12-Octadecadienoic acid (2,2)- | C$_{21}$H$_{42}$O$_2$ | – | – | 35.801 | 47.428 |
| 15     | (cis)-1-Butyl-2-undecylcyclopropane | C$_{16}$H$_{36}$ | – | – | 35.943 | 0.338 |
| 16     | Nonadecane | C$_{19}$H$_{38}$ | 31.28 | 8.011 | 27.362 | 0.719 |
| 17     | Eicosane | C$_{20}$H$_{42}$ | 32.18 | 7.670 | 37.484 | 0.957 |
| 18     | Heneicosane | C$_{21}$H$_{44}$ | 35.074 | 8.335 | – | – |
| 19     | 4-(5'-Nitro-2'-thienyl)-3-iodo-2-butanone | C$_{19}$H$_{24}$O$_2$NO$_3$I | 43.283 | 3.719 | – | – |
| 20     | Ethyl 5-methyl-4(2-phenylimidazol-1-yl)butyl-1,2,4-triazine-6-carboxylate | C$_{30}$H$_{19}$N$_5$O | – | – | 47.605 | 0.335 |
| 21     | Anthraergostetraenol | C$_{18}$H$_{14}$O$_2$ | – | – | 48.479 | 7.331 |
| 22     | hexahydrobenzoate | C$_{6}$H$_{4}$ | 31.28 | 8.011 | 27.362 | 0.719 |

Figure 6. Chemical structure of compounds reported from pseudostipe of \textit{Itajahya rosea} by GC-MS analysis.
and breeding [50]. There is diverse variety of various insects like Coleoptera, Diptera, and more were reported to be obligatory or opportunistic breeders on different mushroom fruit bodies [51–55]. Therefore, the attractiveness of gleba food odor blends containing few key odorants compounds which are responsible for behavioral response of Drosophila and blends of fatty acids.
and pheromones are responsible for feeding and breeding mechanism.

5. Conclusions

To summarize, the *I. rosea* was first time reported in association with *Leucaena leucocephala* from India and molecular phylogenetic analysis revealed that it formed sister clade with *I. galericulata*. During its life cycle, *I. rosea* produced blends of volatile compounds and three abundant fatty acids and pheromone. The mixture of volatiles was responsible for attraction of *Drosophila* species toward the gleba. Three important abundant fatty acids, which activated the olfactory response for searching of food. Thus, flies were feeds on gleba. The pheromones produced by gleba is responsible for breeding program of *Drosophila*. Since the fruiting bodies are short lived, we will in future undertake a prospective study to decipher the volatile compounds of different stages of fruiting and how it affects *Drosophila* interaction and possible role of this interaction in fungal spore dispersal.

Acknowledgments

The authors are thankful to Director, Agharkar Research Institute, Pune, for providing the necessary facilities and support for the research. Authors are thankful to Bhupendra V. Shravage for a fruitful discussion on behavioral studies on *Drosophila*.

Disclosure statement

The authors declare that there are no conflicts of interest. All the experiments undertaken in this study comply with the current laws of the country where they were performed.

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