*Biological Forum – An International Journal* **14(1): 1389-1401(2022)**

**ISSN No. (Print): 0975-1130**
**ISSN No. (Online): 2249-3239**

# Characterization of Twenty One Isolates of *Beauveria bassiana* and Study their Bio-efficacy against *Nodostoma subcostatum*

***B. Temjenmenla[1], K.C. Puzari[1], A. Bhattacharyya[1], P.D. Nath[1] and Pranab Dutta[2*]***
*[1]Department of Plant Pathology, Assam Agricultural University, Jorhat (Assam), India.*
*[2]School of Crop Protection, College of Post Graduate Studies in Agricultural Sciences, Central Agricultural University, Umiam (Meghalya), India.*

*(Corresponding author: Pranab Dutta*)*
*(Received 29 November 2021, Accepted 06 February, 2022)*
*(Published by Research Trend, Website: www.researchtrend.net)*

**ABSTRACT: Considering the negative effects of chemical pesticides have led to emphasis on use of biological control of scarring beetle. *B. bassiana*, the white muscardine fungus was reported to be effective against banana beetle (*N. subcostatum*) in Assam, India. Information on the potentialities of *B. bassiana* for the management of *N. subcostatum* is scanty. Twenty one (21) isolates of *B. bassiana* were made banana leaf and fruit scarring beetle during the survey conducted in four North East States *viz*., Assam, Arunachal, Mizoram and Nagaland and all the isolates were characterized. Out of the different tested concentrations of *B. bassiana* evaluated in laboratory against *N. subcostatum*, concentration of 1 × $10^7$conidia/ml of water was found effective with highest mortality of 89.17 per cent at 20 days post inoculation. All the 21 isolates of *B. bassiana* tested at 1 × $10^7$ conidial concentration was found to be best with highest pathogenic to adult leaf and fruit scarring beetle causing mortalities of between 10.55 to 89.17 per cent. The highest mortality of the beetle 89.17 percent was observed in Bb3 isolated from Horticultural Experimental Farm, AAU, Jorhat. The phylogenetic analysis of twenty one isolates suggested that the *B. bassiana* isolates of North East States were grouped into four clusters, which showed close phylogenetic proximity relationship among isolates from the same host (*N. subcostatum*) but different geographical locality. No correlation was observed in the RAPD analysied clusters and the pathogenicity of *N. subcostatum*. Sequence analysis showed that six *B. bassiana* isolates of North East Region shared 89 to 99 per cent identity with the other known isolates. The isolate Bb15 and Bb4 was found to have the least sequence identity with the other isolates tested and both the isolate showed identity of 89 percent. This showed that genetic variability exists among the *B. bassiana* isolates of North East States. The present study showed that isolates of *B. Bassiana* are highly pathogenic to *N. subcostatum* and studies on molecular characterization of the isolates proved the variability at genetic level among the isolates of *B. bassiana* of North East States. These isolates can be use as a potential bioagent for the management of the pest in organic cultivation of banana, thereby it will reduce the use of chemical pesticides and save the environment.**

**Keywords:** *Beuveria bassiana, Nodostoma subcostatum*, isolate, phylogenetic, genetic variability.

## INTRODUCTION

The diverse agro climatic zones of Assam offer great potential for cultivation of banana. But production is significantly reduced due to attack of insect pests and diseases. Its quality is also affected due to attack of leaf and fruit scarring beetle, *N. subcostatum* and is considered as the specific problem to Assam, rest of N.E states, Bihar and Northern part of West Bengal (Anonymous, 2009). Its ooccurrence is usually high during the rainy season and are the main pest in pockets producing export quality plantain and banana. The adults fly directly after emerging from the soil to the fruit bunches where they feed, scarring the surface and perforating the leaves. The adult beetles feed on the unfurled leaves of the banana, on the skin of the fruits marking the fruits in such a way as to render it unsalable. Fruits infested with the beetle get scars. Severe scarring of fruit skin leads to underdeveloped fruit, that loss commercial value. Most of the scarring occurs on the lower proximal surfaces of the fingers, reflecting the fact that the beetle chooses the most sheltered spots for feeding. Carmona (2008) reported that the pest nibbles along the strings and also through the flat surfaces of tender fingers.

Normal control of scarring beetle includes bagging of fruit and application of chemicals. But these control measures involve high cost, difficulty to perform operations, insect resistance, undesirable residues, environmental pollution, etc. Considering the negative effects of chemical pesticides have led to emphasis on use of biological control of scarring beetle. *B. bassiana*, the white muscardine fungus was reported to be effective against banana beetle (*N. subcostatum*) in Assam (Roy and Puzari, 1979). Information on the potentialities of *B. bassiana* for the management of *N. subcostatum* is scanty. Therefore, the present study has been undertaken with an aim to test the bioefficacy of *B. bassiana* conidia against scarring beetle of banana and sstudy on morphological, virulence and molecular characterization of *Beauveria* isolates collected from North Eastern Region of India.

## MATERIALS AND METHOD

**Natural incidence of *Beauveria* and collection of samples.** Roving survey for the occurrence of the *B. bassiana* infecting leaf and fruit scarring beetle was carried out covering different banana growing areas in North East Region during the cropping season of 2013-14 (Table 1). The organism was isolated in potato dextrose agar media, characterized by following relevant literature. The pure culture was maintained through the investigation period on fresh PDA media.

**Table 1: Survey of natural occurrence of *Beauveria bassiana* infected scarring beetle in N.E Region.**

| Isolate | State | Locations |
|---|---|---|
| Bb1 | Assam | Rajabari, Jorhat |
| Bb2 | Assam | Horticultural Experimental Farm, AAU, Jorhat |
| Bb3 | Assam | Horticultural Experimental Farm, AAU, Jorhat |
| Bb4 | Nagaland | Mokokchung |
| Bb5 | Nagaland | Jharnapani |
| Bb6 | Nagaland | Dimapur |
| Bb7 | Assam | Nagaon |
| Bb8 | Assam | Nagaon |
| Bb9 | Assam | BNCA |
| Bb10 | Assam | BNCA |
| Bb11 | Assam | Singimari, Guwahati |
| Bb12 | Assam | Pub Balitara, Nalbari |
| Bb13 | Assam | KVK, Kahikuchi |
| Bb14 | Assam | KVK, Kahikuchi |
| Bb15 | Assam | KVK, Kachikuchi |
| Bb16 | Arunachal Pradesh | Itanagar |
| Bb17 | Assam | BhokotSapori, Majuli |
| Bb18 | Assam | BhokotSapori, Majuli |
| Bb19 | Mizoram | KVK, Kolasib |
| Bb20 | Mizoram | KVK, Kolasib |
| Bb21 | Assam | Horticultural Experimental Farm, AAU, Jorhat |

**Bioassay of *B. bassiana.*** Conidial suspension of five concentrations *viz*., $1 \times 10^5$, $1 \times 10^6$, $1 \times 10^7$, $1 \times 10^8$ and $1 \times 10^9$ conidia/ml of water were prepared by mixing Tween 80 @ 0.23 g/litre to each suspension (Puzari and Hazarika, 1991). Water along with Tween 80 provided as control. Each concentration was sprayed @ 6.5 ml/ banana plant with atomizer over leaf surface with five replicates. Mortality(%) records were taken daily and was adjusted with Abbott's formula (Abott, 1925).

**Pathogenicity test of *B. bassiana.*** For pathogenicity test, 20 adult leaf and fruit scarring beetle collected from field kept starved for 24 hours were released to the 30 days old banana plant (variety dwarf Cavendish) after 7 days of inoculation. Earthen pots (10 kg capacity) were thereafter caged with plastic cage. Dead insects were carefully picked out from each replication and kept in moist petriplates and incubated at 25$\pm$ 1ºC. Numbers of insects with white *B. bassiana* growth was recorded from the inoculated plants and from the moist Petriplates kept in incubator.

**DNA Isolation.** Genomic DNA of 21 isolates of *B. bassiana* were extracted using the CTAB extraction procedure (Zolan and Pukkila, 1996) with need based modifications. About 100-200 mg of fungal mat was placed on a microfuge tube and grinded thoroughly with a pinch of grinding sand and 750 µl of lysis buffer. Samples were incubated for 30 minutes at 74ºC. The samples were inverted after every 10 minutes. The mixture was centrifuged and 1 vol of Chloroform: Isoamyl alcohol (24:1) was added to the supernatant. The emulsions were centrifuged at 10000 rpm for 10 minutes and the aqueous phase recovered and taken to another tube. DNA was precipitated by adding equal volume of cold Isopropanol. DNA was collected by centrifugation at 14000 rpm for 10 minutes. The supernatant was discarded and the pellet was washed

with 70% ethanol (250 µl) twice and dried at room temperature. DNA was suspended in 50-100 µl TE buffer and stored at -20ºC until further use. DNA concentration was determined by measuring the optical density at $OD_{260}$ and $OD_{280}$ and $OD_{260}/OD_{280}$ ratio was calculated.

**RAPD Analysis.** Thirteen (13) different RAPD primers (Bangalore Genei) were used for DNA finger printing. The primers were RAPD 1, RAPD 2, RAPD 3, RAPD 4, RAPD 5, RAPD 6, RAPD 7, RAPD 8, RAPD 9, RAPD 10, RAPD 11, RAPD 12 and RAPD 13.The PCR reaction was performed in a 25 µl PCR mix containing 2.0 µl of the template DNA , 2.5 µl 10X Buffer, 1.5 µl of 15mM $MgCl_2$, 2.0 µl of 10mM dNTPs, 0.9 µl of *Taq* DNA polymerase, 2.0 µl of RAPD primer and 14.1 µl of double distilled water. A standardized PCR cycle was used and data were analysed based on Jaccard's Coefficient similarity matrix using NTSYS (Numeral Taxonomy and Multivariate Analysis System) software (Rohlf, 1993).

**Primer designing.** A pair of *B. Bassiana* specific primer was used to amplify the DNA of six (6) *B. bassiana* isolates. The isolates were selected randomly with four isolates from Assam and two isolates from Nagaland. The sequences are as follows BBF1-AAGTAG TCGCGGCTAGCAGG andBBR1-TGTTCATTTATCTTGTCAAGCTAAAACTAAT and the expected product length was 501 bp.

**Polymerase Chain Reaction( PCR) for *B. Bassiana* specific primer**

The PCR reaction was performed in a 25 µl PCR mix containing 2.0 µl of the template DNA, 2.5 µl 10X Buffer, 1.5 µl of 15mM $MgCl_2$, 2.0 µl of 10mM dNTPs, 0.9 µl of *Taq* DNA polymerase, 1.0 µl of each BB forward and reverse primers and 14.1 µl of double distilled water. Constituents were mixed well by vortexing and the PCR was run in a thermo cycler with a standardized programme.

**Molecular characterization of *B. bassiana* isolates through gene sequencing.** For gene sequencing, altogether six isolates of *B. bassiana* were selected. Two isolates from Jorhat (Bb2 and Bb3), one isolate from Guwahati (Bb15), one isolate from Majuli (Bb17) of Assam and two isolates from Mokokchung and Jharnapani of Nagaland (Bb4 and Bb5). For molecular characterization the PCR amplified gene was sequenced. Sequencing was done in Bangalore Genei.

**Sequence and phylogenetic analysis.** The sequences were aligned in a global multiple sequence alignment programme, MEGA 5.2.2 and ClustalX version 1.81and edited with BioEdit and aligned. Phylogenetic analysis was performed using TreeView (TreeViewX) software and sequences were subjected to BLAST searches.

## RESULTS AND DISCUSSION

### Morphological and cultural characteristics of fungal isolates

The cadavers of banana scarring beetle were brought carefully to the Mycology Research Section, Department of Plant Pathology, AAU, Jorhat, Assam and were presumed to be infected by *Beauveria* sp. were used for isolation of the fungus. Twenty one indigenous isolates of *B. bassiana* were isolated, characterized (Table 2).

**Table 2: Cultural characters of *Beauveria bassiana* isolates.**

| Isolates | No. of days of sporulation | Colony colour | Colony diameter on 10th DAI (mm) |
|---|---|---|---|
| Bb1 | 12 | Creamish white | 32.24 |
| Bb2 | 10 | Yellowish white | 32.67 |
| Bb3 | 10 | Creamish white | 33.21 |
| Bb4 | 11 | Creamish white | 29.13 |
| Bb5 | 11 | Creamish white | 30.59 |
| Bb6 | 10 | Creamish white | 30.35 |
| Bb7 | 10 | Yellowish white | 30.66 |
| Bb8 | 10 | Yellowish white | 30.15 |
| Bb9 | 15 | Creamish white | 29.84 |
| Bb10 | 14 | Creamish white | 29.72 |
| Bb11 | 10 | Creamish white | 25.38 |
| Bb12 | 14 | Yellowish white | 26.24 |
| Bb13 | 15 | Creamish white | 21.12 |
| Bb14 | 14 | Yellowish white | 22.63 |
| Bb15 | 14 | Yellowish white | 21.32 |
| Bb16 | 15 | Yellowish white | 15.57 |
| Bb17 | 10 | Creamish white | 32.33 |
| Bb18 | 10 | Yellowish white | 31.48 |
| Bb19 | 14 | Creamish white | 18.16 |
| Bb20 | 14 | Creamish white | 20.02 |
| Bb21 | 14 | Creamish white | 31.28 |

DAI – Days after incubation

During this study we have not observed any variation of the hyphal and conidial characters among the isolates but showed variation in time of sporulation and radial growth. Among the different isolates *B. bassiana*, Bb3 collected from AAU, Jorhat recorded rapid growth in PDA medium with highest colony diameter (33.21 mm) while the lowest colony diameter (15.57 mm) after ten days of incubation was observed in Bb16 isolated from Itanagar, Arunachal Pradesh. The number of days of sporulation among the isolates ranged from 10 to 15 days after incubation. These results indicate variability among the isolates of *B. bassiana*. The reason might be a physiological phenomenon of the adaptability of fungus in different environmental parameters prevailed in different surveyed sites of North East Region and the frequency of exposure of the fungus with the host (passing through the host). Das *et al.* (2011) reported variability of twenty three *B. bassiana* strains while screened for biological traits of against rice hispa, *Dicladespa armigera* (Olivier) (Coleoptera: Chrsomeloidae).

**Pathogenicity of *B. bassiana* against *N. subcostatum*.** All the twenty one isolates were evaluated for pathogenicity at five different conidial concentrations ($1\times10^5$ to $1 \times 10^9$ conidia/ ml). At two days after treatment (DAT) significantly, maximum mortality of (11.67%) was recorded in $1\times10^7$ conidia/ ml. After four DAT, $1 \times 10^7$ conidia/ ml was found to cause 15.00 per cent mortality and the other concentrations were statistically significant and at par to each other (Table 3). The highest mortality rate at 6 DAT was 19.59 per cent at $1\times10^7$ conidia /ml and was found to be statistically at par with $1\times 10^8$ (17.84%) and $1 \times 10^9$ (16.71%). The later is however at par with $1\times10^5$ and $1\times10^6$ conidia /ml. Similarly at 8, 10 and 12 DAT, $1 \times 10^7$conidia /ml showed the highest mortality (21.44, 29.63 and 39.65%) followed by $1\times10^8$ conidia /ml (18.33, 24.07 and 33.99%). However, after 14 DAT and 16 DAT, $1\times10^7$ was significantly superior over other concentrations recording 55.56 and 70.37 per cent germination. The remaining concentrations $1\times10^5$, $1\times10^6$, $1\times10^8$ and $1 \times 10^9$conidia /ml were found to be statistically at par with each other. After 20 DAT, $1\times10^7$ conidia /ml exhibited significantly highest mortality percentage of 89.17 per cent over the other concentrations. *B. Bassiana* isolate (Bb3) collected from AAU, Jorhat recorded the highest pathogenicity (89.17%).

**Table 3: Corrected mortality percentage of leaf and fruit scarring beetle inoculated with *B. bassiana* at different days and concentrations.**

| Concentration (Conidia ml$^{-1}$) | Corrected mortality (%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 2 DAI | 4 DAI | 6 DAI | 8 DAI | 10 DAI | 12 DAI | 14 DAI | 16 DAI | 18 DAI | 20 DAI |
| Control | 0.00±0.00 (0.00)$^{a}$ | 0.00±0.00 (0.00)$^{a}$ | 0.00±0.00 (0.00)$^{a}$ | 0.00±0.00 (0.00)$^{a}$ | 0.00±0.00 (0.00)$^{a}$ | 0.00±0.00 (0.00)$^{a}$ | 0.00±0.00 (0.00)$^{a}$ | 0.00±0.00 (0.00)$^{a}$ | 0.00±0.00 (0.00)$^{a}$ | 0.00±0.00 (0.00)$^{a}$ |
| $1 \times 10^5$ | 5.00±0.00 (12.92)$^{b}$ | 6.67±2.89 (14.76)$^{b}$ | 11.67±2.89 (19.88)$^{b}$ | 12.47±2.89 (20.60)$^{b}$ | 18.52±3.21 (25.43)$^{b}$ | 26.32±2.45 (27.9)$^{b}$ | 34.49±3.95 (25.46)$^{b}$ | 37.04±3.21 (32.35)$^{b}$ | 44.36±9.02 (44.13)$^{b}$ | 44.58±10.18 (47.00)$^{b}$ |
| $1 \times 10^6$ | 6.67±2.89 (14.76)$^{bc}$ | 8.33±2.89 (16.59)$^{b}$ | 13.33±2.89 (21.34)$^{ab}$ | 12.47±2.89 (20.60)$^{b}$ | 20.37±6.41 (26.66)$^{b}$ | 32.02±2.26 (31.89)$^{bc}$ | 38.19±6.62 (29.12)$^{b}$ | 45.92±17.56 (33.83)$^{b}$ | 49.65±10.30 (45.56)$^{b}$ | 59.44±5.29 (50.50)$^{bc}$ |
| $1 \times 10^7$ | 11.67±2.89 (19.88)$^{d}$ | 15.00±0.00 (22.79)$^{c}$ | 19.59±0.00 (26.56)$^{c}$ | 20.00±2.53 (26.24)$^{d}$ | 29.63±3.21 (32.96)$^{c}$ | 39.65±1.32 (36.79)$^{d}$ | 55.56±5.56 (42.26)$^{c}$ | 70.37±6.41 (51.20)$^{c}$ | 81.79±6.40 (59.83)$^{d}$ | 89.17±10.10 (75.29)$^{d}$ |
| $1 \times 10^8$ | 10.00±0.00 (18.43)$^{cd}$ | 11.67±2.89 (19.88)$^{b}$ | 17.84±2.89 (25.30)$^{c}$ | 18.33±2.82 (24.94)$^{cd}$ | 24.07±6.41 (29.24)$^{bc}$ | 33.99±1.13 (33.10)$^{cd}$ | 40.28±3.67 (30.51)$^{b}$ | 54.44±7.29 (38.95)$^{b}$ | 75.49±8.38 (54.35)$^{cd}$ | 87.08±6.88 (61.27)$^{c}$ |
| $1 \times 10^9$ | 8.33±5.77 (16.21)$^{bcd}$ | 10.00±5.00 (18.05)$^{b}$ | 16.67±2.89 (24.05)$^{bc}$ | 14.23±2.70 (22.10)$^{bc}$ | 25.92±3.21 (30.58)$^{bc}$ | 32.13±3.90 (31.82)$^{bc}$ | 37.96±12.53 (29.27)$^{b}$ | 50.37±10.56 (35.86)$^{b}$ | 71.68±5.63 (50.84)$^{bc}$ | 85.00±4.33 (54.67)$^{bc}$ |
| S.Ed(±) | 2.33 | 2.38 | 1.57 | 1.66 | 2.44 | 2.16 | 2.60 | 3.62 | 3.75 | 6.11 |
| C.D (P=0.05) | 5.08 | 5.19 | 3.42 | 3.63 | 5.32 | 4.71 | 5.66 | 7.87 | 8.17 | 13.32 |

DAI = Days after inoculation

Figures within parentheses are angular transformed values

In column mean followed by common letter do not different significantly at 5% level of probability

The entomopathogenicity is determined by fungal growth and its sporulation on the surface of the dead targeted insect. The entomopathogen, *B. bassiana* kill the targeted host by hyphae that germinate from inoculam like spores that penetrate the exocuticle, invade to the insect body tissues and ramify within the body tissues and destroy the internal tissues (Schaerfenberg, 1964). The scarring beetles in the present were probably infected by the same way. About 3-7 days after death, whitish mycelial growth was seen covering the whole dead insect body. The fungus was found to sporulate on the infected cadaver on ten to eleven days. Similar findings was reported by Puzari *et al.* (1994) that white frosty mycelial growth of *B. bassiana* covered the entire body surfaces of *Dicladispa armigera* and hyphae emerged through the intersegmental sutures, dorso- lateral as well as latero-ventral sides of the thorax and abdomen of the adults. Phukan *et al*. (2008) reported that with the increase in *B. bassiana* infection time in *Dicladispa armigera*, total haemocyte count (THC) decreased with an increase in granulocytes population, which reacted in disintegration of the plasmamembrane. They also observed formation of fine pseudopod-like cytoplasmic extensions and finally clumping of cells. Carruthers and Soper (1987) reported that beetles are more susceptible to fungal pathogens than other insect groups. Moreover, rougher the body surface, better for spore anchorage and mycelial ramification. Spores germinate on the body surface but grow profusely inside the haemocoel, utilizing haemolymph (Puzari *et al*., 1994). Our SEM study showed that another enotomopathogen *Metrahizium anisopliae* can penetrate into the body of *Aphis cracivora*, ramify and spore germinate on the

body and causes deformation of body part and ultimately death of the host (Boruah *et al.,* 2016; Dutta *et al.,* 2018). Hazarikia *et al*., (2016) observed that entomopathogen *Nomurea rileyi* can causes significant damage to the *Helicoverpa armigera* up on its infection and causes death.

In this study, the pathogenicity of the most potential isolate Bb3 was tested against *N. subcostatum* in the laboratory using conidial concentration $1 \times 10^5$ to $1 \times 10^9$ conidia/ml. At conidial concentration $1 \times 10^7$ conidia/ml, mortality was found more than the uninoculated control and the rate was found significant in terms of conidial concentration and incubation periods of the entomopathogen or we can say the mortality rate of *N. subcostatum* was found to proportional (directly to the incubation period of the fungus till 20 days because of the population of inoculam present in the respective concentration of the entomopathogen which was incubated at a particular period of time for determination entomopathogenicity. The per cent mortality (89.17) was highest in $1 \times 10^7$ concentration progressively increased from 11.67 at 2 days after inoculation (DAI) to 20 DAI. Virulence is a function of pathogenic organism. 100 per cent mortality of larvae of *Scolytus scolytus* and adults of *Cyla formicarius* after seven days exposure in more than $1 \times 10^6$ conidia/ml of *B. bassiana* observed by Barson (1977). In the present study, the maximum mortality was observed at 20 days after inoculation when $1 \times 10^7$ conidia/ml inoculums was used. Burdeos and Villacarlos (1989) while working on sweet potato weevil, *Cylas formicarius* (F.) reported that *B. Bassiana* required higher spore concentration and shorter exposure period to kill 50 per cent of the worker population. The fungus, *B. bassiana* was was found to be more effective at a concentration of $10^7$ to $10^9$ spores/ml against *Leptispa pygmaea* Baly (Coleoptera: Chrysomelidae) had been observed in the studies carried out by Karthikeyan and Jacob (2010). The present findings on increased mortality due to increase in concentration of conidia upto a certain extent ($1 \times 10^7$ conidia/ml in water) are in line with the works of Ramos *et al.* (2004) who reported that isolates of *B. bassiana*, *M. anisopliae*, *Paecilomyces* spp. and *Verticillium lecanii* were found pathogenic to nymphs of *Bemisia tabaci* and caused 10 to 89 per cent mortality when treated at a concentration of $10^7$ conidia/ml. Vanmathi *et al*. (2011) showed that *B. bassiana* tested at different concentrations ($1 \times 10^4$ to $1 \times 10^8$ conidia/ml) against the pulse beetle, *Callosobruchus maculatus* was effective at higher concentration causing 99.44 per cent of adult mortality. Boruah and Dutta (2014) also showed successful result of *M. anisopliae* based bioformulation against cowpea aphid, *Aphis craccivora.*

**Testing of efficacy of *B. bassiana* isolates against *N. subcostatum*.** The bioefficacy test of twenty one *B. bassiana* isolates against *N. subcostatum* was done at conidial concentration $1 \times 10^7$ conidia/ml. The range of pathogenecity was highly variable ranging from 10.55 to 89.17 per cent (Table 4). Earlier worker reported the differences in virulence among isolates of entomopathogens like, *B. bassiana* on *Plutella xylostella* (Vandenberg *et al*., 1998) and *L. decemlineata* (Todorova *et al*., 1994).

**Table 4. Corrected mortality percentage of leaf and scarring beetle inoculated with *B. bassiana* isolates.**

| ***B. bassiana* isolates** | **Corrected mortality (%)** |
|---|---|
| Control | 0.00±0.00(0.00)$^{a}$ |
| Bb1 (Rajabari, Jorhat) | 78.75±3.31(62.59)$^{ghi}$ |
| Bb2 (AAU, Jorhat) | 85.14±9.47(68.03)$^{ghi}$ |
| Bb3 (AAU, Jorhat) | 89.17±10.10(74.24)$^{hi}$ |
| Bb4 (Mokokchung, Nagaland) | 87.22±6.27(69.48)$^{ghi}$ |
| Bb5 (Jharnapani, Nagaland) | 82.64±14.18(66.51)$^{ghi}$ |
| Bb6 (Dimapur, Nagaland) | 46.53±11.84(42.95)$^{ef}$ |
| Bb7 (Nagaon, Assam) | 44.58±5.05(41.88)$^{ef}$ |
| Bb8 (Nagaon, Assam) | 40.28±6.01(39.36)$^{def}$ |
| Bb9 (BNCA, Assam) | 27.50±6.49(31.51)$^{cde}$ |
| Bb10 (BNCA, Assam) | 25.42±10.48(29.99)$^{bcd}$ |
| Bb11 (Singimari, Guwahati) | 29.58±8.87(32.77)$^{cde}$ |
| Bb12 (Pub Balitara, Nalbari) | 33.75±15.15(35.18)$^{def}$ |
| Bb13 (Kahikuchi, Guwahati) | 76.39±10.48(61.34)$^{ghi}$ |
| Bb14 (Kahikuchi, Guwahati) | 72.64±12.54(58.94)$^{g}$ |
| Bb15 (Kahikuchi, Guwahati) | 78.75±9.44(62.91)$^{ghi}$ |
| Bb16 (Itanagar, Arunachal Pradesh) | 53.19±3.13(46.83)$^{f}$ |
| Bb17 (BhokotSapori, Majuli) | 87.03±11.20(72.59)$^{hi}$ |
| Bb18 (BhokotSapori, Majuli) | 83.05±6.99(65.96)$^{ghi}$ |
| Bb19 (Kolasib, Mizoram) | 14.86±3.39(22.59)$^{bc}$ |
| Bb20 (Kolasib, Mizoram) | 10.55±3.37(18.79)$^{b}$ |
| Bb21 (AAU, Jorhat) | 74.44±6.32(59.75)$^{g}$ |
| S.Ed(±) | 5.83 |
| C.D (P=0.05) | 11.75 |

Figures within parentheses are angular transformed values

In column mean followed by common letter do not different significantly at 5% level of probability

The level of effectiveness obtained in the present study compares favourably with Omukoko *et al*. (2011) who reported that isolates of *B. bassiana* @ $1 \times 10^8$ conidia/ml tested were found to be pathogenic to *Cosmopolites sordidus* causing mortalities of between 20-50 per cent by 40 days post exposure. Todorova *et al*. (2000) observed the significant differences in mortality between the ten *B. bassiana* isolates tested on *L. decemlineata*, *M. persicae* and *C. maculatalengi* at a concentration of $1 \times 10^7$ conidia/ml. Proteins is the major constituent of the insect cuticle, so fungi usage a combined enzymatic process and mechanical forces to disintegrate insect host cuticle (by proease enzyme) (Butt, 2002). The growth and development as well as virulence of entomopathogenic hyphomycetes fungi like *B. bassiana*, *M. anisopliae* (Shah *et al*., 2005) are influenced by nutritional conditions and especially the carbon: nitrogen content (Safavi *et al*., 2007). From the results it is quite evident that the *B. bassiana* was able to inflict mortality on *N. subcostatum* and this can be used as a biocontrol agent for banana scarring beetle. The present study confirms to earlier findings of Puzari *et al*., (1994); Hazarika and Puzari (1995); Pegu *et al*., (2013); Dutta *et al*., (2014); Boruah and Dutta, (2014); Puzari *et al*. (2015), while working on entomopathogenic fungi alone different insect pests. In an another study Hazarika and Puzari (1995) reported the efficacy of *B. bassiana* @ 10 million spores as on par with neem oil and monocrotophos @1% against coleopteran pests, Rice hispa. *B. bassiana* used in the formulation was found effective in field tests for the management of cotton leaf folder. Under field conditions, the fungus *B. bassiana* brought about 61 to 72 per cent reduction of leaf damage over untreated control and was superior to all tested biopesticides, paraffin oil, neem oil and azadirachtin against the rice blue beetle (Karthikeyan and Jacob 2010). Recently, the antagonistic potential and antifungal fungal properties of *B. bassiana* against *Pythium myriotylum* causing damping off of tomato and *Curvularia lunata* causing leaf spot of rice has also been reported (Deb and Dutta, 2021; Deb *et al.*, 2021).

**Quantitative analysis by Spectrophotometer.** Results of Spectrophotometric analysis presented in the Table 5 revealed that majority (90%) of the samples were having $A_{260}/A_{280}$ ratio above 1.7 with DNA concentration as high as 2657.2 ng/µl and as low as 1093.6 ng/µl.

**Table 5: Quantification and purity determination of genomic DNA using CTAB method.**

| Sr. No. | $A_{260}$ | $A_{280}$ | $A_{260}/A_{280}$ | $A_{280}/A_{260}$ | Concentration (ng/µl) |
|---|---|---|---|---|---|
| 1. | 48.22 | 23.96 | 2.01 | 2.03 | 2411.1 |
| 2. | 51.43 | 26.54 | 2.05 | 2.07 | 2426.7 |
| 3. | 52.38 | 27.76 | 2.06 | 2.09 | 2507.1 |
| 4. | 44.99 | 22.21 | 2.03 | 1.94 | 2249.8 |
| 5. | 43.31 | 21.96 | 1.97 | 1.89 | 2165.8 |
| 6. | 46.92 | 23.22 | 2.02 | 2.07 | 2346.2 |
| 7. | 41.59 | 20.36 | 1.95 | 1.87 | 2059.1 |
| 8. | 43.21 | 21.90 | 1.96 | 1.87 | 2158.3 |
| 9. | 55.76 | 29.34 | 2.09 | 2.11 | 2657.2 |
| 10. | 54.25 | 28.43 | 2.08 | 2.10 | 2604.8 |
| 11. | 45.63 | 22.41 | 1.99 | 1.89 | 2271.4 |
| 12. | 54.61 | 29.48 | 2.13 | 2.08 | 2579.8 |
| 13. | 39.45 | 18.24 | 1.92 | 1.85 | 2059.1 |
| 14. | 45.73 | 22.56 | 2.01 | 2.06 | 2286.7 |
| 15. | 44.50 | 21.78 | 1.99 | 1.86 | 2209.8 |
| 16. | 23.19 | 11.49 | 1.78 | 1.81 | 1098.4 |
| 17. | 51.38 | 25.55 | 2.01 | 1.99 | 2569.0 |
| 18. | 21.87 | 11.64 | 1.88 | 1.76 | 1093.6 |
| 19. | 25.52 | 13.48 | 1.84 | 1.87 | 1159.8 |
| 20. | 49.87 | 27.00 | 2.02 | 2.04 | 2439.2 |
| 21. | 52.11 | 27.54 | 2.06 | 2.08 | 2496.5 |

**Molecular variability by using RAPD technique.** Random Amplified Polymorphic DNA (RAPD) analysis of all the twenty one (21) samples have been used to detect the variation among the isolates of *B. bassiana*. Thirteen different RAPD primers were used to determine the molecular variation between isolates collected from different regions of North East India. All the bands produced in the present study were scored for the analysis. RAPD data analysis revealed that all the thirteen (13) primers were found to be polymorphic to twenty one (21) *B. bassiana* isolates (Table 6 and Fig. 1-6). Data analysis of *B. bassiana* isolates revealed a total of 52 bands were obtained based on the result of amplification with the thirteen selected primers among which RAPD 2 amplified the highest amount of bands (6 bands). The number of fragments generated by each

primer varied from 3 to 6. The primer RAPD 7 gave the highest polymorphism percentage of 80 per cent, lowest being 33 per cent by RAPD 5. Out of the 52 fragment generated by 13 RAPD primers, 31 fragment were polymorphic while 21 were monomorphic.

**Table 6: Primers sequence and details of amplification generated by each primer for *B. bassiana* isolates.**

| Primers code | Total bands | Monomorphic bands | Polymorphic bands | Polymorphism percentage % |
|---|---|---|---|---|
| RAPD 1 | 4 | 2 | 2 | 50 |
| RAPD 2 | 6 | 3 | 3 | 50 |
| RAPD 3 | 5 | 2 | 3 | 60 |
| RAPD 4 | 5 | 2 | 3 | 60 |
| RAPD 5 | 3 | 2 | 1 | 33 |
| RAPD 6 | 4 | 2 | 2 | 50 |
| RAPD 7 | 5 | 1 | 4 | 80 |
| RAPD 8 | 4 | 1 | 3 | 75 |
| RAPD 9 | 4 | 2 | 2 | 50 |
| RAPD 10 | 2 | 1 | 1 | 50 |
| RAPD 11 | 3 | 1 | 2 | 66 |
| RAPD 12 | 3 | 1 | 2 | 66 |
| RAPD 13 | 4 | 1 | 3 | 75 |
| **Total** | **52** | **21** | **31** | **59.61** |

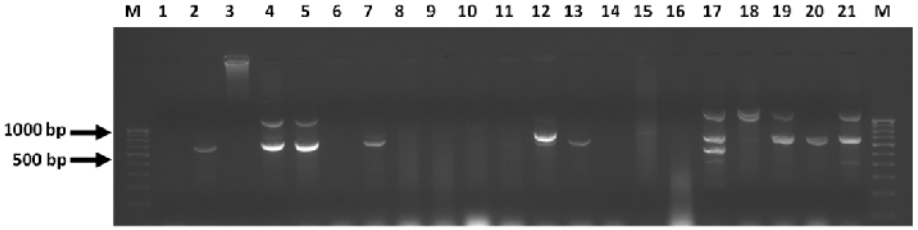


**Fig. 1.** Agarose Gel Electrophoresis of PCR product with RAPD primer 1; M- 100 Bp DNA ladder.

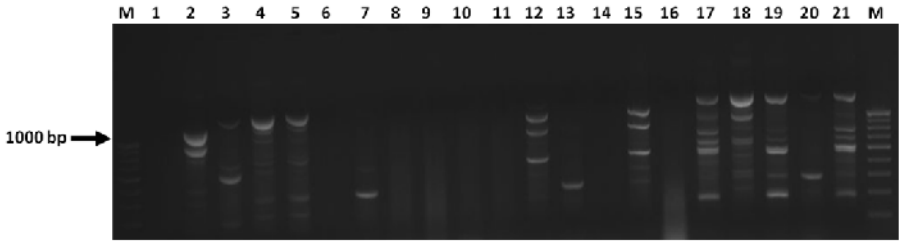


**Fig. 2.** Agarose Gel Electrophoresis of PCR product with RAPD primer 2; M- 100 Bp DNA ladder.

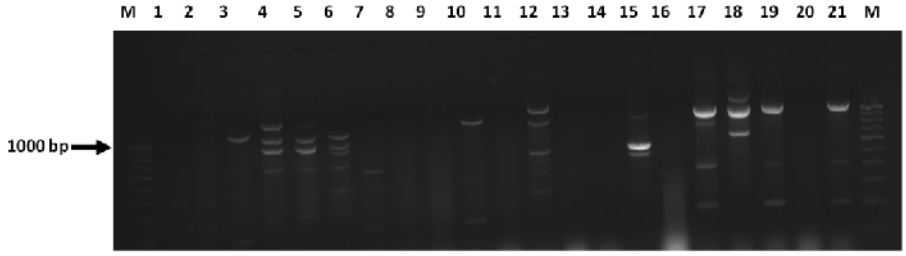


**Fig. 3.** Agarose Gel Electrophoresis of PCR product with RAPD primer 3; M- 100 Bp DNA ladder.

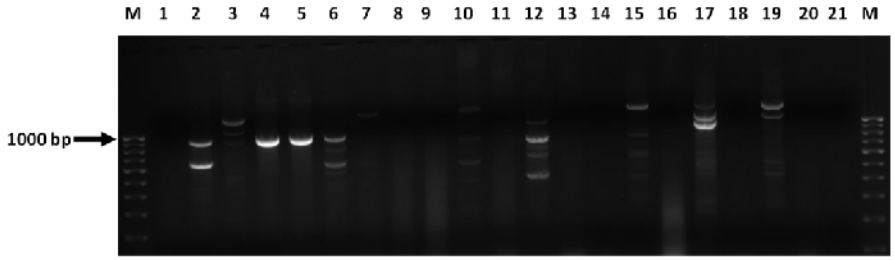


**Fig. 4.** Agarose Gel Electrophoresis of PCR product with RAPD primer 7; M- 100 Bp DNA ladder.

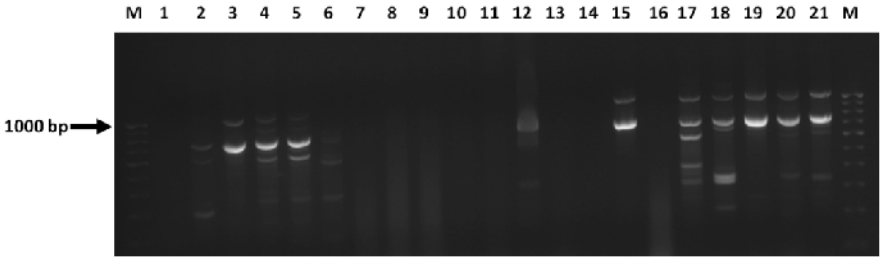


**Fig. 5.** Agarose Gel Electrophoresis of PCR product with RAPD primer 9; M- 100 Bp DNA ladder.

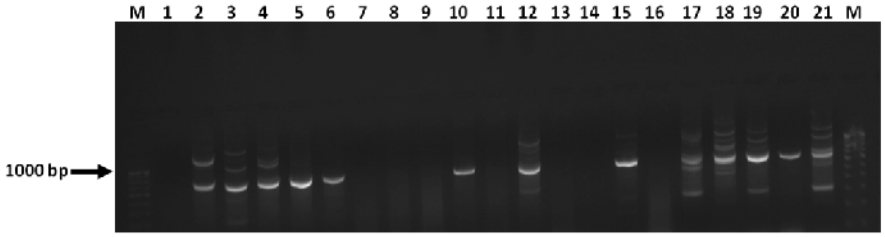


**Fig. 6.** Agarose Gel Electrophoresis of PCR product with RAPD primer 13; M- 100 Bp DNA ladder.

**Cluster analysis of twenty one *B. bassiana* isolates.** Binary data was obtained for all the ten indigenous isolates were used for cluster analysis and to get at the dendogram and simple matching coefficients. Cluster analysis of all the isolates under the study was performed using MEGA 5.05 software and their phylogenetic tree was shown in Fig. 7.

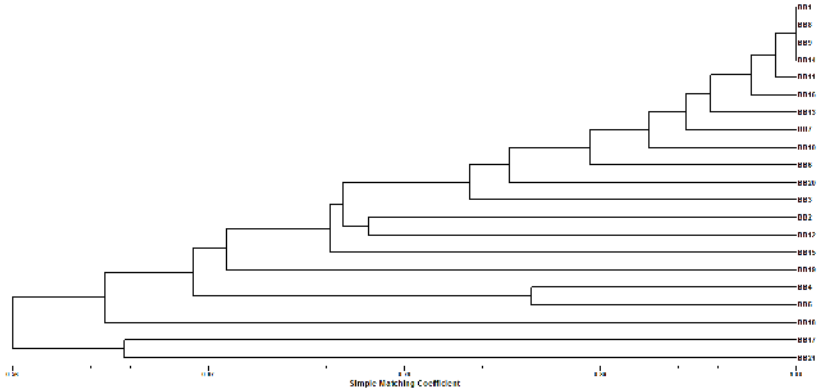


**Fig. 7.** Cluster analysis of 21 *Beauveria bassiana* isolates by using RAPD markers.

The simple matching coefficient values were generated using Jaccard's similarity coefficient and the data in Table 7 shows the similarity values between the isolates. The similarity coefficients among RAPD profiles of the isolates of *B. bassiana* were found higher than 47.1 per cent. The maximum similarity value of 1.00 or 100 per cent was observed between isolates Bb1 (Jorhat) – Bb8 (Nagaon), Bb1 (Jorhat) – Bb9 (BNCA), Bb1 (Jorhat) –Bb4 (Mokokchung), Bb8 (Nagaon) – Bb9 (BNCA), Bb8 (Nagaon) – Bb14 (Kahikuchi) and Bb9 (BNCA) – Bb14 (Kahikuchi), followed by similarity value of 0.98 (98%) was observed between isolates Bb1 (Jorhat) – Bb11 (Guwahati), Bb8 (Nagaon) – Bb11 (Guwahati), Bb9 (BNCA)– Bb11(Guwahati) and Bb11 (Guwahati) – Bb14 (Kahikuchi). The lowest similarity value 0.47 was recorded between isolates Bb11 (Guwahati) – Bb21 (AAU, Jorhat). The rest of the isolates showed values ranging from 0.49 to 0.98.

**Table 7: Simple matching coefficients of 21 *B. bassiana* isolates.**

| | Bb1 | Bb2 | Bb3 | Bb4 | Bb5 | Bb6 | Bb7 | Bb8 | Bb9 | Bb10 | Bb11 | Bb12 | Bb13 | Bb14 | Bb15 | Bb16 | Bb17 | Bb18 | Bb19 | Bb20 | Bb21 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Bb1 | 1.00 | | | | | | | | | | | | | | | | | | | | |
| Bb2 | 0.77 | 1.00 | | | | | | | | | | | | | | | | | | | |
| Bb3 | 0.83 | 0.72 | 1.00 | | | | | | | | | | | | | | | | | | |
| Bb4 | 0.58 | 0.67 | 0.60 | 1.00 | | | | | | | | | | | | | | | | | |
| Bb5 | 0.71 | 0.73 | 0.73 | 0.85 | 1.00 | | | | | | | | | | | | | | | | |
| Bb6 | 0.89 | 0.73 | 0.78 | 0.64 | 0.77 | 1.00 | | | | | | | | | | | | | | | |
| Bb7 | 0.94 | 0.73 | 0.80 | 0.59 | 0.72 | 0.86 | 1.00 | | | | | | | | | | | | | | |
| Bb8 | 1.00 | 0.77 | 0.83 | 0.58 | 0.71 | 0.89 | 0.94 | 1.00 | | | | | | | | | | | | | |
| Bb9 | 1.00 | 0.77 | 0.83 | 0.58 | 0.71 | 0.89 | 0.94 | 1.00 | 1.00 | | | | | | | | | | | | |
| Bb10 | 0.93 | 0.79 | 0.81 | 0.58 | 0.71 | 0.89 | 0.89 | 0.93 | 0.93 | 1.00 | | | | | | | | | | | |
| Bb11 | 0.98 | 0.75 | 0.82 | 0.57 | 0.70 | 0.88 | 0.93 | 0.98 | 0.98 | 0.92 | 1.00 | | | | | | | | | | |
| Bb12 | 0.73 | 0.75 | 0.75 | 0.62 | 0.67 | 0.72 | 0.70 | 0.73 | 0.73 | 0.75 | 0.72 | 1.00 | | | | | | | | | |
| Bb13 | 0.95 | 0.74 | 0.79 | 0.60 | 0.71 | 0.85 | 0.94 | 0.95 | 0.95 | 0.88 | 0.96 | 0.73 | 1.00 | | | | | | | | |
| Bb14 | 1.00 | 0.77 | 0.83 | 0.58 | 0.71 | 0.89 | 0.94 | 1.00 | 1.00 | 0.93 | 0.98 | 0.73 | 0.95 | 1.00 | | | | | | | |
| Bb15 | 0.74 | 0.65 | 0.74 | 0.63 | 0.64 | 0.71 | 0.73 | 0.74 | 0.74 | 0.81 | 0.73 | 0.73 | 0.72 | 0.74 | 1.00 | | | | | | |
| Bb16 | 0.97 | 0.77 | 0.81 | 0.58 | 0.69 | 0.87 | 0.92 | 0.97 | 0.97 | 0.90 | 0.96 | 0.71 | 0.93 | 0.97 | 0.72 | 1.00 | | | | | |
| Bb17 | 0.54 | 0.49 | 0.60 | 0.58 | 0.62 | 0.55 | 0.55 | 0.54 | 0.54 | 0.54 | 0.52 | 0.55 | 0.54 | 0.54 | 0.51 | 0.56 | 1.00 | | | | |
| Bb18 | 0.63 | 0.54 | 0.58 | 0.58 | 0.62 | 0.59 | 0.59 | 0.63 | 0.63 | 0.60 | 0.62 | 0.57 | 0.63 | 0.63 | 0.60 | 0.60 | 0.58 | 1.00 | | | |
| Bb19 | 0.66 | 0.62 | 0.73 | 0.62 | 0.70 | 0.65 | 0.72 | 0.66 | 0.66 | 0.71 | 0.65 | 0.67 | 0.69 | 0.66 | 0.71 | 0.64 | 0.66 | 0.64 | 1.00 | | |
| Bb20 | 0.85 | 0.73 | 0.78 | 0.62 | 0.72 | 0.79 | 0.83 | 0.85 | 0.85 | 0.82 | 0.83 | 0.72 | 0.85 | 0.85 | 0.73 | 0.82 | 0.55 | 0.62 | 0.67 | 1.00 | |
| Bb21 | 0.529 | 0.52 | 0.55 | 0.64 | 0.58 | 0.51 | 0.56 | 0.52 | 0.52 | 0.50 | 0.51 | 0.47 | 0.55 | 0.52 | 0.57 | 0.52 | 0.62 | 0.62 | 0.67 | 0.60 | 1.00 |

The dendrogram generated with the similarity data grouped the isolates into four clusters with most bifurcation at 0.56 similarities (Fig. 7). The first cluster had twelve isolates Bb1 (Jorhat), Bb8 (Nagaon), Bb9 (BNCA), Bb 14 (Kahikuchi), Bb11 (Guwahati), Bb16 (Itanagar), Bb13 (Kahikuchi), Bb7 (Nagaon), Bb10 (BNCA), Bb6 (Dimapur), Bb20 (Kolasib) and Bb3 (AAU, Jorhat). The second cluster comprised of four isolates Bb2 (AAU, Jorhat), Bb12 (Nalbari), Bb15 (Kahikuchi) and Bb19 (Kolasib). In the third cluster two isolates Bb4 (Mokokchung) and Bb5 (Jharnapani) were included, while the fourth cluster consisted of three isolates Bb17 (Majuli), Bb18 (Majuli) and Bb21 (AAU, Jorhat).

***B. bassiana* specific PCR and DNA Sequence Analysis.** Altogether 6 isolates from different North Eastern Region were selected and sequenced at Eurofins Genomics Pvt. Ltd, Bangalore. DNA sequences were assembled using Bioedit software. They were designated as Bb2 (AAU, Jorhat), Bb3 (AAU, Jorhat), Bb4 (Mokokchung), Bb5 (Jharnapani), Bb15 (Guwahati) and Bb17 (Majuli). PCR analysis revealed PCR products of 500 bp against *B. bassiana* specific primer pair BBF1-BBR1 for the six *B. bassiana* isolates *viz.*, Bb2, Bb3, Bb4, Bb5, Bb15 and Bb17 (Fig. 8).

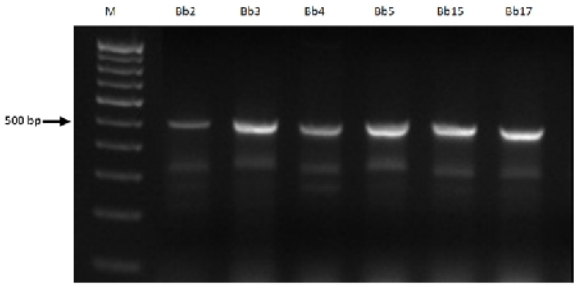


**Fig. 8.** Agarose Gel Electrophoresis of PCR product with *B. bassiana* specific primer; M- 100 bp DNA ladder.

Multiple sequence alignments of the six *B. bassiana* isolates clearly indicated the difference in their nucleotide sequences (Fig. 9). The sequenced amplified products size varied approximately in the range of 387 to 393 bp in all the isolates. The nucleotide sequences obtained were compared with each other for sequence similarity to reveal genetic diversity amongst them (Table 8). Sequence analysis showed that six *B. bassiana* isolates of North East Region shared 89 to 99 per cent identity with the other known isolates. This suggested that genetic variability exist among the *B. bassiana* isolates of North East States.

The cluster analysis of the 6 isolates were performed using MEGA 5.05 software (Fig. 10). The distance matrix of the isolates presented in Table 9. Out of the six *B. bassiana* isolates, Bb 4 (Mokokchung) and Bb5 (Jharnapani) had shown the highest similarity of 1.0 or 100%. This was followed by Bb2 (AAU, Jorhat) – Bb4 (Mokokchung) and Bb2 (AAU, Jorhat) – Bb5 (Jharnapani) showing 99.7 per cent similarity. The lowest similarity percentage of 91.3 per cent was observed between isolates Bb4 (Mokokchung) – Bb15 (Guwahati) and Bb5 (Jharnapani) – Bb15 (Guwahati) followed by 91.6 per cent between isolate Bb2 (AAU, Jorhat) – Bb15 (Guwahati).

The dendogram generated (Fig. 10) clusterd the *B. bassiana* isolates into three groups, while the sample Bb15 (Guwahati) was clearly separated from the other samples. A second group was formed by Bb3 (AAU, Jorhat) and Bb17 (Majuli) and in the third group, three samples were found Bb4 (Mokokchung), Bb5 (Jharnapani) and Bb2 (AAUs, Jorhat).

**Table 8: Sequence similarity of 6 isolates of *B. bassiana* with other known Bb isolates.**

| Sr. No. | Sequence Name | Strain Name | Maximum Identity (%) | Accession No. | Similarity base Pair | Matching of query and subject sequence |
|---|---|---|---|---|---|---|
| 1. | BB2 | *Cordyceps bassiana* | 99% | EU100742.1 | 436/440 | 5571-6010 |
| | BB2 | *Beauveria bassiana* | 96% | EU371503.2 | 435/451 | 5581-6030 |
| | BB2 | *Cordyceps brongniartii* | 94% | EU100743.1 | 160/191 | 7263-7442 |
| 2. | BB3 | *Beauveria bassiana* | 98% | EU371503.2 | 441/448 | 5583-6027 |
| | BB3 | *Cordyceps bassiana* | 96% | EU100742.1 | 428/448 | 5573-6007 |
| | BB3 | *Cordyceps brongniartii* | 90% | EU100743.1 | 157/190 | 7265-7442 |
| 3. | BB4 | *Cordyceps bassiana* | 98% | EU100742.1 | 441/452 | 5591-6042 |
| | BB4 | *Beauveria bassiana* | 95% | EU371503.2 | 440/463 | 5601-6062 |
| | BB4 | *Cordyceps brongniartii* | 89% | EU100743.1 | 139/170 | 7283-7442 |
| 4. | BB5 | *Cordyceps bassiana* | 98% | EU100742.1 | 430/437 | 5597-6032 |
| | BB5 | *Beauveria bassiana* | 96% | EU371503.2 | 429/448 | 5607-6052 |
| | BB5 | *Cordyceps brongniartii* | 92% | EU100743.1 | 134/164 | 7289-7442 |
| 5. | BB15 | *Beauveria bassiana* | 90% | EU371503.2 | 361/401 | 5633-6027 |
| | BB15 | *Cordyceps bassiana* | 89% | EU100742.1 | 328/369 | 5652-6007 |
| 6. | BB17 | *Beauveria bassiana* | 98% | EU371503.2 | 443/451 | 5583-6030 |
| | BB17 | *Cordyceps bassiana* | 95% | EU100742.1 | 430/451 | 5573-6010 |
| | BB17 | *Cordyceps brongniartii* | 94% | EU100743.1 | 158/190 | 7265-7442 |

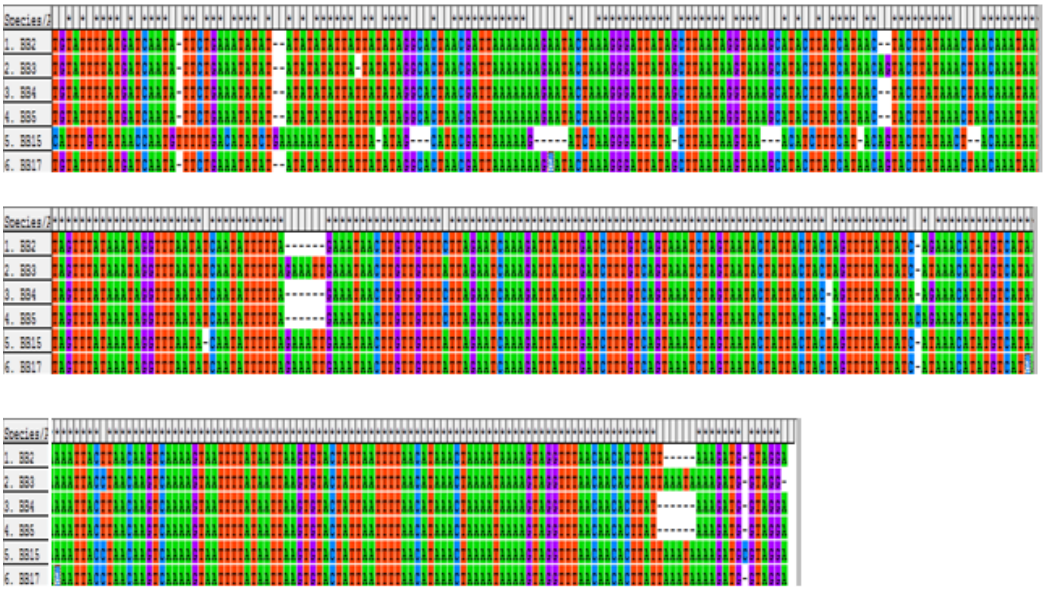

**Fig. 9.** Sequence alignment of 6 isolates of *B. bassiana.*

Kosir *et al.* (1991) reported that molecular characterization of *B. bassiana* is important to understand the organization at genomic level, variation at genetic level and stability of gene of the isolates. Many a times, morphological or biochemical parameters based characterization may not be sufficient to understand the genetic basis for differentiation among the isolates. In an study conducted by Tigano *et al*. (1995) showed that the variation at genetic level is revealed by DNA markers. They also mentioned that genetic study done by DNA markers results finer taxonomic resolution than morphological characterization. Further, PCR-based technology yields an extra means to solve the problems arises during taxonomic identification of entomopathogenic fungi (Fegan *et al*., 1993). Earlier, PCR based technology was used to study the genomic variability among 24 isolates of two entomopathogenic fungi *viz*., *Metarhizium* spp. and *B. bassiana*, and found that with specific markers the taxonomic classification of the species can be done accurately (Bidochka *et al*., 1994).

**Table 9. Distance matrix of 6 *B. bassiana* isolates.**

| | Bb2 | Bb3 | Bb4 | Bb5 | Bb15 | Bb17 |
|---|---|---|---|---|---|---|
| Bb2 | 0.00 | | | | | |
| Bb3 | 0.011 | 0.00 | | | | |
| Bb4 | 0.003 | 0.014 | 0.00 | | | |
| Bb5 | 0.003 | 0.014 | 0.00 | 0.00 | | |
| Bb15 | 0.084 | 0.071 | 0.087 | 0.087 | 0.00 | |
| Bb17 | 0.011 | 0.00 | 0.014 | 0.014 | 0.071 | 0.00 |

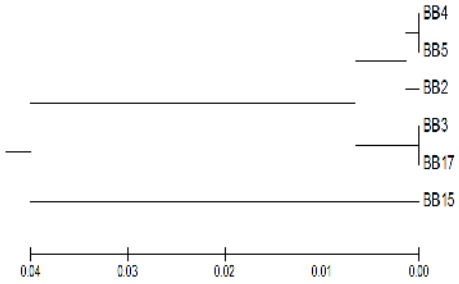


**Fig. 10.** Cluster analysis of 6 *B. bassiana* isolates by using *B. bassiana* specific primer.

To determine the genetic variability of *B. bassiana* associated with *N. subcostatum*, twenty one *B. bassiana* samples were studied using RAPD. Thirteen random sequence RAPD primers were used for the twenty one*B. bassiana* isolates, no identical banding patterns were found. The total 52 bands were scored for the 13 RAPD primers out of which 21 bands were monomorphic. The present result is in agreement with the report of Costa *et al*. (2011) who studied on 11 *B. bassiana* and RAPD-PCR based analysis showed to have diverse polymorphism. This result reflects that there is which possibility of variability among the isolates at genetic level. Similarly, isozymes and RAPD analysis showed variation among 24 isolated of the *B. bassiana* (Castrillo *et al*., 1998), with possibilities of better resolution by RAPD markers. Earlier, it was reported that genetical dissimilarities can be obtained among the *B. bassiana* isolates of same insect species and from the same region (Bidochka *et al*., 1994; Berretta *et al*., 1998). The number of genetic loci detected with RAPD markers are much higher than detected with morphological and chemical or biochemical markers (Kongkiatngam *et al*., 1995).

The dendrogram, based on genetic similarities (NTYSIS) of all the isolates (21) of *B. bassiana* showed the presence of four distinct groups representing a relatively close relationship amongst the isolates though they were from the same host but geographically from different locality. Similarities at genetic level amongst isolates ranged from 0.49 to 1.0. Similar phenomenon was also observed by Wang *et al*. (2003). In an study on 56 isolates, when assessed by three molecular methods. Earlier, Castrillo and Brooks, (1998) reported this can be justified by the fact that *Beauveria* spp. is an haploid fungi and reproduces mainly asexually, so the major genetic variation is due to mutations or parasexual recombination.

In the present study, no correlation was observed between the clusters obtained by RAPD analysis and the pathogenecity of *N. subcostatum*. Similar results was reported by Piatti *et al*. (1998) who showed that the isolates of *B. brongniartii* was separated into five groups which contained both strong and weak pathogenic isolates, but no correlation between pathogenicity and grouping according to RAPD patterns was found. Earlier work reported that no correlation was observed between the isolates aggressiveness and the relatedness of the *S. Litura* Fab, the original insect host.

Six *B. bassiana* isolates of the North East Region were selected and amplified using specific primer and the amplified PCR products were sequenced. Amplified products size varied approximately in the range of 387 to 393 bp in all the isolates under the study. The nucleotide sequences obtained were compared with each other for sequence similarity to reveal genetic diversity amongst them. Sequence analysis showed that six *B. bassiana* isolates of North East Region shared 89 to 99 per cent identity with the other known isolates. The isolate Bb15 from Kahikuchi, Guwahati district of Assam and Bb4 from Mokokchung, district of Nagaland was found to have the least sequence identity with the other isolates tested and both the isolate showed identity of 89 per cent. This suggested that genetic variability exist among the *B. bassiana* isolates of North East States. Shin *et al*. (2011) carried out a study on P1-P3 primer set specific to *Beauveria* spp. amplified an approximately 500 bp product from MsW1 (*Monochamus saltuarius*). The PCR product of about 330 bp was amplified from MsW1,

suggesting that MsW1 is *B. bassiana*. Costa *et al*. (2011) reported that the two primer *viz*., ITS4 and ITS5 amplification for 11*B. bassiana* isolates resulted in a single product of approximately 600 bp. The alignment of the partial sequences corresponding to the ITS1 region, 5.8S gene and ITS2 region revealed similarity among the 11 isolates.

## CONCLUSION AND FUTURE SCOPE

The present study showed that isolates of *B. bassiana* are highly pathogenic to *N. subcostatum* and studies on molecular characterization of the isolates proved the variability at genetic level among the isolates of *B. bassiana* of North East States. These isolates can be use as a potential bioagent for the management of the pest in organic cultivation of banana, thereby it will reduce the use of chemical pesticides and save the environment.

**Acknowledgement.** The author acknowledges the Director of PG studies, Director of Research (Agri), Head, Department of Plant Pathology, Assam Agricultural University, Jorhat, Assam for the facility provided for the research work, consent support and guidance during the research work. The financial support received by Dr P. Dutta through project funded by DST, GoI; PCIL, West Bengal for survey is also duly acknowledged.

**List of abbreviation:**

AAU: Assam Agricultural University, Bb: *Beauveria bassiana*, RAPD: Rapid Amplified Polymorphic Deoxyribose Nucleic Acid, DPGS: Director of Post Graduate Studies, PCR: Polymerase Chain Reaction, ITS: Internal Transcribed Spacer, NTSYS: Numeral Taxonomy and Multivariate Analysis System, CTAB: Cetyl trimethylammonium bromide, DNA: Deoxyribo nucleic acid, DAT: Days after treatment, DAI: Days after inoculation.

**Conflict of interest.** None.

## REFERENCES

Abott, W. S. (1925). A method of computing the effectiveness of an insecticide. *J Econ Entomol*., *18*: 265-267.

Anonymous (2009). Biennial Research Report AICRP on Tropical Fruits, p 246.

Barson, G. (1977). Laboratory evaluation of *Beauveria bassiana* as a pathogen of larva stage of large elm bark beetle, *Scolytus scolytus. J. Invertebr. Pathol., 29*: 361-366.

Bidochka, M. J., McDonald, M. A., St. Leger, R. J., Roberts, D. W. (1994). Differentiation of species and strains of entomopathogenic fungi by random amplification of polymorphic DNA (RAPD). *Curr Genet*., *25*: 107-113.

Boruah, S. and Dutta, P. (2014). Preliminary evaluation of bioformulation of *Metahizium anisopliae* against cowpea aphid, *Aphis craccivora*. *Insect Environment*. *20*(2): 54-56.

Boruah, S., Dutta, Pranab; Pegu, J. R., Kaushik, H., Gogoi, N., Puzari, K. C. and Hazarika, G. N. (2016). SEM study on morphological changes in *Metarhizium anisopliae* infected *Aphis cracivora. Journal of Biological Control, 30*(1): 329-333.

Butt, T. M. (2002). Use of Entomogenous fungi for the control of insect pests. In: The Mycota: Agricultural Applications, Kemken, F (Eds.). Publ. by Springer-Verlag, Berlin, Heidelberg, pp 111-134.

Carmona, A. (2008). Fungal pathogenecity test on immature bioregulators, *Colaspi*s sp. under field conditions in the region of Uraba. Grade thesis. National University Medellin Colombia Faculty of Agriculture Sciences, Department of Agricultural Sciences.

Carruthers, R. I. and Soper, R. S. (1987). Fungal diseases. In: Epizootiology of insect disease. Fuxa, JR, Tanada Y (Eds.) Publ. by John Wiley and Sons, New York, pp 357-416.

Castrillo, L. A., Brooks, W. M. (1998). Differentiation of *Beauveria bassiana* isolates from the darkling beetle, *Alphitobius diaperinus*, using isozyme and RAPD analyses. *J. Inverteb Pathol., 72*(3): 190-196.

Castrillo, L. A., Vanderberg, J. D., Wraight, S. P. (2003). Strain specific detection of induced *Beauveria bassiana* in agricultural fields by use of sequence-characterized amplified region markers. *Beauveria bassiana. J. Inverteb. Pathol., 82*(2): 75-83.

Costa, E. M., de, M. B., Pimenta, F. C., Luz, C., Oliveira, V. de., Oliveira, M., Bueno, E., Petrofeza, S. (2011). *Beauveria bassiana*: Quercetinase production and genetic diversity. *Braz J. Microbiol., 42*(1): 12-21.

Das, P., Hazarikia, L. K., Bora, D. S., Puzari, K. C. and Dutta, P. (2011). Screening of biological traits of twenty three *Beauveria bassiana* strains against rice hispa, *Dicladespa armigera* (Olivier) (Coleoptera: Chrsomeloidae). *Insect Environment, 17*(3): 128-130.

Deb, L., Devi, T. R. K., Majumder, D., Dutta Pranab and Thakuria, D and Rajesh, T and Ningthoujam, K. (2021b). Antifungal ability of *Beauveria bassiana* (Balsam) Vullemi against *Curvularia lunata* causing leaf spot of rice. *The Pharma Innovation Journal*, *10* (4): 298-303.

Deb, L., & Dutta, P. (2021). Antagonistic potential of *Beauveria bassiana* (Balsamo) Vuillemin against *Pythium myriotylum* causing damping off of tomato. *Indian Phytopathology*, *74*(3), 715-728.

Dutta, Pranab, Pegu, J., Kaushik, H., Kaman, P. and Das, Ankita (2018). Scanning Electron Microscopy (SEM) Study Showed the Mode Action of *Metarhizium anisopliae* on *Odontotermes obesus*. *International Journal of Current Microbiology and Applied Sciences*, *7*(10): 901-906.

Dutta, Pranab, Patgiri, P., Pegu, J., Kaushik, H. and Boruah, S. (2014). First record of *Nomuraea rileyi* (Farlow) Samson on *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) from Assam, India. *Current Biotica*, *8*(2): 187-190.

Fegan, M., Manners, J. M., Maclean, D. J., Irwin, J. A.G., Samuels, K. D. Z., Holdon, D. G., and Li, D. P. (1993). Random amplified polymorphic DNA markers reveal a high degree of genetic diversity in the entomopathogenic fungus, *Metarhizium anisopliae* var. *anisopliae. J. Gen. Microbiol., 139*: 2075-2081.

Hazarika, L. K., Puzari, K. C. (1995). White muscardine fungus (*Beauveria bassiana*) pathogenic to different stages of rice hispa (*Dicladispa armigera*). *Indian J. Agril. Sci., 65*: 368-372.

Hazarika, S., Patgiri, P., Dutta, Pranab, Borkataki, S. and Das, K. (2016). Efficacy of local isolates of *Nomura earileyi* (Farloe) Sampson against *Helicoverpa armigera* (Hubner). *Journal of Entomology and Zoological Studies*, *4*(3): 167-169.

Karthikeyan, K., and Jacob, S. (2010). Biological efficacy of *Beauveria bassiana* against rice blue beetle, *Leptispa pygmaea* Baly (Coleoptera: Chrysomelidae). *Intl. Res. J. Plant. Sci., 1*(2): 26-33.

Kongkiatngam, P., Waterway, M. J., Fortin, M. G., Coulman, B. E. (1995). Genetic variation within and between two cultivars of red clover (*Trifolium protense* L.): Comparison of morphological, isozymes and RAPD markers. *Euphytica, 84*: 237-246.

Kosir, J. M., Macpherson, J. M., and Khachatourians, G. G. (1991). Genomic analysis of a virulent and a less virulent strain of the entomopathogenic fungus *Beauveria bassiana* using RFLP. *Can. J. Microbiol., 37*: 534-538.

Omukoko, C. A., Maniania, K. N., Wesnoga, J. M., Kahangi, E. M., and Wamocho, L. S. (2011). Pathogenicity of isolates of *Beauveria bassiana* to the banana weevil *Cosmopolites sordidus. J. Agril. Sci. Techol., 13*(2):4-14.

Pegu, J. R., Dutta, Pranab, Puzari, K. C. and Nath, P. D. (2013). Natural incidence of entomopathogenic fungi in North East India. *Journal of Mycology and Plant Pathology*, *43*(2): 243-245.

Phukan, M., Hazarika, L. K., Barooah, M., Puzari, K. C. & Kailta, S. (2008). Interaction of *Dicladispa armigera* (Coleoptera: Chrysomelidae) haemocytes with *Beauveria bassiana. Intl. J. Trop. Insect. Sci., 28*(2): 88-97.

Piatti, P., Cravanzola, F., Bridge, P. D. & Ozino, O. I. (1998). Molecular characterization of *Beauveria brongniartii* isolates obtained from *Melolontha melolontha* in Valle d' Aosta (Italy) by RAPD-PCR. *Lett. Appl. Microbiol. 26*: 317-324.

Puzari, K. C. & Hazarika, L. K. (1991). Study on the efficacy of *Beauveria bassiana* (Bals.) Vuill. combined with various stickers or spreaders against rice hispa (RH). *IRRN16*: 21.

Puzari, K. C., Hazarika, L. K. & Deka, N. (1994). Pathogenecity of *Beauveria bassiana* on rice hispa (*Dicladispa armigera*). *Ind J Agril Sci., 64*(2): 123-125.

Puzari, K.C., Dutta, Pranab, Hazarika, L.K., Hazarika, G.N. and Kaushik, H. (2015). *Beauveria bassiana*– a potential entomopathogen against rice hispa, *Dicladispa armigera*. *International Journal of Applied biology and Pharmaceutical Sciences, 6*(2): 94-99.

Ramos, E. Q., Alves, S. B., Dementrio, C. G. B., and Costa, S. C. (2004). Selection of entomopathogenic fungi to control *Bemisia tabaci* biotype B. *Manenjo integrado de Plagas Agroecol., 73*: 21-28.

Rohlf, F. J. (1993). NTSYS-pc: Numeral Taxonomy and Multivariate Analysis System. Version 2.02 Exeter software, New York.

Roy, A. K., and Puzari, K. C. (1979). Infection of banana leaf beetle by *Beauveria bassiana*. *Curr. Sci., 48*(3):115-116.

Safavi, S. A., Kharrazi, A., Rasoulian, G. R., and Bandani, A. R. (2010). Virulence of some isolates of entomopathogenic fungus, *Beauveria bassiana* on *Ostrinianubilalis* (Lepidoptera: Pyralidae) larvae. *J Agric Sci Technol 12*: 13-21.

Safavi, S. A., Shah, F. A., Pakdel, A. K., Rasoulian, G. R., Bandani, A. R., and Butt, T. M. (2007). Effect of nutrition on growth and virulence of the entomopathogenic fungus *Beauveria bassiana. FEMS Microbiol. Lett., 270*: 116-123.

Shah, F. A., Wang, C. S., and Butt, T. M. (2005). Nutrition influences growth and virulence of the insect-pathogenic fungus *Metarhizium anisopliae. FEMS Microbiol Lett., 251*: 259-266.

Shin, T. Y., Choi, J. B., Bae, S. M., Koo, H. N., Roh, J. Y., Je, Y. H., ... & Woo, S. D. (2011). Characterization of *Beauveria bassiana* MsW1 isolated from pine sawyers, *Monochamus saltuarius*. *Journal of basic microbiology*, *51*(5), 531-539.

Tigano, M. S., Ana, C. M. M., Gomes, B., and Sobral, W. S. (1995). Genetic variability among Brazilian isolates of the entomopathogenic fungus *Metarhizium anisopliae*. *J Invertbr. Pathol., 65*: 206-210.

Todorova, S. I., Coderre, D., and Cote, J. C. (2000). Pathogenicity of *Beauveria bassiana* isolates toward, *Leptinotarsa decemlineata* (Say) (Col.: Chrysomelidae), *Myzuspericae* (Homoptera: Aphididae) and their predator *Coleomegilla maculate lengi* (Coleoptera: Coccinellidae). *Phytoprotection, 81*:15-22.

Todorova, S. I., Cote, J. C.,Martel, P., and Coderre, D. (1994). Heterogeneity of two *Beauveria bassiana* strains revealed by biochemical tests, protein profiles and bio-assays on *Leptinotarsa decemlineata* (Coleotera: Chrysomelidae) and *Coleomegilla maculate lengi* (Coleoptera: Coccinellidae) larvae. *Entomophaga, 39*: 159-169.

Wang, C., Shah, F. A., Patel, N., Li, Z., and Butt, T. (2003). Molecular investigation on strain genetic relatedness and population structure of *Beauveria bassiana*. *Environ Micicrobiol., 5*: 908-915.

Zolan, M. E., Pukkila, P. J. (1986). Inheritance of DNA methylation in *Coprinus cinereus*. *Mole. Cell. Biol., 6*: 195-200.

**How to cite this article:** B. Temjenmenla, K.C. Puzari, A. Bhattacharyya, P.D. Nath and Pranab Dutta (2022). Characterization of Twenty One Isolates of *Beauveria bassiana* and Study their Bio-efficacy Against *Nodostoma subcostatum*. *Biological Forum – An International Journal*, *14*(1): 1389-1401.