

## INTERACTION OF SOME FUNGAL ENTOMOPATHOGENS OF RICE PESTS WITH PHYLLOPHYTIC MICROORGANISMS OF FOUR RICE GENOTYPES CULTIVATED IN COASTAL ODISHA, INDIA

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### ABSTRACT

Interactions of six entomopathogenic fungi viz. *Cordyceps* (2 strains), *Beauveria* (1 strain) and *Metarhizium* (3 strains) spp. of rice leaf folder (LF, *Cnaphalocrocismedinalis*) with the phyllophytic (phyllospheric and phylloplanic) bacteria (n=35) and fungi (n=4) isolated at pre-flowering stages of four cultivated rice (*Oryza sativa* L.) var. Lalat, Swarna, Swarna-Sub1 and Naveen were assessed to reveal possibility of intergroup inhibition in the field. Dynamics of the phyllophytic microbes revealed that the phyllospheric bacterial population (3.59 to 4.10 log CFU/cm<sup>2</sup>) was more than those of the phylloplane (1.56 to 1.75 log CFU/cm<sup>2</sup>) of different plants. The phyllophytes of the four rice genotypes decreased in the order of Swarna-Sub1 > Swarna > Lalat > Naveen. The fungal pathogens of LF viz. *C. brongniartii* (TF6 and TF6-1A), *B. bassiana* (TF6-1B) and *M. anisopliae* (TF19, TF19-3A and TF19-3B) were not inhibited by any of the phyllophytic organisms which proved that they can be applied on the canopy of the rice plants to control the pests.

**Key words:** Entomopathogenic fungi, Interaction, Leaf folder, Rice phyllophytes.

### INTRODUCTION

Among the microbial pathogens such as bacteria, virus, fungi and protozoa of the insect pests, the fungal pathogens are earlier but lesser emphasized pathogenic bioagents (Krattiger 1997). In the field, interactions of the abiotic factors like UV radiation, temperature, rainfall, humidity, and the biotic factors like microbes with synergistic or antagonistic effect would cause the inconsistent infectivity, survival and persistence of the biopesticides (Villani *et al.* 1992). Therefore,

analysis of interaction of the entomopathogenic fungi with the epiphytic organisms is essential prior to application of the biopesticides which has not yet been investigated for the rice entomopathogens.

Phyllophytic, i.e., phyllospheric (loosely attached microbial community of leaves) and phylloplanic (firmly attached microbial community of leaves) bacteria and fungi are important for agricultural and environmental functionality as they can affect ecological balance, plant growth,

suppress or stimulate colonization and infection of the plant pathogens (Lindow and Brandl 2003, Rasche *et al.* 2006). However, diversity and dynamics of the phyllophytic bacterial populations vary among and within different crop species, growth period and leaf ages (Kinkel *et al.* 1995, Yadav *et al.* 2004, Lambais *et al.* 2006, Yadav *et al.* 2011). Evidently, Mwijita *et al.* (2013) recorded 77 phyllospheric, 119 rhizoplastic and 54 rhizospheric plant growth promoting (PGP) bacteria and fungi from Kenyan rice and the leaf imprints which showed that the epiphytic microbes are not distributed uniformly across the leaf surfaces (McCaig *et al.* 1999). Nevertheless, collate information showed that, like other habitats, phyllospheric and phylloplanic microbial community analysis is a complex proposition, and despite culture independent molecular and community physiology analysis would comprehend overall diversity of the microbes (Garland *et al.* 2001, Preston-Mafhan *et al.* 2002, Yadav *et al.* 2008) but culture methods (solid and broth culture, leaf imprints etc.) are essential for analysis of microbial functions and interactions of different habitats (Ritz 2007, Yadav *et al.* 2010).

Entomopathogenic fungi have been used against a broad range of insect pests (Lacey *et al.* 2001, Ansari *et al.* 2004) and the *Beauveria* and *Metarhizium* spp. are established pathogens of rice leaf folder (LF), *Cnaphalocrocis medinalis* (Guenee) (Lepidoptera: Pyralidae) (Dangar 1998, Sahoo *et al.* 2013) which is a major yield-limiting factor of rice. As resident microbes would interfere with the biocides which are generally used externally, their interactions with the phyllophytic organisms should be evaluated to ascertain their success for sustainable rice production. As the epiphytic microbes have PGP properties, it is desirable that biocides should not displace the resident microbiome also (Cook *et al.* 1996). The interactions of the entomopathogens with

phyllophytic organisms of rice and vice versa have not been studied to date. Therefore, the study was undertaken to disclose the interactions of 3 virulent pathogens to LF viz. *C. brongniartii*, *B. bassiana* and *M. anisopliae* with the resident phyllophytic microbiota of four cultivated rice genotypes var. Lalat, Swarna, Swarna-Sub1 and Naveen.

## MATERIALS AND METHODS

### Selection of entomopathogens for the study

The entomopathogenic fungi viz. *Cordyceps* (anamorph *Beauveria*), *Beauveria*, *Metarhizium*, *Nomuraea*, *Fusarium*, *Verticillium*, *Trichoderma* and *Paecilomyces* spp. were isolated from naturally infected rice (*Oryza sativa* L.) insect pest including the leaf folder (LF, *C. medinalis*) larvae (Sahoo *et al.* 2013). Virulence of the pathogens were assessed and the six selected effective and virulent (infected > 50% LF larvae in the laboratory and net house) pathogens of LF viz. *C. brongniartii* (TF6 and TF6-1A), *B. bassiana* (TF6-1B) and *M. anisopliae* (TF19, TF19-3A and TF19-3B) (Sahoo *et al.* 2013) which were subsequently genotyped using ITS 1, 4 sequencing to confirm phenotypic identification.

### Isolation, characterization and identification of the phyllophytic organisms

The phyllophytic (phyllospheric and phylloplanic) organisms (bacteria and fungi) were isolated from four cultivated rice (*Oryza sativa* L.) var. Lalat, Swarna, Swarna-Sub1 and Naveen of different growth duration and water regimen (hydrology) for cultivation (Table 1). The phyllospheric and phylloplanic microbes were isolated by dilution plating and leaf imprint methods, respectively (Aneja 2003, Yadav *et al.* 2010). Ten uninfested and healthy apical leaves were collected from each variety at panicle initiation stage (pre-flowering stage), cut into 5 cm long pieces and rinsed 10-15 times with tap water

followed by sterilized (autoclaved in 1.1 kg/cm<sup>2</sup> pressure at 121 ± 0.1°C for 15 min) double distilled water under a laminar air flow hood to remove externally loosely attached dusts and microbes. To estimate the phyllospheric microbes, one of the leaf pieces was aseptically cut into 5 × 1 cm pieces and put in a conical flask (100 ml) with 10 ml sterilized distilled water containing 0.001% tween 80, 0.1 M MgSO<sub>4</sub> and 0.15% glycerine; incubated for 12 h on a rotary shaker at 80±1 rpm at 37 ± 0.1°C. 1 ml of each leaf wash was mixed separately with each of 100 ml nutrient agar, NA (g/l: peptone 5, beef extract 1, yeast extract 2, NaCl 5, agar 15, pH 7.4) for bacteria and potato dextrose agar, PDA (g/l: potato infusion 200, dextrose 20, agar 15, pH 5.6) for fungi, plated separately in five petridishes and incubated in a BOD incubator at 30 ± 0.1°C. To estimate the phylloplanic microbes, the leaf pieces (1 cm length × 1-2 cm width) were taken out from the flasks, blotted to dryness on sterile blotting papers, washed 3 times with sterile distilled water, blotted the excess water to dryness and each surface of a leaf piece was held pressed alternatively for 2 h on separate places on a NA plate, as well as, similar impressions of another leaf piece were taken on PDA plates. The leaf pieces were removed from the plates and incubated in a BOD (biological oxygen demand) incubator at 30± 0.1°C. Each experiment was repeated 3 times taking three leaf pieces for each media. Different colonies grown from leaf washes (phyllospheric microbes) and along the imprints (phylloplanic microbes) were isolated, purified and the bacteria and fungi were preserved on NA or PDA slants, respectively, at 4 ± 0.1°C.

The microbial populations (bacteria and fungi) were enumerated by dilution plating of the leaf washes and leaf imprints, and expressed as log colony forming units (CFU) /cm<sup>2</sup> leaf (Yadav *et al.* 2004). For the leaf imprints, the CFUs on adaxial

and abaxial leaf surfaces were added to calculate the total bacterial population on the phylloplane. The population data were analyzed by ANOVA (Table 3) with errors of the means of three replications. The phyllophytic bacteria and fungi were phenotyped by cultural (colony), morphological, staining (cellular characters were observed under 40-100X LM) and biochemical character grown on NA and PDA. The bacteria (Smibert and Krieg 1994) and fungi (Samson *et al.* 1988) were tentatively identified following standard identification protocols. Fluorescence pigment production by the bacteria was checked under a 312 nm UV lamp.

**Table 1. Hydrology and duration of growth.**

| Rice variety | Hydrology of cultivation  | Total growth duration (d) | PI stage <sup>a</sup> period (d) |
|--------------|---|---------------------------|----------------------------------|
| Lalat        | Irrigated land, water depth ≤ 30 cm                                   | 125                       | 100-105                          |
| Swarna       | Rainfed shallow lowland, water depth ≥ 30 cm                          | 145                       | 115-120                          |
| Swarna-Sub1  | Rainfed shallow lowland, flooded, submerged land, water depth ≥ 30 cm | 145                       | 115-120                          |
| Naveen       | Irrigated, medium/up land, water depth ≤ 30 cm                        | 120                       | 90-95                            |

<sup>a</sup>PI (panicle initiation) stage can vary from 3-5d depending on the season and environment, and the period indicates time scale of the growth phase.

#### Test for antagonism

Antagonism assay among the biocides and phyllophytes was carried out by co-culturing the mon tryptose soya agar (TSA) medium (g/l: pancreatic digest of casein 15, papaic digest of

soyabean meal 5, NaCl 5, agar 15, pH 7.3) (Ansari *et al.* 2005) with minor modification. The entomopathogenic fungi, i.e., two isolates each of *M. anisopliae*, *B. bassiana* and *C. brongniartii* were streaked along two parallel lines 1 cm away from opposite margins of the TSA plates (9 cm dia). The phylogenetic bacteria were streaked along equidistant parallel lines perpendicularly between the streaks of two entomopathogenic fungi without touching the streaks (0.5 cm away the fungal streaks). The plates were incubated in a BOD incubator at 30 ± 0.1°C, growth of the organism was checked after 3-4 d and antagonism between the organisms was determined from inhibition of growth of the organisms. The inhibition of growth was measured by measuring the length and breadth of the colonies on co-culture plate comparing with the control, i.e., without co-culture.

## RESULTS

### Identification of the entomopathogenic fungi

The virulent fungal pathogens of the LF viz. TF6, TF6-1A and TF6-1B were phenotyped as *Beauveria* spp., TF19, TF19-3A and TF19-3B were phenotyped as *Metarhizium* spp. (Sahoo *et al.* 2013). Besides phenotyping, the ITS 1, 4 sequencing showed that the TF6 and TF6-1A as *Cordycepsbrongniartii* (NCBI Acc. No. JX122734 and JX122735) which is a teleomorph of *B. bassiana* and TF6-1B as *B. bassiana* (NCBI Acc. No. JX122736) (Table 2). The ITS 1, 4 phylogenetic characters of the three isolates, i.e., TF19, TF19-3A and TF19-3B identified them as *Metarhiziumanisopliae* (NCBI Acc. No. JX122737, JX122738 and JX122739, respectively) (Table 2).

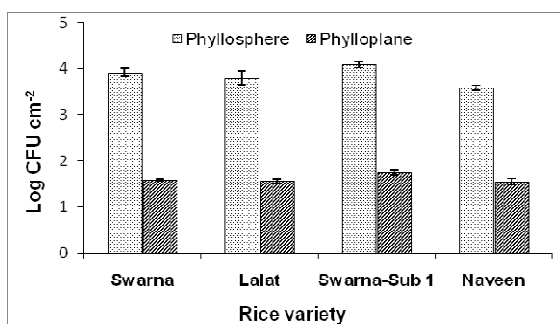
**Table 2. The phenotypic and genetic identity of the virulent entomopathogenic fungi of LF.**

| Isolate No. | Cultural, morphological and spore character on PDA*   | Phenotypic identity*         | Genetic identity by ITS 1, 4 sequence (NCBI Acc.No.) |
|-------------|---|------------------------------|--|
| TF6         | Colonies woolly white from upper and lower sides; conidiophores with cluster of short, ovoid and flask-shaped conidiogenous cells with narrow long zigzag filamentous extension (rachis) formed after each conidial attachment; conidia in chains single-celled, spherical, white, diameter 2.5-2.7 µm, and hydrophobic.                            | <i>Beauveriabassiana</i>     | <i>Cordycepsbrongniartii</i> (JX122734)              |
| TF6-1A      | Colonies woolly white from upper and pale yellow from lower side; conidiophores and conidia same as TF6   | <i>B.bassiana</i>            | <i>C. brongniartii</i> (JX122735)                    |
| TF6-1B      | Morphology same as TF6-1A   | <i>B.bassiana</i>            | <i>B.bassiana</i> (JX122736)                         |
| TF19        | Colonies white woolly initially and became light green after sporulation from upper side and yellow-green from lower side of colony; the conidiophores erect with 2-3 repeated branches from each node, conidiogenous cells cylindrical and conidia (6-7.8) x (2.2-2.5) µm, green, cylindrical with a slight central constriction (groundnut shape) | <i>Metarhiziumanisopliae</i> | <i>M. anisopliae</i> (JQ766113)                      |
| TF19-3A     | Morphology same as TF19 but more woolly growth than TF19; late sporulation than TF19  | <i>M. anisopliae</i>         | <i>M. anisopliae</i> (JX122738)                      |
| TF19-3B     | Morphology same as TF19-3A  | <i>M. anisopliae</i>         | <i>M. anisopliae</i> (JX122739)                      |

\*Sahoo *et al.* 2013. Presented here for comparison

### Phyllophytic microbial population density and diversity

The phyllophytic microbial pool population of the four rice varieties counted by serial dilution plating and leaf imprint methods are presented in Fig. 1. Populations of the phyllophytic (phyllospheric and phylloplanic) microbes varied significantly and the size of phyllospheric population was greater than that of phylloplanic ones (Table 3). Average phyllospheric bacterial population (estimated from leaf washes) was higher (4.10 log CFU/cm<sup>2</sup>) for Swarna-Sub1 and lower (3.59 log CFU/cm<sup>2</sup>) for Naveen, whereas, Swarna and Lalat leaves harboured intermediate levels of populations (3.92 and 3.81 log CFU/cm<sup>2</sup>, respectively). Similarly, phylloplanic bacterial population (estimated from leaf imprint) was higher for Swarna-Sub1 (1.75 log CFU/cm<sup>2</sup>) and lower for Naveen (1.56 log CFU/cm<sup>2</sup>) and that of Swarna and Lalat attained intermediate population levels, i.e., 1.59 and 1.56 log CFU/cm<sup>2</sup>, respectively. The phyllospheric microbial (bacteria and fungi) population among the 4 rice genotypes was significantly different, whereas, phylloplanic population among the rice genotypes was not significantly different (Table 3).



**Fig. 1. Phyllophytic (phyllospheric and phylloplanic) microbial population of the rice varieties.**

From the four rice varieties, 11 bacteria (a1-a11) and two fungi (f1 and f2) were isolated from Swarna, 11 bacteria (b1-b11) and one fungus (f3) were isolated from Lalat, 6 bacteria (c1-c6) and one fungus (f4) were isolated from Swarna-Sub1 and 7 bacteria (d1-d7) were isolated from Naveen (Tables 4 and 5). Among the isolates, a1, a2, a4, a5, b1, b5, b8, b9 and d7 produced pale/light creamy; a3 and a10 produced light yellow; b7 produced fluorescent yellow; d1 produced fluorescent green; a8, b2, b3, b4 and b11 produced off-white; a9, b10 and c2 produced yellow; b6 and d6 produced yellowish green and a6, a7, a11, c1, c3, c4, c5, c6, d2, d3, d4 and d5 produced white colonies on NA plates. The bacterial cell shapes varied from rod to oval and some (a1, a2, a4, a6, b1, b4, b9, c1, c4, c5, d2-d5 and d7) of them produced spores. The a1-a4, a6, b1, b4, b9, c1, c3-c5, c10, d2-d5 and d7 were Gram positive, whereas, remainder bacteria were Gram negative. Besides, b6, b7, d1 and d6 produced green fluorescence under the UV lamp grown on King's B medium. Based on the phenotypic characters the bacteria were tentatively identified (Smibert and Krieg 1994) as *Bacillus* spp. (a1, a2, a4, a6, b1, b4, b9, c1, c4, c5, d2-d5 and d7), *Pseudomonas* spp. (b6, b7, d1 and d6) and a3, a5, a8- a11, b2, b3, b5, b8, b10, b11, c2, c3 and c6 remained unidentified (Table 4).

The phyllophytic fungi of the cultivars were few, i.e., only 3 genera (Table 5). The phenotypic identities (tentative) of leaf epiphytic fungi were *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp.

**Table 3. Comparison of phyllophytic microbial populations using one-way ANOVA.**

| Variables    | Source of variation | Sum of squares | d. f. | Mean square | F value | P value | F critical |
|--------------|---------------------|----------------|-------|-------------|---------|---------|------------|
| Phyllosphere | Between groups      | 0.407          | 3     | 0.136       | 4.966   | 0.031   | 4.066      |
|              | Within groups       | 0.218          | 8     | 0.027       |         |         |            |
|              | Total               | 0.625          | 11    |             |         |         |            |
| Phylloplane  | Between groups      | 0.077          | 3     | 0.026       | 3.091   | 0.089   | 4.066      |
|              | Within groups       | 0.066          | 8     | 0.008       |         |         |            |
|              | Total               | 0.143          | 11    |             |         |         |            |

**Table 4. The phenotypic characters of phylophytic bacterial isolates on NA plates.**

| Isolate no.  | Colony morphology on NA   | Cell shape  | Motility   | Spore  | Gram stain                      | On UV | Nearest Genus           |
|--|---|---|--|--|---------------------------------|-------|-------------------------|
| a1, a2, a4, a6, b1, b4, b9, c1, c4, c5, d2-d5, d7    | Circular, pale yellow (a1), white (a7, c1, c4, c5, d2, d4), pale/light cream (a2, a4, a6, b1, b9, d5, d7), off white (b4, d3)                                     | Oval (b1, b9, d4, d7), rods (others)              | Non motile (a2, a4, b9, d3, d5, d7), motile (others)   | Produced   | +ve                             | NF    | <i>Bacillus</i> spp.    |
| b6, b7, d1, d6                                       | Amoeboid, yellowish-green (b6, d6), diffused green (d1), diffused, fluorescent-yellow (b7)  | Rod   | Motile   | Not produced   | -ve                             | F     | <i>Pseudomonas</i> spp. |
| a3, a5, a8-a11, b2, b3, b5, b8, b10, b11, c2, c3, c6 | Circular, light yellow (a3, a5, a9, a10, c3), off white (a8, b2, b3, c6) spreading off white (a11, b11) very small, pale/light cream (b5 b8) and yellow (b10, c2) | Rod (a8, a11, b2, b3, b8, c6, b11), oval (others) | Non motile (a9, a10, b3, b8, c2, b11), motile (others) | Produced (a5, a9, b5, b10, c2) Not produced (others) | +ve (a3, c3, a10), -ve (others) | NF    | Unidentified            |

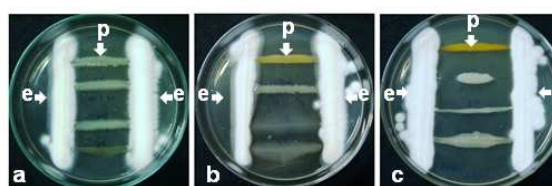
F = fluorescent and NF = non-fluorescent

**Table 5. The phenotypic characters of phylophytic fungal isolates on PDA plates.**

| Isolate no. | Cultural, morphological and spore character on PDA   | Nearest Genus           |
|-------------|--|-------------------------|
| f1 and f4   | Upper side of the colony white, lower side yellow, dark brown at sporulation, conidiophores terminate in a vesicle covered with metulae which bear small whorls of phialides. Conidia are single celled, hyaline and formed long chains which aggregated in compact columns (columnar).                                | <i>Aspergillus</i> spp. |
| f2          | Fast growing dark green colonies from upper side, consisting of dense conidiophores, chains of single-celled conidia produced from conidiogenous cells (phialide) which were produced from branched metulae, brush-like appearance. Conidia ellipsoidal or spherical, greenish, smooth.                                | <i>Penicillium</i> spp. |
| f3          | Colony wooly, whitish to light-pink from upper side, conidiophores short, simple, bearing apical conidiogenous cells (phialides) which were cylindrical to much elongated, macroconidia canoe-shaped, hyaline, 2-5 celled, fusiform to sickle-shaped and number increased at maturity, microconidia 1-2 celled, ovoid. | <i>Fusarium</i> spp.    |

### Interaction of entomopathogens with epiphytic organisms

Interactions of the entomopathogens with phylophytic organisms are given in Table 6. Interactions depicted inhibition of growth of a5 by TF19-3B; a6, d4 and d5 by TF6, TF6-1A, TF6-1B, TF19 and TF19-3A; a8 and a10 by TF19; f2 by TF6-1B; and f3 by TF6-1B, TF19 and TF19-3A (Fig. 2, Table 6). However, growth of the six effective fungal entomopathogens was also intermediately inhibited by the phylophytes a3, a11, b6, c4, c6, d1, d6 and f1 only (Fig. 2, Table 6). But the entomopathogens did not inhibit growth of both phyllospheric and phylloplanic microbes except for a6, d4, d5 and f3 (Table 6).



**Fig. 2. Representative photograph of interaction between entomopathogens (e) and phylophytes (p):**

- (a) No inhibition of either entomopathogens or phylophytes,
- (b) intermediate inhibition of entomopathogens,
- (c) inhibition and intermediate inhibition of phylophytes.

**Table 6. Interaction of the phyllophytes and entomopathogenic fungi.**

| Test organism  | Effect on growth of the organism (given are the isolate numbers)            |   |                         |
|--|---|---|-------------------------|
|  | Zero inhibition   | Intermediate inhibition                                       | Inhibition              |
| TF6  | a1, a3, a4, a7, a9-a11, b1-b7, b9-b11, c1, c2, c4-c6, d1-d3, d6, d7, f1, f3 | a5, a8, b8, f2, f4  | a2, a6, c3, d4, d5      |
| TF6-1A   | a3, a7, a9-a11, b1-b11, c4-c6, d1-d3, d6, f1, f3                            | a1, a2, a4, a5, a8, c1-c3, d7, f2, f4                         | a6, d4, d5              |
| TF6-1B   | a1, a3, a4, a9-a11, b1-b4, b6-b11, c1, c2, c4-c6, d1, d3, d6, d7, f1, f4    | a2, a5, a7, a8, b5, c3, d2                                    | a6, d4, d5, f2, f3      |
| TF19   | a1-a3, a5, a7, a11, b1-b4, b6-b11, c1, c3-c6, d1-d3, d6, d7, f1             | a4, a9, b5, c2, d7, f2, f4                                    | a6, a8, a10, d4, d5, f3 |
| TF19-3A  | a1-a3, a5, a7, a9-a11, b1-b8, b10, b11, c1, c3-c6, d1-d3, f1                | a4, a8, b9, c2, d6, d7, f2, f4                                | a6, d4, d5, f3          |
| TF19-3B  | a2, a3, a9-a11, b1-b4, b7-b11, c3-c6, d1, d3, d6, f1                        | a1, a4, a6-a8, b5, b6, c1, c2, d2, d4, d5, d7, f2-f4          | a5                      |
| a1, a2, a4-a10, b1, b3, b4, b7, b9-b11, c1-c3, c5, d2-d5, d7, f3 | All entomopathogens had normal growth                                       | Nil   | Nil                     |
| a3, b6, c4   | TF6, TF6-1A, TF6-1B, TF19-3A  | TF19, TF19-3B   | Nil                     |
| a11, c6  | TF19-3B   | All entomopathogen except TF19-3B had intermediate inhibition | Nil                     |
| b2, f2   | All entomopathogens except TF19 had normal growth                           | TF19  | Nil                     |
| b5   | All entomopathogens except TF19-3B had normal growth                        | TF19-3B   | Nil                     |
| b8   | All entomopathogens except TF19-3A had normal growth                        | TF19-3A   | Nil                     |
| f4   | All entomopathogens except TF6-1B had normal growth                         | TF6-1B  | Nil                     |
| d1, d6, f1   | Nil   | All entomopathogens had intermediate inhibition               | Nil                     |

**DISCUSSION**

The results of phyllophytic microbes indicated that, like the microbial population density and type of different other habitats, phyllophytic microbes were also variable among the rice genotypes, but they did not follow similar trends among the

cultivars. For example, Swarna-Sub1 harboured more bacteria but types of microbes were lesser (6 bacteria and 1 fungus) than Swarna (11 bacteria and 2 fungi), whereas, the population size and diversity of microbial load were comparable for other two cultivars (Lalat and Naveen). The results

indicated that support of the plants on the resident phyllophytic microbes is complex and it would probably depend on the nutrition supply by the plants, interactions among the phyllophytes and the environmental factors. Only 3 genera of the phyllophytic fungi could be obtained from the 4 rice cultivars on PDA media which indicated that the culture medium would not support all epiphytic fungi which dependent on the nutritional support from the plants. Alike the present study (Table 5), several similar or more epiphytic fungi (39 isolates) including *Penicillium*, *Aspergillus*, *Trichoderma*, *Eupenicillium*, *Isaria*, *Leptosphaerulina*, *Hypocrea* and *Fusarium* spp. were recorded from Keniyan rice (Mwajita *et al.* 2013). Naveen (irrigated, medium/up land variety) had lower phyllospheric and phylloplanic microbial population, while Swarna-Sub1 (rainfed shallow lowland and flooded, submergence tolerant variety) had higher phyllospheric and phylloplanic populations. The results proved that phyllophytic microbial load vary widely among the rice cultivars and microbial load would be lesser on the genotypes of shorter growth period, i.e., there grown in drier cropping habitat, i.e., lesser water (moisture) regime. Wide variations of the phyllophytic microbes of four cultivated rice genotypes supported De Costa *et al.* (2006) who observed significant variation of total epiphytic microbial (bacteria and fungi) populations ranging from  $(0.591 \text{ to } 14.976) \times 10^3 \text{ CFU/cm}^2$  on fifteen traditional and high-yielding rice varieties grown in Srilanka under both planthouse and field conditions. However, microbial population of the Srilanka cultivars was similar in both high yielding and traditional varieties, but diversity of microbes was more in traditional rice varieties than high-yielding varieties (De Costa *et al.* 2006).

The natural entomofungal pathogens which could infect > 50% LF were planned to be used for mass production and formulation. Therefore, their interactions with the phyllophytes were assessed to understand the possible effects of the pathogens

with the phyllophytes and *vice versa*. The interactions among the entomopathogens and phyllophytic organisms was not always antagonistic except for a few cases like inhibition of the a2, a5, a6, a8, a10, c3, d4, d5, f2 and f3 by the pathogens. The results supported the postulate that the entomopathogens might not inhibit all the epiphytic microbes (Cook *et al.* 1996) and vice versa. Zhang *et al.* (2008) did not find significant effect of the biological control agent, *Bacillus thuringiensis*, on the phyllospheric microbial community and biomass of pepper. The findings of the study favoured Sylla *et al.* (2013) who observed no significant negative impact of the biocontrol agents viz. *B. amyloliquefaciens*, *Tricodermaharzia* and *B. bassiana* on the phyllophytic microbial diversity of strawberry, i.e., the antimicrobial secondary metabolites of the entomopathogenic fungi (*Beauveria*, *Fusarium*, *Gliocladium*, *Metarhizium*, *Paecilomyces* and *Verticillium* spp.) would not have antimicrobial properties against all microbes (Amiri *et al.* 1999, Kershaw *et al.* 1999, Strasser *et al.* 2000, Vey *et al.* 2001).

As growth of most of the phyllophytic organisms was not inhibited by the fungal entomopathogens and growth of the entomopathogenic fungi (*Beauveria*, *Codyceps* and *Metarhizium* spp.) was not inhibited by the leaf epiphytic bacteria and fungi therefore, the results suggested that the pathogens can be used as effective biocontrol agents against the target pests without affecting or nominally affecting the natural microbial diversity of the rice phyllosphere system.

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