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*Full Length Research Paper*

# Effects of carbon and nitrogen sources and ratio on the germination, growth and sporulation characteristics of *Metarhizium anisopliae* and *Beauveria bassiana* isolates

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**The entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* are ubiquitous and widespread with well documented broad- spectrum myco-pesticidal effects. It has been reported that nutrient source is an integral determinant of growth and virulence of entomopathogenic fungi. Nutrient based studies also impart light towards the identification of most commendable media for laboratory assays as well as selection of the tolerant isolates. With such a perspective, an *in-vitro* study was undertaken to evaluate conidial germination, mycelial growth and spore yield of fourteen *M. anisopliae* and seventeen *B. bassiana* isolates and the effect of different carbon and nitrogen sources on these characteristics were studied. The different isolates showed significant variation with respect to the differing preference for the nutrient sources. From this study it was inferred that isolate UM2 (*M. anisoplaie*) and AB1 ( *B. bassiana*) exhibited commendable growth potentials on almost all the nutrient sources studied, and were basically more robust isolates and holds high affirmations for commercial usage as biopesticides.**

**Key words:** *Metarhizium anisopliae*, nutrition, germination, growth, sporulation, *Beauveria bassiana*.

## INTRODUCTION

The last century has been witness to both the keynote augmentation of the chemical pesticides as well as its backlashes, so much so, that the development of entomopathogenic fungi as environmentally friendly alternative is fast gaining momentum (Inglis et al., 2001). Several microorganisms are currently under consideration as control agents of insects, with particular emphasis on *Metarhizium anisopliae*, probably because of their high host-specificity, non-persistence and non-toxicity to environment, unique mode of action and appreciable shelf life. Mycelial forms that bear asexual resting spores called 'conidia' that serves as the infective propagule characterizes them. Good indicators of virulence include factors like germination rate, growth and sporulation (Chandler et al., 1993; Altre et al., 1999). It has been reported that nutrient source is an integral of determinant of growth and virulence of entomopathogenic fungi (Shah et al., 2005). Nutrients are substances used in biosynthesis and energy release and therefore serve as cardinal impetus towards the viability, survival and sustainance of any organism (Safavi et al., 2007). For elite cultivation of microorganism, it is imperative to have an in-depth knowledge of their nutritional requirements. The macro-elements like carbon, oxygen, hydrogen, nitrogen, sulfur and phosphorus are integral components of carbohydrates, lipids, proteins and nucleic acids and these metabolically active groups are directly/ indirectly involved in host-pathogen interaction and self-defense and perpetuation mechanisms. Carbon is required as the skeletal element of all organic molecules, and molecules serving as carbon sources normally also contribute both oxygen and hydrogen. Gao et al. (2007) studied the effect of different nutrient sources on the growth and sporulation of several biocontrol agents. The mycelial growth and spore yield on artificial media depends upon the fungal isolates and the components used in the culture media. Although saprophytic fungi are able to utilize a wide range

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**Table 1.** Source of *M. anisopliae* isolates.

| Isolates | Code No. / Accession No. ARSEF/ Local | Host insect | Geographical location |
|---|---|---|---|
| UM1 | USDA-ARS (1745) | *Nilaparvata lugens* | India |
| UM2 | USDA-ARS (2735) | *Spodoptera sp.* | Philippines |
| UM3 | USDA-ARS (2153) | *Nephotettix virescens* | Indonesia |
| UM4 | USDA-ARS (2424) | *Lepidoptera larva* | Indonesia |
| UM5 | USDA-ARS (3210) | *Coleoptera* | India |
| UM6 | USDA-ARS(2596) | *Pyrausta machaeralis* | India |
| UM7 | USDA-ARS(1080) | *Helicoverpa zea* | USA |
| UM8 | USDA-ARS(1724) | *Nilaparvata lugens* | India |
| UM9 | USDA-ARS(1727) | *Nilaparvata lugens* | India |
| UM10 | USDA-ARS(3295) | *Anticarsia gemmatalis* | Mexico |
| UM11 | USDA-ARS(1729) | *Nilaparvata lugens* | India |
| UM12 | USDA-ARS(1744) | *Nilaparvata lugens* | India |
| UM13 | USDA-ARS(1823) | *Nilaparvata lugens* | India |
| AR1 | Local | Unknown | India |

range of nutrient sources (Liu and Chen, 2002), but simple and less expensive media are needed to permit their mass- production and commercialization (Shah et al., 2005). For commendable flourishment, the microorganism must be able to incorporate appreciable quantities of nitrogen, phosphorus and sulfur, which are the essential ingredients of growth factors viz., amino acids, purines and pyrimidines and vitamins. Although these elements may be acquired from the same sources that supply carbon, the microbes have the capacity of employing inorganic sources as well (Li and Holdom, 1995). The CN ratio is said to significantly affect the number of conidia produced and conidial characteristics of *Colletotrichum truncatum* (Jackson and Bothast, 1990; Jackson and Schisler, 1992; Jackson and Slininger, 1993). Shah et al. (2005) studied the influence of nutrition on growth and virulence of *M. anisopliae*. Li and Holdom (1995) characterized two *M. anisopliae* isolates on basis of various carbohydrate and nitrogen supplements. Doust et al. (1983) studied the effect of growth substrates on conidial viability and virulence. Engelkes et al. (1997) reported the effect of CN ratio on growth, sporulation and biocontrol efficacy of *Taloromyces flavus*. An understanding of the growth characteristic with respect to the growth substrates shall also be handy in tolerance-selection studies.

Laboratory culture medium be it in solid (Shah and Tariq, 2005) or liquid form (Adour et al., 2002), are preparations used to grow, transport and store microorganisms. To be effective, the medium must contain all the nutrients, the microorganisms require for growth. This study on variable nutrient source had been conducted for fourteen *M. anisopliae* and seventeen *Beauveria bassiana* isolates so as to evaluate the fungi for their conidial germination, mycelial growth and sporulating potential on different media containing carbon and nitrogen sources and ratios. The culture media which supports better germination growth and sporulation promises to the key to the effective, low cost development of biopesticides. The cardinal objective of this work has been the development of cost effective methods for fungal propagation so as to yield effective inoculum levels towards mass production and formulation studies.

## MATERIALS AND METHODS

### Fungal cultures

The different isolates of the fungus *M. anisopliae* and *B. bassiana* were either procured from ARSEF (USDA-ARS Plant Protection Unit) or isolated locally in India (Tables 1 and 2). The isolates were routinely sub cultured on SDA (Sabouraud dextrose Agar) slants at 28°C in incubators and maintained at 4°C.

### Culture media

Culture media representing disparate carbon and nitrogen sources and ratios were used in this study. They included;

1. Sabouraud Dextrose Agar (SDA) with intermediate CN ratio (35:1) consisting of glucose (4%) and peptone (1%)
2. Low CN (10:1) medium consisting of glucose (0.6%) and peptone (1%)
3. High CN source (75:1) consisting of glucose (9.1%) and peptone (1%)
4. Nutrient poor media, peptone (2%) with CN ratio (8:1)
5. Yeast extract (1%) with CN ratio (3.6:1),
6. Potato Dextrose Agar (PDA) with CN ratio (10:1) and finally
7. 'Osmotic stress' medium (OSM) consisting of glucose (8%), peptone (2%), KCl (5.5%) and an exceptionally higher level of agar (5.5%) for solidification. The rest of the media were solidified in agar (2%). The pH of all the media was maintained at 7.0 and was sterilized at 121°C at 15 psi for 15 min and 15 ml poured into 9 cm dia-

**Table 2.** Source of *B. bassiana* isolates.

| Isolates | Code No. / Accession No. ARSEF/ Local | Host Insect | Geographical location |
|---|---|---|---|
| UB1 | USDA-ARS (1788) | *Helicoverpa virescens* | Spain |
| UB2 | USDA-ARS (2041) | *Cnaphalocrocis medinalis* | Philippines |
| UB3 | USDA-ARS (5278) | *Bemicia tabaci* | USA |
| UB4 | USDA-ARS (2417) | *Emmalocera depressella* | India |
| UB5 | USDA-ARS (2597) | *Hyblaea puer* | India |
| UB6 | USDA-ARS (6646) | *Spodoptera litura* | India |
| UB7 | USDA-ARS(4027) | *Coccinella septumpunctata* | Denmark |
| UB8 | USDA-ARS(1166) | *Helicoverpa armigera* | Spain |
| UB9 | USDA-ARS(2033) | *Coccinella* sp. | USA |
| UB10 | USDA-ARS(2034) | *Coccinella* sp. | USA |
| UB11 | USDA-ARS(4018) | *Coccinella septempunctata* | Denmark |
| UB12 | USDA-ARS(1886) | *Chilo infuscatellus* | India |
| UB13 | USDA-ARS(2412) | *Xyloryctes jamaicensis* | India |
| UB14 | USDA-ARS(8250) | *Basilepta fulvicornis* | India |
| UB15 | USDA-ARS(6650) | *Spodoptera litura* | India |
| UB16 | USDA-ARS(2660) | Adult Coleoptera | India |
| AB1 | Local | Unknown | India |

meter Petri plates.

#### Assay of germination

Agar slide technique was used for studying the rate of germination. Petri plates were lined with blotting paper discs and 2 glass slides were placed in each of the plates and autoclaved. 1 ml medium was evenly spread on each of the glass slides using micropipette. Conidial suspension was prepared from seven to ten day old cultures with concentrations maintained at $10^6$ conidia/ml. Around 100 µl of $10^6$ spores of fungal isolates were spread on the different media coated slides. The slides were placed back in the Petri plates and the blotting paper discs were moistened with sterilized water. The Petri plates were kept for incubation at 28°C. The slides were observed under compound microscope (40X) for germination, every 2 h starting after 3 h. A conidium was considered germinated when a distinct germ tube projected from it, and was at least twice the diameter of the conidium (Milner et al., 1991). Approximately 300 conidia were scored per replicate for each of the treatments.

#### Assay of colony growth and sporulation

Seven day old cultures on SDA slants were used for preparing spore suspension in 0.02% Tween 80 solution at 1 x $10^6$ spores/ml. 200 l of $10^6$ spores were spread plated on SDA medium and were incubated for 3 days at 28°C. At the end of 3 day, 5 mm agar disc with mycelia was retrieved with the help of a cork borer and placed in middle of fresh 9 cm Petri plates and incubated at 28°C. This was done for all the nutrient source media and five replicates were maintained for each media and for each isolate. Radial growth was measured every $2^{nd}$ till $8^{th}$ day. Radial growth rate ($mmd^{-1}$ ) was calculated from the linear portions of the curves plotted from these values. At end of $8^{th}$ day, 5 mm agar discs were randomly taken with the help of a cork borer. The discs were placed in 10 ml of 0.02% (v/v) Tween 80 solution and vortexed to suspend the spores. Spore yield was determined using a Haemocytometer.

#### Statistical analysis

Statistical analysis of all the data for fungal growth, sporulation and germination were subjected to one-way analysis of variance (ANOVA) and the means were separated by Student -Newman-Keuls multiple range test of comparisons of means at P = 0.05.

## RESULTS

The effect of different carbon and nitrogen sources on the conidial germination, growth and sporulation of *M. anisopliae* and *B. bassiana* isolates showed variations among the isolates studied. The results are presented in Tables 3 and 4 (*M. anisoplaie*) and Tables 5 and 6 (*B. bassiana*).

### Effect of CN sources and ratio on germination potential of *M. anisopliae* and *B. bassiana* isolates

Germination potential of *M. anisopliae* isolates on Sabouraud Dextrose Agar (CN = 35:1) showed isolate UM2 with 75% germination potential at $8^{th}$ h of incubation amongst the 14 isolates of *M. anisopliae* studied. This was followed by isolate UM3, UM4 and AR1 with germination potential of ≥ 65%. All of these isolates showed 100% germination potential at $16^{th}$ h of incubation. Amongst the 17 isolates studied for *B. bassiana*, isolate AB1 was identified to have up to 62% germination potential at $8^{th}$ h of incubation. This was followed by isolate UB2 and UB5 with germination potential of about 40%. In case of low CN media (CN = 10:1), *M. anisopliae* isolate UM2 exhibited germination potential of more than 70% at

**Table 3.** Germination percentage of *M. anisopliae* isolates on various media at 8th and 16th hour of incubation.

| Isolates | CN ratio – 35:1 (Glucose (4%) and Peptone (1%)) | | CN ratio – 10:1 (Glucose (0.6%) and Peptone (1%)) | | CN ratio – 75:1 (Glucose (9.1%) and Peptone (1%)) | | CN ratio – 8:1 Peptone (2%) | | CN ratio – 3.6:1 Yeast extract (1%) | | CN ratio – 10:1 (Potato Dextrose agar) | | OSM (Glucose (8%), Peptone (2%), KCl (5.5%)) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 8th h | 16th h | 8th h | 16th h | 8th h | 16th h | 8th h | 16th h | 8th h | 16th h | 8th h | 16th h | 8th h | 16th h |
| UM1 | 40.15$^{d*}$ | 100.00 $^{a}$ | 40.71$^{e}$ | 100.00$^{a}$ | 40.97$^{e}$ | 100.00 $^{a}$ | 40.84$^{d}$ | 100.00$^{a}$ | 44.42$^{d}$ | 100.00$^{a}$ | 27.39$^{g}$ | 90.00$^{b}$ | 60.42$^{b}$ | 100.00$^{a}$ |
| UM2 | 75.00$^{a}$ | 100.00 $^{a}$ | 72.00$^{a}$ | 100.00$^{a}$ | 61.57$^{a}$ | 100.00 $^{a}$ | 68.22$^{a}$ | 100.00$^{a}$ | 51.20$^{c}$ | 100.00$^{a}$ | 71.17$^{a}$ | 100.00$^{a}$ | 68.87$^{a}$ | 100.00$^{a}$ |
| UM3 | 68.98$^{b}$ | 100.00 $^{a}$ | 49.42$^{d}$ | 100.00$^{a}$ | 55.60$^{b}$ | 87.98$^{b}$ | 48.00$^{c}$ | 100.00$^{a}$ | 50.93$^{c}$ | 100.00$^{a}$ | 44.60$^{d}$ | 100.00$^{a}$ | 50.40$^{c}$ | 100.00$^{a}$ |
| UM4 | 67.92$^{b}$ | 99.50$^{a}$ | 64.52$^{b}$ | 100.00$^{a}$ | 62.43$^{a}$ | 76.00$^{d}$ | 49.00$^{c}$ | 100.00$^{a}$ | 64.95$^{a}$ | 83.80$^{c}$ | 51.31$^{c}$ | 81.20$^{c}$ | 47.45$^{d}$ | 78.80$^{b}$ |
| UM5 | 29.82$^{e}$ | 100.00 $^{a}$ | 36.80$^{f}$ | 100.00$^{a}$ | 23.20$^{f}$ | 100.00 $^{a}$ | 49.20$^{c}$ | 86.20$^{b}$ | 41.20$^{e}$ | 100.00$^{a}$ | 41.80$^{e}$ | 100.00$^{a}$ | 43.40$^{f}$ | 100.00$^{a}$ |
| UM6 | 9.60$^{j}$ | 47.50$^{e}$ | 0$^{j}$ | 27.60$^{f}$ | 0$^{i}$ | 21.80$^{i}$ | 22.20$^{g}$ | 65.00$^{d}$ | 23.40$^{h}$ | 53.20$^{f}$ | 20.70$^{n}$ | 44.30$^{g}$ | 0$^{g}$ | 17.30$^{c}$ |
| UM7 | 12.50$^{i}$ | 79.70$^{b}$ | 15.20$^{g}$ | 82.28$^{c}$ | 14.00$^{h}$ | 100.00 $^{a}$ | 18.10$^{i}$ | 100.00$^{a}$ | 17.11$^{i}$ | 100.00$^{a}$ | 0$^{j}$ | 67.60$^{d}$ | 0$^{g}$ | 0$^{e}$ |
| UM8 | 0$^{k}$ | 0$^{g}$ | 0$^{j}$ | 0$^{i}$ | 22.00$^{g}$ | 33.80$^{g}$ | 0$^{j}$ | 0a | 0$^{k}$ | 0$^{h}$ | 35.07$^{f}$ | 55.20$^{e}$ | 0$^{g}$ | 0$^{e}$ |
| UM9 | 0$^{k}$ | 0$^{g}$ | 0$^{j}$ | 0$^{i}$ | 0$^{i}$ | 53.20$^{f}$ | 0$^{j}$ | 32.20$^{h}$ | 0$^{k}$ | 0$^{h}$ | 0$^{j}$ | 0$^{j}$ | 0$^{g}$ | 0$^{e}$ |
| UM10 | 25.40$^{f}$ | 59.50$^{c}$ | 15.60$^{g}$ | 42.10$^{d}$ | 0$^{i}$ | 68.4740$^{e}$ | 34.20$^{e}$ | 83.57$^{c}$ | 28.20$^{g}$ | 76.92$^{d}$ | 0$^{j}$ | 25.10$^{i}$ | 0$^{g}$ | 0$^{e}$ |
| UM11 | 17.10$^{h}$ | 100.00$^{a}$ | 0$^{j}$ | 13.52$^{h}$ | 50.80$^{c}$ | 86.20$^{c}$ | 20.20$^{h}$ | 50.60$^{f}$ | 54.00$^{b}$ | 90.20$^{b}$ | 0$^{j}$ | 33.40$^{h}$ | 0$^{g}$ | 0$^{e}$ |
| UM12 | 0$^{k}$ | 43.44$^{f}$ | 11.00$^{i}$ | 39.20$^{e}$ | 0$^{i}$ | 26.40$^{h}$ | 30.20$^{f}$ | 62.80$^{e}$ | 29.00$^{g}$ | 67.40$^{e}$ | 14.70$^{i}$ | 50.80$^{f}$ | 0$^{g}$ | 14.60$^{d}$ |
| UM13 | 23.40$^{g}$ | 50.50$^{d}$ | 13.60$^{h}$ | 23.40$^{g}$ | 0$^{i}$ | 21.55$^{i}$ | 0$^{j}$ | 38.42$^{g}$ | 7.04$^{j}$ | 41.40$^{g}$ | 0$^{j}$ | 24.20$^{i}$ | 0$^{g}$ | 0e |
| AR1 | 64.40$^{c}$ | 100.00$^{a}$ | 50.80$^{c}$ | 96.80$^{b}$ | 44.20$^{d}$ | 100.00 $^{a}$ | 60.60$^{b}$ | 100.00$^{a}$ | 37.60$^{f}$ | 100.00$^{a}$ | 55.56$^{b}$ | 100.00$^{a}$ | 44.80$^{e}$ | 100.00$^{a}$ |

*Values followed by the same lower case alphabets in the same column are statistically equivalent ($p < 0.05$) according to the Newman-Keul's multiple range test.

**Table 4**.Growth rate and spore yield of *M. anisopliae* isolates on various media

| Isolates | CN ratio – 35:1 (Glucose (4%) and Peptone (1%)) | | CN ratio – 10:1 (Glucose (0.6%) and Peptone (1%)) | | CN ratio – 75:1 (Glucose (9.1%) and Peptone (1%)) | | CN ratio – 8:1 Peptone (2%) | | CN ratio – 3.6:1 Yeast extract (1%) | | CN ratio – 10:1 (Potato Dextrose agar) | | OSM (Glucose (8%), Peptone (2%), KCl (5.5%)) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Growth rate (mm/d) | Spore yield (x $10^7$) | Growth rate (mm/d) | Spore yield (x $10^7$) | Growth rate (mm/d) | Spore yield (x $10^7$) | Growth rate (mm/d) | Spore yield (x $10^7$) | Growth rate (mm/d) | Spore yield (x $10^7$) | Growth rate (mm/d) | Spore yield (x $10^7$) | Growth rate (mm/d) | Spore yield (x $10^7$) |
| UM1 | 2.04$^{b*}$ | 1.75$^{f}$ | 2.05$^{b}$ | 2.10$^{de}$ | 2.04$^{bc}$ | 1.75$^{g}$ | 1.30$^{h}$ | 4.40$^{b}$ | 1.66$^{f}$ | 3.75$^{b}$ | 1.02$^{e}$ | 1.50$^{gh}$ | 1.54$^{d}$ | 1.50$^{d}$ |
| UM2 | 1.99$^{b}$ | 3.30$^{d}$ | 2.02$^{b}$ | 1.00$^{i}$ | 1.97$^{cd}$ | 1.00$^{hi}$ | 1.60$^{ef}$ | 1.90$^{g}$ | 1.62$^{f}$ | 2.00$^{e}$ | 0.88$^{f}$ | 2.50$^{d}$ | 1.54$^{d}$ | 1.20$^{e}$ |
| UM3 | 1.68$^{c}$ | 1.00$^{h}$ | 1.92$^{c}$ | 1.60$^{g}$ | 1.72$^{e}$ | 1.65$^{g}$ | 2.12$^{c}$ | 2.00$^{g}$ | 2.06$^{c}$ | 2.00e | 1.42$^{b}$ | 3.95$^{c}$ | 1.13$^{e}$ | 1.00$^{ef}$ |
| UM4 | 1.48$^{d}$ | 2.55$^{e}$ | 1.50$^{d}$ | 3.00$^{c}$ | 1.16$^{h}$ | 3.30$^{d}$ | 2.17$^{c}$ | 1.60$^{h}$ | 2.50$^{b}$ | 1.65$^{f}$ | 1.47$^{b}$ | 2.60$^{d}$ | 2.50$^{b}$ | 2.65$^{c}$ |
| UM5 | 1.37$^{e}$ | 3.60$^{c}$ | 1.98$^{bc}$ | 3.25$^{b}$ | 1.67$^{ef}$ | 2.40$^{e}$ | 2.18$^{c}$ | 2.00$^{g}$ | 2.00$^{cd}$ | 1.25$^{g}$ | 1.39$^{b}$ | 4.60$^{b}$ | 2.15$^{c}$ | 2.50$^{c}$ |
| UM6 | 2.52$^{a}$ | 1.10$^{g}$ | 2.58$^{a}$ | 1.30$^{h}$ | 3.10$^{a}$ | 3.60$^{c}$ | 2.60$^{a}$ | 2.25$^{f}$ | 2.86$^{a}$ | 2.50$^{d}$ | 1.90$^{a}$ | 1.85$^{ef}$ | 1.00$^{ef}$ | 1.05$^{ef}$ |

**Table 4**. Contd.

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UM7 | 1.39$^{e}$ | 1.15$^{g}$ | 1.04$^{f}$ | 0.85$^{f}$ | 2.10$^{b}$ | 2.00$^{f}$ | 1.66$^{e}$ | 1.15$^{f}$ | 1.62$^{f}$ | 1.30$^{g}$ | 1.04$^{e}$ | 4.15$^{c}$ | 0.50$^{h}$ | 0.60$^{g}$ |
| UM8 | 0$^{h}$ | 0$^{i}$ | 0$^{g}$ | 0$^{j}$ | 0.60$^{i}$ | 1.00$^{hi}$ | 0$^{j}$ | 0$^{j}$ | 0$^{g}$ | 0$^{j}$ | 1.28$^{c}$ | 1.05$^{i}$ | 0$^{i}$ | 0$^{h}$ |
| UM9 | 0$^{h}$ | 0$^{i}$ | 0$^{g}$ | 0$^{j}$ | 1.52$^{g}$ | 0.90$^{i}$ | 1.48$^{g}$ | 1.55$^{h}$ | 0$^{g}$ | 0$^{j}$ | 0$^{g}$ | 0$^{j}$ | 0$^{i}$ | 0$^{h}$ |
| UM10 | 1.07$^{f}$ | 5.10$^{b}$ | 1.50$^{d}$ | 5.50$^{a}$ | 1.90$^{d}$ | 5.20$^{a}$ | 1.88$^{d}$ | 5.80$^{a}$ | 1.76$^{e}$ | 5.60$^{a}$ | 1.42$^{b}$ | 6.10$^{a}$ | 2.80$^{a}$ | 3.15$^{b}$ |
| UM11 | 0.95$^{g}$ | 1.35$^{g}$ | 0.99$^{f}$ | 1.70$^{fg}$ | 1.55$^{fg}$ | 1.15$^{hi}$ | 1.14$^{i}$ | 3.00$^{d}$ | 1.99$^{cd}$ | 0.55$^{i}$ | 1.13$^{d}$ | 2.10$^{e}$ | 0.80$^{g}$ | 0.85$^{fg}$ |
| UM12 | 1.04$^{f}$ | 1.20$^{g}$ | 1.45$^{d}$ | 1.90$^{ef}$ | 1.59$^{fg}$ | 1.30$^{h}$ | 1.49$^{g}$ | 2.50$^{e}$ | 2.01$^{cd}$ | 0.75$^{h}$ | 1.08$^{de}$ | 1.70$^{fg}$ | 0.75$^{g}$ | 0.85$^{fg}$ |
| UM13 | 0.99$^{f}$ | 1.80$^{f}$ | 1.34$^{e}$ | 2.20$^{d}$ | 1.49$^{g}$ | 1.55$^{g}$ | 1.52$^{fg}$ | 2.35$^{ef}$ | 1.95$^{d}$ | 0.55$^{i}$ | 1.24$^{c}$ | 1.35$^{h}$ | 0.89$^{fg}$ | 1.00$^{ef}$ |
| AR1 | 1.50$^{d}$ | 5.40$^{a}$ | 1.94$^{c}$ | 5.50$^{a}$ | 1.52$^{g}$ | 4.70$^{b}$ | 2.41$^{b}$ | 3.60$^{c}$ | 2.50$^{b}$ | 3.10$^{c}$ | 1.42$^{b}$ | 6.15$^{a}$ | 1.07$^{e}$ | 4.40$^{a}$ |

*Values followed by the same lower case alphabets in the same column are statistically equivalent ($p < 0.05$) according to the Newman-Keul's multiple range test.

**Table 5.** Germination percentage of *B. bassiana* isolates on various media at 8$^{th}$ and 16$^{th}$ h of incubation.

| Isolates | CN ratio – 35:1 (Glucose (4%) and Peptone (1%)) | | CN ratio – 10:1 (Glucose (0.6%) and Peptone (1%)) | | CN ratio – 75:1 (Glucose (9.1%) and Peptone (1%)) | | CN ratio – 8:1 Peptone (2%) | | CN ratio – 3.6:1 Yeast extract (1%) | | CN ratio – 10:1 (Potato Dextrose agar) | | OSM (Glucose (8%), Peptone (2%), KCl (5.5%)) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 8$^{th}$ h | 16$^{th}$ h | 8$^{th}$ h | 16$^{th}$ h | 8$^{th}$ h | 16$^{th}$ h | 8$^{th}$ h | 16$^{th}$ h | 8$^{th}$ h | 16$^{th}$ h | 8$^{th}$ h | 16$^{th}$ h | 8$^{th}$ h | 16$^{th}$ h |
| UB1 | 31.00$^{d}$ | 100$^{a}$ | 31.60$^{e}$ | 100$^{a}$ | 25.50$^{e}$ | 100$^{a}$ | 36.20$^{d}$ | 100$^{a}$ | 30.00$^{g}$ | 100$^{a}$ | 27.60$^{e}$ | 100$^{a}$ | 20.50$^{f}$ | 100$^{a}$ |
| UB2 | 38.60$^{b}$ | 100$^{a}$ | 45.70$^{b}$ | 100$^{a}$ | 35.00$^{d}$ | 100$^{a}$ | 45.80$^{b}$ | 100$^{a}$ | 35.60$^{e}$ | 100$^{a}$ | 32.10$^{d}$ | 100$^{a}$ | 30.00$^{d}$ | 100$^{a}$ |
| UB3 | 36.64$^{c}$ | 100$^{a}$ | 36.00$^{d}$ | 100$^{a}$ | 36.90$^{c}$ | 100$^{a}$ | 36.40$^{d}$ | 100$^{a}$ | 36.10$^{d}$ | 100$^{a}$ | 36.00$^{c}$ | 100$^{a}$ | 40.60$^{b}$ | 100$^{a}$ |
| UB4 | 26.97$^{e}$ | 100$^{a}$ | 26.50$^{f}$ | 100$^{a}$ | 25.60$^{e}$ | 100$^{a}$ | 24.40$^{f}$ | 100$^{a}$ | 31.00$^{f}$ | 100$^{a}$ | 28.00$^{e}$ | 100$^{a}$ | 23.01$^{e}$ | 100$^{a}$ |
| UB5 | 40.00$^{b}$ | 100$^{a}$ | 39.80$^{c}$ | 100$^{a}$ | 41.40$^{b}$ | 100$^{a}$ | 38.00$^{d}$ | 100$^{a}$ | 40.80$^{c}$ | 100$^{a}$ | 40.10$^{b}$ | 100$^{a}$ | 37.50$^{c}$ | 100$^{a}$ |
| UB6 | 30.20$^{d}$ | 100$^{a}$ | 26.10$^{f}$ | 100$^{a}$ | 24.40$^{e}$ | 100$^{a}$ | 32.70$^{e}$ | 100$^{a}$ | 27.62$^{h}$ | 100$^{a}$ | 25.40$^{f}$ | 100$^{a}$ | 20.45$^{f}$ | 100$^{a}$ |
| UB7 | 0$^{g}$ | 92.70$^{b}$ | 0$^{i}$ | 45.40$^{c}$ | 0$^{i}$ | 35.00$^{f}$ | 14.40$^{g}$ | 60.20$^{c}$ | 12.10$^{k}$ | 73.00$^{c}$ | 10.60$^{j}$ | 73.60$^{b}$ | 0$^{j}$ | 49.50$^{c}$ |
| UB8 | 1.40$^{g}$ | 54.20$^{e}$ | 2.20$^{h}$ | 24.60$^{g}$ | 1.00$^{hi}$ | 20.40$^{i}$ | 11.50$^{hi}$ | 36.80$^{f}$ | 13.00$^{j}$ | 41.60$^{j}$ | 11.50$^{i}$ | 38.80$^{h}$ | 0$^{j}$ | 27.60$^{g}$ |
| UB9 | 2.20$^{g}$ | 67.80$^{d}$ | 2.20$^{h}$ | 25.80$^{g}$ | 1.60$^{h}$ | 27.50$^{g}$ | 5.00$^{k}$ | 36.80$^{f}$ | 12.00$^{k}$ | 51.60$^{i}$ | 6.00$^{k}$ | 26.50$^{i}$ | 1.80$^{i}$ | 19.40$^{j}$ |
| UB10 | 6.60$^{f}$ | 35.80$^{i}$ | 2.60$^{h}$ | 29.00$^{f}$ | 3.50$^{g}$ | 25.20$^{h}$ | 9.40$^{ij}$ | 55.20$^{d}$ | 7.60$^{m}$ | 69.00$^{e}$ | 6.10$^{k}$ | 52.00$^{g}$ | 4.00$^{h}$ | 27.60$^{g}$ |
| UB11 | 7.00$^{f}$ | 28.20$^{j}$ | 6.00$^{g}$ | 43.00$^{d}$ | 5.80$^{f}$ | 45.60$^{c}$ | 7.70$^{j}$ | 47.20$^{e}$ | 10.70$^{i}$ | 83.00$^{b}$ | 6.00$^{k}$ | 23.30$^{j}$ | 0$^{j}$ | 20.50$^{i}$ |
| UB12 | 0$^{g}$ | 49.00$^{f}$ | 0$^{i}$ | 51.20$^{b}$ | 0$^{i}$ | 42.00$^{d}$ | 0$^{i}$ | 59.80$^{c}$ | 0$^{n}$ | 66.40$^{f}$ | 0$^{i}$ | 61.20$^{e}$ | 0$^{j}$ | 51.70$^{b}$ |
| UB13 | 0$^{g}$ | 46.20$^{g}$ | 0$^{i}$ | 45.90$^{c}$ | 0$^{i}$ | 42.50$^{d}$ | 13.60$^{gh}$ | 52.96$^{d}$ | 12.00$^{k}$ | 60.60$^{g}$ | 15.10$^{g}$ | 66.60$^{d}$ | 0$^{j}$ | 40.00$^{e}$ |
| UB14 | 0$^{g}$ | 44.00$^{h}$ | 0$^{i}$ | 41.80$^{d}$ | 0$^{i}$ | 37.90$^{e}$ | 0$^{i}$ | 46.20$^{e}$ | 0$^{n}$ | 54.60$^{h}$ | 0$^{i}$ | 53.42$^{f}$ | 0$^{j}$ | 45.00$^{d}$ |
| UB15 | 0$^{g}$ | 46.40$^{g}$ | 0$^{i}$ | 31.60$^{e}$ | 0$^{i}$ | 26.90$^{g}$ | 10.80$^{i}$ | 69.40$^{b}$ | 15.10$^{i}$ | 70.60$^{d}$ | 12.10$^{h}$ | 68.50$^{c}$ | 0$^{j}$ | 21.50$^{h}$ |
| UB16 | 30.40$^{d}$ | 83.40$^{c}$ | 39.20$^{c}$ | 100$^{a}$ | 25.60$^{e}$ | 73.40$^{b}$ | 41.20$^{c}$ | 100$^{a}$ | 45.90$^{b}$ | 100$^{a}$ | 40.60$^{b}$ | 100$^{a}$ | 5.10$^{g}$ | 35.40$^{f}$ |
| AB1 | 61.80$^{a}$ | 100$^{a}$ | 51.30$^{a}$ | 100$^{a}$ | 56.00$^{a}$ | 100$^{a}$ | 67.40$^{a}$ | 100$^{a}$ | 54.40$^{a}$ | 100$^{a}$ | 47.40$^{a}$ | 100$^{a}$ | 50.20$^{a}$ | 100$^{a}$ |

*Values followed by the same lower case alphabets in the same column are statistically equivalent ($p < 0.05$) according to the Newman-Keul's multiple range test

**Table 6.** Growth rate and spore yield of *B. bassiana* isolates on various media.

| Isolates | CN ratio – 35:1 (Glucose (4%) and Peptone (1%)) | | CN ratio – 10:1 (Glucose (0.6%) and Peptone (1%)) | | CN ratio – 75:1 (Glucose (9.1%) and Peptone (1%)) | | CN ratio – 8:1 Peptone (2%) | | CN ratio – 3.6:1 Yeast extract (1%) | | CN ratio – 10:1 (Potato Dextrose agar) | | OSM (Glucose (8%), Peptone (2%), KCl (5.5%) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Growth rate (mm/d) | Spore yield (x $10^7$) | Growth rate (mm/d) | Spore yield (x $10^7$) | Growth rate (mm/d) | Spore yield (x$10^7$) | Growth rate (mm/d) | Spore yield (x$10^7$) | Growth rate (mm/d) | Spore yield (x $10^7$) | Growth rate (mm/d) | Spore yield (x $10^7$) | Growth rate (mm/d) | Spore yield (x $10^7$) |
| UB1 | $1.00^{e}$ | $3.60^{b}$ | $1.03^{h}$ | $3.75^{b}$ | $0.84^{g}$ | $3.05^{cd}$ | $0.70^{k}$ | $4.40^{bcd}$ | $1.23^{i}$ | $4.08^{d}$ | $1.15^{h}$ | $3.04^{c}$ | $1.00^{f}$ | $2.75^{c}$ |
| UB2 | $1.52^{abc}$ | $3.00^{c}$ | $1.53^{e}$ | $3.00^{c}$ | $0.99^{ef}$ | $3.50^{c}$ | $1.10^{i}$ | $3.75^{d}$ | $1.85^{g}$ | $6.02^{a}$ | $1.03^{i}$ | $5.56^{a}$ | $1.02^{ef}$ | $3.00^{c}$ |
| UB3 | $1.13^{de}$ | $2.25^{ef}$ | $1.33^{f}$ | $2.95^{c}$ | $1.19^{d}$ | $2.50^{de}$ | $1.94^{d}$ | $3.60^{d}$ | $1.95^{e}$ | $4.12^{d}$ | $1.43^{de}$ | $2.02^{de}$ | $1.06^{e}$ | $2.80^{c}$ |
| UB4 | $1.22^{d}$ | $2.60^{d}$ | $1.02^{h}$ | $2.10^{de}$ | $0.83^{g}$ | $4.20^{b}$ | $1.67^{g}$ | $4.85^{bc}$ | $1.92^{ef}$ | $5.06^{c}$ | $1.74^{a}$ | $2.54^{d}$ | $1.35^{b}$ | $3.15^{c}$ |
| UB5 | $1.40^{c}$ | $3.10^{c}$ | $1.78^{d}$ | $4.10^{ab}$ | $1.09^{e}$ | $3.40^{c}$ | $1.95^{d}$ | $1.30^{f}$ | $2.03^{d}$ | $3.06^{f}$ | $1.57^{b}$ | $3.52^{c}$ | $1.14^{d}$ | $3.05^{c}$ |
| UB6 | $1.59^{ab}$ | $4.05^{a}$ | $1.52^{e}$ | $4.40^{a}$ | $0.93^{f}$ | $5.50^{a}$ | $1.62^{g}$ | $1.80^{f}$ | $2.01^{d}$ | $2.02^{g}$ | $1.52^{c}$ | $4.58^{b}$ | $1.19^{c}$ | $4.20^{a}$ |
| UB7 | $0.75^{f}$ | $1.90^{f}$ | $1.45^{e}$ | $1.80^{e}$ | $1.18^{d}$ | $1.10^{h}$ | $1.24^{h}$ | $2.00^{f}$ | $1.38^{k}$ | $3.00^{f}$ | $0.95^{j}$ | $2.35^{d}$ | $0.63^{i}$ | $1.30^{e}$ |
| UB8 | $1.49^{bc}$ | $0.90^{i}$ | $1.20^{g}$ | $1.05^{f}$ | $1.22^{d}$ | $4.30^{b}$ | $1.04^{ij}$ | $4.50^{bcd}$ | $2.09^{c}$ | $5.00^{c}$ | $0.62^{k}$ | $4.35^{b}$ | $0.94^{g}$ | $3.55^{b}$ |
| UB9 | $1.57^{abc}$ | $1.55^{g}$ | $2.01^{c}$ | $1.75^{e}$ | $1.35^{c}$ | $2.40^{def}$ | $1.83^{f}$ | $5.05^{b}$ | $2.03^{d}$ | $4.50^{d}$ | $1.03^{i}$ | $2.10^{de}$ | $0.65^{i}$ | $2.05^{d}$ |
| UB10 | $1.18^{de}$ | $2.15^{ef}$ | $2.02^{c}$ | $2.10^{de}$ | $1.50^{b}$ | $2.10^{efg}$ | $2.00^{cd}$ | $4.10^{cd}$ | $2.25^{b}$ | $4.10^{d}$ | $1.33^{g}$ | $3.15^{c}$ | $0.75^{h}$ | $2.10^{d}$ |
| UB11 | $1.40^{c}$ | $1.30^{gh}$ | $1.56^{e}$ | $1.70^{e}$ | $1.42^{bc}$ | $2.25^{def}$ | $1.85^{ef}$ | $4.05^{cd}$ | $2.29^{b}$ | $4.10^{d}$ | $1.42^{de}$ | $1.85^{de}$ | $0.53^{j}$ | $0.90^{e}$ |
| UB12 | $1.09^{de}$ | $1.00^{hi}$ | $2.23^{b}$ | $1.05^{f}$ | $1.85^{a}$ | $1.40^{gh}$ | $1.18^{h}$ | $2.75^{e}$ | $1.72^{h}$ | $5.05^{c}$ | $1.40^{ef}$ | $1.10^{f}$ | $1.00^{f}$ | $1.80^{d}$ |
| UB13 | $1.00^{e}$ | $0.95^{hi}$ | $1.00^{h}$ | $1.10^{f}$ | $1.00^{ef}$ | $1.60^{fgh}$ | $1.00^{j}$ | $1.90^{f}$ | $1.45^{j}$ | $1.50^{h}$ | $1.00^{i}$ | $1.50^{ef}$ | $0.75^{h}$ | $1.05^{e}$ |
| UB14 | $1.03^{e}$ | $1.25^{ghi}$ | $1.48^{e}$ | $1.55^{ef}$ | $1.27^{d}$ | $1.05^{h}$ | $3.00^{a}$ | $4.35^{bcd}$ | $1.88^{fg}$ | $4.60^{cd}$ | $1.37^{f}$ | $2.05^{de}$ | $0.38^{k}$ | $1.05^{e}$ |
| UB15 | $1.67^{a}$ | $2.50^{de}$ | $3.01^{a}$ | $2.55^{cd}$ | $1.48^{b}$ | $4.70^{b}$ | $2.05^{c}$ | $5.70^{a}$ | $2.98^{a}$ | $5.65^{b}$ | $1.47^{d}$ | $3.35^{c}$ | $2.04^{a}$ | $3.60^{b}$ |
| UB16 | $1.48^{bc}$ | $3.05^{c}$ | $1.09^{h}$ | $3.95^{ab}$ | $1.50^{b}$ | $4.35^{b}$ | $1.92^{de}$ | $1.95^{f}$ | $1.62^{i}$ | $2.35^{g}$ | $1.02^{i}$ | $4.35^{b}$ | $1.04^{ef}$ | $2.00^{d}$ |
| AB1 | $1.13^{de}$ | $2.20^{ef}$ | $1.11^{h}$ | $3.00^{c}$ | $1.00^{ef}$ | $2.50^{de}$ | $2.13^{b}$ | $3.80^{d}$ | $2.27^{b}$ | $3.56^{e}$ | $1.76^{a}$ | $2.10^{de}$ | $1.01^{ef}$ | $3.05^{c}$ |

*Values followed by the same lower case alphabets in the same column are statistically equivalent (P<0.05) according to the Newman-Keul's multiple range test.

$8^{th}$hincubation. This was followed by germination rate of > 60% by isolate UM4 and > 50% by isolate AR1. Among the *B. bassiana* isolates, isolate AB1 exhibited germi-nation potential of more than 50% at $8^{th}$ h of incubation. This was followed by germination rate of > 45% by isolate UB2 and ≥ 40% by isolate UB5. For High CN source (CN = 75:1), isolates UM2 and UM4 with germination rate of > 60% at $8^{th}$ h incubation, was followed by isolates UM3 and UM11 with germination rate of > 50%, and isolate UM1, UM5 and AR1 with > 40% germination potential among the *M. anisopliae* isolates. All these isolates exhibited an appreciable germination in the range of 75 - 100% at $16^{th}$ h of incubation. Among the *B. bassiana* isolates, isolate AB1 with germination rate of ≥ 56% at $8^{th}$ h incu-bation, was followed by isolate UB5 and UB3 with germination rate of about 40%. For the media containing Yeast extract (1%) (CN = 3.6:1), isolate UM4 showed maximum germina-tion rate of 65% at $8^{th}$ h of incubation among the *M. anisopliae* isolates. Isolates UM3 and UM11 exhibited > 50% germination potential and this was followed by isolate UM1, UM5 and AR1 with around 40% germination at $8^{th}$ h of incubation. Among the *B. bassiana* isolates, isolate AB1 showed maximum germination rate of 67% at $8^{th}$ h of incubation. Isolates UB2 and UB16 ex -hibited > 40% germination potential at $8^{th}$ h of incubation, and had all their conidia germinated at $16^{th}$ h observation. In the medium containing Peptone (2%) (CN = 8:1), it was observed

that isolate UM2 and AR1 had germination rate > 60%, isolates UM3, UM4 and UM5 with around 50% germination rate and isolate UM1 with 40% germination potential among the *M. anisopliae* isolates at $8^{th}$ h of incubation. Among the *B. bassiana* isolates at $8^{th}$ h of incubation, it was observed that isolate UB16 and AB1 had germination rate > 45%, followed by isolates UB5 with 40% germination potential. Among the *M. anisopliae* isolates at $8^{th}$ h of incubation, it was observed that isolate UM2 exhibited > 70% germination potential, followed by isolate UM4 and AR1 with > 50% germination rate and isolate UM3 with germination potential of > 45% for Potato Dextrose Agar (CN = 10:1). For isolate UM9, PDA was a stress media. Among the *B. bassiana* isolates at $8^{th}$ h of incubation, it was observed that isolate AB1 exhibited > 45% germination potential, followed by isolate UB16 and UB5 with > 40% germination rate and isolate UB3 with germination potential of > 35%; all these isolates showed complete germination at $16^{th}$ h of incubation. For osmotic stress media, among the *M. anisopliae* isolates at $8^{th}$ h of incubation, isolate UM1 and UM2 demonstrated > 60% germination potential, followed by isolate UM3 and UM4 with around 50% and isolate UM5 and AR1 with > 40% germination potential. Among the *B. bassiana* isolates at $8^{th}$ h of incubation, isolate AB1 demonstrated > 50% germination potential, followed by isolate UB3 with around 40% and isolate UB5 with > 38% germination.

**Effect of CN sources and ratio on growth rate and spore yield of *M. anisopliae* and *B. bassiana* isolates**

The study on colony growth rate on SDA (CN = 35:1), showed isolates UM2, UM3 UM4 and AR1 exhibiting an appreciable growth rate of ≥ 1.5 mm/d and spore yield in the varying range of 1 - 6 x $10^7$ spores/ml at $8^{th}$ d of incu-bation. Isolate UM6 though showed profuse growth rate of 2.6 mm/d which was considered to be highest on SDA media for the isolates studied, had less than 50% of its conidia germinated at $16^{th}$ h of incubation. The colony growth rate of the good germinating *B. bassiana* isolates like UB2, UB5 and AB1 also exhibited an appreciable colony growth rate of ≥ 1.0 mm/d and spore yield in the varying range ≥ 2 x $10^7$ spores/ml at $8^{th}$ day of incubation. Isolate UB15 though showed profuse growth rate of 1.67 mm/d which was considered to be highest on SDA media among isolates studied, had none of its conidia germinated at $16^{th}$ h of incubation and so it was not consi-dered to be good candidate. The growth rate in the low CN source (CN = 10:1) showed isolates UM2, UM4 and AR1 in the range 1.5 - 2.0 mm/d but highly varying spore yield (1 - 6 x $10^7$ spores/ml). Isolate UM10 and AR1 showed similar sporulation potential of 5.5 x $10^7$ spores/ ml. Here again isolate UM6 showed maximum growth rate (2.58 mm/d) but had poor germination potential (< 50%) even at $16^{th}$ h of incubation. The growth rate of isolate AB1, UB2 and UB5 were in the range 1.1 - 1.7 mm/d and spore yield of 3 - 4 x $10^7$ spores/ml. Isolate UB 15 showed maximum growth rate of 3.0 mm/d but it did not initiate any germination at $8^{th}$ h of incubation. Isolate UB6 showed best spore yield of 4.40 x $10^7$ spores/ml but had poor germination rate of only 26% at $8^{th}$ h of incubation. The isolates UM1, UM2, UM4 and AR1 exhibited growth rate in the range1 - 2 mm/d and variable sporulation potential (1 - 4.5x$10^7$ spores/ml) in high CN source (CN = 75:1). Isolate UM8 and UM9, for which SDA and low CN source was a stress media, showed some amount of growth on high CN nutrient source. Isolate UM6 and UM7 had an exceptionally high growth rate (> 2.0 mm/d) and spore yield (≥ 2.0x$10^7$ spores/ml) on $8^{th}$ day of incubation, but had poor germination potential. Isolate UB12 had maximum growth rate of 1.85 mm/d but showed poor germination and spores yield. In the media containing Yeast extract (1%) (CN = 3.6:1), the growth rate of all the isolates studied was quite appreciable in the range 1.5 - 2.5 mm/d with the only exception of isolates UM8 and UM9 for which this media was nutrient poor. The spore yield was maximum for isolate UM10 (5.60 x $10^7$ spores/ml), followed by isolates UM1 - 7 and AR1 in the range of 1.25 - 3.75 x $10^7$ spores/ml. The growth rate of all the *B. bassiana* isolates studied was quite variable in the range 0.7 - 3.0 mm/d with maximum growth rate shown by isolate UB14. Isolate UB15 sporulated best on Yeast extract (1%) and this was followed by isolate UB9. All the *M. anisopliae* isolates studied showed an appreciable growth rate in the range 1.0 - 2.5 mm/d, with maximum growth potential shown by isolate UM6 in Peptone (2%) (CN = 8:1). The best sporulating isolate was UM10 (5.8 x $10^7$ spores/ml) followed by isolates UM1 (4.4 x $10^7$ spores/ml) and AR1 (3.6 x $10^7$ spores/ml). All the *B. bassiana* isolates studied, showed an appreciable growth rate in the range 1.5 - 3.0 mm/d, with maximum growth potential shown by isolate UB15. The best sporulating isolate was UB2 (6.02 x $10^7$ spores/ml) followed by isolates UB15 (5.65 x $10^7$ spores/ml) and UB8 (5.0 x $10^7$ spores/ml). For Potato Dextrose Agar (CN = 10:1), all *M. anisopliae* isolates showed good growth rate in the range 0.8 - 2.0 mm/d, the best being for isolate UM6 (1.90 mm/d), followed by isolate UM3, UM4, UM5, UM10 and AR1 (≥1.4 mm/d). Isolates UM10 and AR1 exhibited maximum spore yield (> 6 x $10^7$ spores/ml) and isolate UM3 and UM7 with ≥4 x $10^7$ spores/ml. All *B. bassiana* isolates showed good growth rate in the range 1.0 - 1.8 mm/ d, the best being for isolate AB1 (1.76 mm/d) and UB4 (1.74 mm/d), followed by isolate UB5 and UB6 (≥1.5 mm/ d) . Isolate UB2 exhibited maximum spore yield (5.56 x $10^7$ spores/ml), followed by isolate UB6, UB8 and UB16 with ≥ 4 x $10^7$ spores/ml. Amongst *M. anisopliae* isolates, maximum growth potential was demonstrated by isolate UM10 (2.8 mm/d), followed by isolate UM4 (2.5 mm/d) and UM5 (2.15 mm/d) in Osmotic Stress media. Isolate AR1 exhibited maximum spore yield (4.4 x $10^7$ spores/ml) followed by isolate UM10 (3.15 x $10^7$ spores/ml) and for rest of the isolates OSM was a stress media. Amongst

*B. bassiana* isolates maximum growth potential was demonstrated by isolate UB15 (2.04 mm/d), followed by isolate UB4 (1.35 mm/d) and UB6 (1.19 mm/d) . Isolate UB6 exhibited maximum spore yield (4.20 x $10^7$ spores/ml) followed by isolate UB15 (3.60 x $10^7$ spores/ml). In general OSM was a stress media for *B. bassiana* isolates too like *M. anisopliae*.

## DISCUSSION

Such is the utility of nutrients in the survival ability of microorganisms, that, finally it must be emphasized that they require it in a balanced mix. If an essential element is below threshold in supply, then microbial growth will be limited regardless of the concentrations of other nutrients. The different *M*. *anisopliae* isolates showed significant variation ($p < 0.05$) with respect to the differing preference for the carbon sources. One of the most remarkable characteristics of microorganisms is their extraordinary flexibility with respect to carbon sources. Laboratory experiments indicate that most naturally occurring organic molecules can be utilized by most microbes but with varying success rate of germination, growth and sporulation. Gao et al. (2007) concluded that the requirement for carbon and CN ratio varied among fungal species and isolates and that the growth characteristics might be strain dependent. In this study we observed that each of the isolate had specific preference for a particular nutrient source. Isolates UM8 and UM9 were the most susceptible isolates and it was observed that the original lyophilized cultures of these were difficult to grow on almost all the nutrient media studied and so these isolates were screened out of further studies. It has been reported that nutrient source have significant effects on the spore yield of fungus (Jackson and Bothast, 1990). Jackson and Schisler (1992) reported that the nutritional environment alters the composition and attributes of fungal spores. Schisler et al. (1991) reported the influence of nutrition on conidial germination. We observed that SDA, low CN source, high CN source, peptone (2%), PDA and OSM supported maximum germination for isolate UM2, while Yeast extract (1%) did the job for isolate UM4. It was seen that isolate UM6 had maximum growth rate on almost all the six media investigated, but had poor germination potential on all of them, even after 16 h of incubation, and hence it was considered to be a poor isolate. Shah et al. (2005) observed that an increase in radial growth did not result in simultaneous increase in conidial yield. Our study too showed that the best sporulation supporting media was not the same as the media, which induced best colony growth in most *M*. *anisopliae* isolates, for most nutrient sources. We observed that SDA that produced maximum sporulation in isolate UM2, UM3 and UM4, did not produce maximum radial growth in them. Although there is a tendency for more sporulation on carbon rich source, the threshold varies with the species, strains and the nature of the CN source (Engelkes et al., 1997). Wyss et al. (2001) evaluated agar and grain media for mass production of conidia and found varying spore yield on different substrates. In this study, it was observed that OSM was a stress medium for spore harvestation in most isolates. Several authors have reported that an intermediate CN source achieves best yield in terms of growth. Shah et al. (2005) achieved maximum yields in 35:1 CN medium (similar to SDA). Similarly, Jackson and Schisler (1992) observed maximum spore yield in 30:1 CN medium and not at higher or lower CN ratios. In contrast, we found that SDA (intermediate CN source) which is the routine culture media for entomopathogenic fungi did not always support ample growth and sporulation; and high CN source and low CN source were not always marginal to the intermediate source.

In *B*. *bassiana* isolates, different nutrient media had a significant impact on conidial germination. It was observed that at $8^{th}$ h of incubation, all the CN sources showed capability of initiating germination in all the *B. bassiana* isolates except isolates UB7 and UB12 - 15 and hence these were considered poor isolates. Isolate AB1 was the most robust isolate in terms of germination potential on all the nutrient stress media used. It germinated best on Yeast extract (1%) (up to 67% at $8^{th}$ h of incubation). Isolate UB2, UB3, UB5 and UB16 also depicted commendable germination potential on different nutrient sources. A general trend observed was that, Yeast extract (1%) induced a higher germination rate in most of the *B*. *bassiana* isolates. The different *B*. *bassiana* isolates showed significant difference ($p < 0.05$) in con-text of radial growth with respect to the nutrient source. The colony growth also varied significantly ($P < 0.05$) with respect to the observation time. Isolate UB15, UB16 and AB1 were better isolates in terms of specific growth rate and spore yield on most of the nutrient sources studied. Yeast extract (1%) supported better mycelia growth than any other media. OSM was a stress medium for most isolates as it did not induce appreciable colony growth in these isolates when compared to other nutrient source. Like *M*. *anisopliae* isolates, in *B*. *bassiana* isolates too, the best sporulation supporting media was not the same as the media which induced best colony growth in them.

Upon field application, the major impending challenges that the entomopathogens face is survival against fluctuating physical environment (Tang and Hou, 2001). The germination and acclimatization of the fungal conidia, before its subsequent growth and sporulation, is the prerequisite threshold for their efficacy, and we considered germination potential as the major criteria for isolate selection. It may be stated that some of the nutrient sources used in this study were the stress media which might mimic the on-field abiotic stress such as osmotic stress and/or nutrient poor soil. The knowledge of entomopathogenic fungal ecology such as tolerance to environmental stress an contribute to a better understand of the effect of optimum factors on the survival and distribution of these fungi in filed. This in turn can enable

prediction or promote habitats that encourage amplification of natural inoculum and the induction of epizootics. The growth characteristic of vast majority of entomopathogenic fungi is clearly affected by the supply of nutrients (Humber, 2008). Our findings show that isolates UM2 ( *M. anisoplaie*) and AB1(*B. bassiana*) was the fastest germinating isolate on almost all nutrient sources studied and possessed commendable growth rate and sporulation potential on them. High germination ability on nutrient poor sources as well as osmotic stress sources highlights the endurance of the infective propagule of the isolates UM2 and AB1.

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