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Evaluation of biological compatibility of *Beauveria bassiana* with fungicides and botanicals

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Abstract
Beauveria bassiana is the most widely studied entomopathogenic fungi which have drawn attention worldwide not only as an insect-pest control but also as management of plant pathogenic fungi and bacteria along with their plant growth promotion activities. The compatibility of *Beauveria* isolate BP1.1 was evaluated with different fungicides namely Carbendazim (50 WP) and Copper Oxychloride (50 WP) and botanicals such as Aqueous Neem Leaf Extract (ANLE) (1% w/v), Aqueous Garlic Extract (AGE) (1% w/v) and Neem Seed Kernel Extract (NLKE) (5% w/v) at three different concentrations. The isolate of *Beauveria* BP1.1 showed maximum growth in Copper Oxychloride (80.50 mm) followed by Neem Leaf Extract (74.75 mm) and Neem Seed kernel Extract (70.75 mm), whereas, the least growth was observed in Garlic extract (60.50 mm) and total inhibition was observed in carbendazim.

Keywords: *Beauveria bassiana*, Entomopathogenic, Compatibility, Fungicide, Botanicals

Introduction
Beauveria bassiana (Balsamo) Vuillemin is a soil dwelling necrotrophic fungus and is widely known as white muscardine fungus. In addition to its entomopathogenic properties, this fungus is also being exploited for its other beneficial properties like plant endophytes, antagonists for plant diseases, beneficial rhizosphere colonizers and plant growth promoters (Vega *et al.*, 2009). Different mechanisms involved by *Beauveria* spp. as a biocontrol agent are antibiosis, competition, mycoparasitism, endophytism and induced systemic resistance (ISR), production of specific or non-specific metabolites like antibiotics, bioactive volatile organic compounds, lytic agents, enzymes and toxic substances which play an important role in pathogenesis. The wide input of chemicals either as sole or in combination are usually expensive having negative impact on the environment and human health which has alarmed the public concern over potential risks caused in the form of health risk, environmental pollution, pesticide resistance, pesticide residue *etc* (Griffin, 2007). A potential alternative is to the use of bio control agents. The use of biological control methods reduces the reliance on high risk chemicals, eco-friendly in nature and goal for long term sustainability (Howell, 2003). The biological compatibility of *B. bassiana* was tested with different fungicides such as carbendazim & copper oxychloride and botanicals such as aqueous neem leaf extract, aqueous garlic extract & neem leaf kernel extract. The best pesticide formulation in combination with entomopathogenic fungi to be used in Integrated Pests Management (IPM) programme.

Materials and Methods
The compatibility of *Beauveria* isolate with fungicides *viz.*, carbendazim (Bavistin 50 WP) and copper oxychloride (Blitox 50 WP) and botanicals *viz.*, neem neaf extract (1% w/v), neem seed kernel extract (NSKE 5% w/v) and garlic clove extract (1% w/v) were studied by using poisoned food technique (Nene and Thapliyal, 1979). The experiments were carried out at three different concentrations in Sabouraud Dextrose Agar (SDA) media. The screened *Beauveria* isolate was grown in SDA medium for seven days. A 7 mm of mycelial disc was cut with the help of cork borer from the periphery of actively growing culture plate and inoculated in respective poisoned medium. Three replicates were maintained along with the control plate without any treatment as a check.

Fungal Isolate
About 20 isolates were obtained from different regions of Meghalaya *viz.*, Ri-Bhoi district (Umrynjah), East Khasi Hills district (Puriang and Laitmynsaw), East Jaintia hills (Jaintia) and West Jaintia Hills districts (Jowai). The collected cadaver samples were surface sterilised with 0.1% sodium hypochlorite for one minute, followed by washing twice with sterilized distilled

water for one minute each. The samples were then blot dried and placed on centre of petri plate containing selective media *viz.*, Sabouraud Dextrose Agar (SDA) medium amended with 0.05% streptomycin sulphate (Senthamizhlselvan *et al.*, 2010). Microscopic identification of *Beauveria* was done by observing conidia under 10X (0.25), 40X (0.65) and 100X (0.80) magnification of LEICA microscope as single celled, hyaline, round to oval in shape and (1.5-4) X (1.5-3) µm in size and were arranged either singly or in group on geniculate rachis. The base of the conidia was observed bulgy with ovoid shape and was produced sympodially at the tip of mother cell. Screening of best potential isolate was done on the basis of their functional & biocontrol attributes such as production of hydrolytic enzymes *viz.*, amylase, lipase, caseinase, cellulase, chitinase and protease. Out of 20, isolate BP1.1 have been selected potential strain on the basis of its functional attributes and selected further for conducting compatibility test.

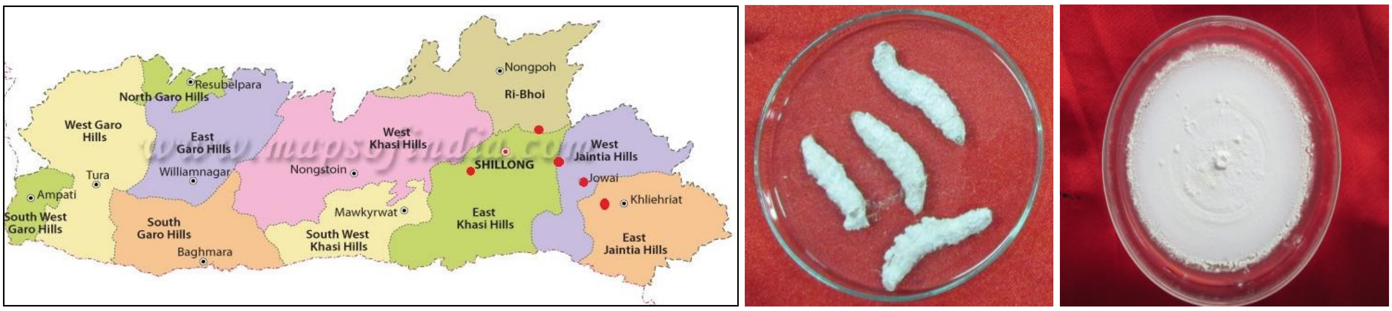

Fig 1: Collection and Isolation of *B. bassina* isolates from silkworm cadavars from different regions of Meghalaya Compatibility test

The compatibility of *Beauveria* isolate was studied with fungicides *viz.*, carbendazim (Bavistin 50 WP) at three concentrations *i.e.*, 0.05%, 0.1% and 0.15% and copper oxychloride (Blitox 50 WP) at three concentrations *i.e.*, 0.15%, 0.2% and 0.25% were studied. The compatibility of *Beauveria* isolate was also studied with three different botanicals *viz.*, aqueous neem leaf extract (1% w/v), neem seed kernal extract (NSKE) (5% w/v) and aqueous garlic clove extract (1% w/v) at three different concentrations *i.e.*, 3%, 5% and 7% respectively.

Quantity of chemical required (g) = (% Concentration X Volume desired) / a.i. of commercial formulation

Table 1: Chemicals used to study compatibility with *Beauveria*

Sl. No.	Chemicals	Trade name	Active ingredient	Different concentrations
1.	Carbendazim	Bavistin	50 WP	0.05%, 0.1%, 0.15%
2.	Copper oxychloride	Blitox	50 WP	0.15%, 0.2%, 0.25%
3.	Aqueous Neem Leaf extract	-	1 % w/v	3%, 5%, 7%
4.	Aqueous Garlic Clove extract	-	1% w/v	3%, 5%, 7%
5.	Neen Seed Kernel Extract	-	5% w/v	3%, 5%, 7%
6.	Untreated control	-	-	-

The quantity of required chemicals were filter sterilized with the help of sterilized syringe filters with Mixed Cellulose Ester (MCE) membrane of 0.22 µm pore size (Maximum pressure: 4.5 bars; 67.5 psi.) into molten media and rotated in circular motion till it get mixed properly. The poisoned media was then poured into petri plates (20 ml) and 7 mm of actively growing mycelia disc of *Beauveria* was inoculated at the centre of the petriplate and incubated for seven days at 25 ±1 ℃

Observation

Observations were taken when control plates attained full growth. Compatibility of *Beauveria* isolate was studied on the basis of growth of mycelia as well as production of aerial conidia on the treated media. The radial growth of screened *Beauveria* isolate was measured on the day when control plates attained full growth. Per cent inhibition of *Beauveria* isolate was calculated based on the diameter of growth of the colony by using the formula given by Vincent (1947),

$I = (C-T)/C \times 100$

Where, I = Per cent inhibition (%),

C = Growth of *Beauveria* isolate in control (mm),

T = Growth of *Beauveria* isolate in treatment (mm).

Results and Discussions

The *Beauveria* isolate BP1.1 was grown on SDA media amended with three different concentrations of fungicides and botanicals. The mycelial growth of isolate BP1.1 was either partially or completely inhibited by all the chemicals and botanicals at all three concentrations except the control plate.

Observations were taken and results are presented in Figures and Table 2. The significant inhibition percentage was observed in all treatments at sub normal concentration (C1) at seventh day after incubation and results are presented in Table 2. The total inhibition of *Beauveria* was observed in Carbendazim which was followed by Garlic extract (32.78 %) and neem seed kernel extract (21.39 %). The lowest inhibition percentage at C1 concentration was observed in Copper oxychloride (10.74%) followed by neem leaf extract (16.94 %). At Normal concentration (C2), significant inhibition percentage was observed in all treatments. The total inhibition was observed in carbendazim which was followed by garlic extract (63.06 %) and neem seed kernel extract (50.56 %). The significantly lowest inhibition percentage was observed in copper oxychloride (32.22 %) followed by neem leaf extract (39.17%).

The significant inhibition percentage was observed in all treatments at higher concentration (C3) and results were presented in Table 2. The total inhibition was observed in carbendazim which was followed by garlic extract (78.33 %) and neem leaves extract (68.33 %). The significantly lowest inhibition percentage was observed in copper oxychloride (51.94 %) followed by neem seed kernel extract (66.67 %). Among all the treatments, *Beauveria* isolate BP1.1 was found highly compatible with copper oxychloride, moderately compatible with neem seed kernel extract, neem leaf extract and garlic extract but non compatible with carbendazim at all three concentrations.

The mycelial growth and conidial germination of *B. bassiana* was affected either partially or completely by all the concentrations of selected pesticides and botanicals. Copper oxychloride have multisite activity which inhibits the fungal growth by interfering with enzyme system of spores and mycelium. Challa and Savinda (2014) reported that *B. bassiana* was found highly compatible with copper oxycloride as the copper binding protein (CuBp) of *B. bassiana* is a member of metallothioneins which is a cysteine-rich protein lacking aromatic residues and consists of copper (I)- thiolate cluster. This is the reason that *B. bassiana* has a stronger affinity for copper. Therefore, copper based fungicide (Dodine, $CuCl_2$) is also used in selective isolation of *B. bassiana* from soil (Akello *et al.*, 2007; Shin *et al.*, 2010). Similarly, in the present study, copper oxychloride 50WP was found as the most compatible fungicide at all concentrations by exhibiting an inhibition percentage of 10.56 % (80.50 mm), 32.22% (61.00 mm) and 51.94 % (43.25 mm) respectively.

Challa and Savinda (2014) also reported that the complete inhibition (100%) of conidial germination of *B. bassiana* take place in response to carbendazim. Carbendazim inhibits the development of fungus by interfering with spindle formation at mitosis during cell division, thus, inhibiting germtube development, formation of appresorium and growth of mycelia. Butters *et al*. (2003) has also reported the abnormal bent appearance of the germination peg of *B. bassiana* with bursting at the tip in the presence of bavistin. Similarly, in the present study, the total inhibition of mycelial growth was observed in carbendazim 50WP at all three concentrations.

Table 2: Mean colony diameter and per cent inhibition of *B. bassiana* BP1.1 in SDA media treated with different fungicides and botanicals

Treatments	**Mean colony diameter (mm)***			**% Inhibition***		
	C_1	**C_2**	**C_3**	**C_1**	**C_2**	**C_3**
Carbendazim	0.00^{d} (0.71)	0.00^{f} (0.71)	0.00^{f} (0.71)	100.00^{a} (10.02)	100.00^{a} (10.02)	100.00^{a} (10.02)
Copper oxychloride	80.50^{b} (9.00)	61.00^{b} (7.84)	43.25^{b} (6.61)	10.56^{d} (3.37)	32.22^{e} (5.72)	51.94^{d} (7.24)
Neem leaf extract	74.75^{b} (8.67)	54.75^{c} (7.43)	28.50^{d} (5.38)	16.94^{d} (4.17)	39.17^{d} (6.30)	68.33^{c} (8.30)
Garlic extract	60.50^{c} (7.81)	33.25^{e} (5.81)	19.50^{e} (4.47)	32.78^{b} (5.77)	63.06^{b} (7.97)	78.33^{b} (8.88)
Neem seed kernel extract	70.75^{b} (8.69)	44.50^{d} (6.71)	30.00^{c} (5.52)	21.39^{c} (4.68)	50.56^{c} (7.15)	66.67^{c} (8.20)
Control	90.00^{a} (9.51)	90.00^{a} (9.51)	90.00^{a} (9.51)	0.00^{e} (0.71)	0.00^{f} (0.71)	0.00^{e} (0.71)
SEm ±	0.11	0.03	0.04	0.04	0.03	0.04
CD (P=0.05)	0.31	0.08	0.12	0.11	0.09	0.11

Note: Figures in the table are mean values of four replications
SEm: Standard error mean
CD: Critical difference
*Figures written in parenthesis are square root transformed values

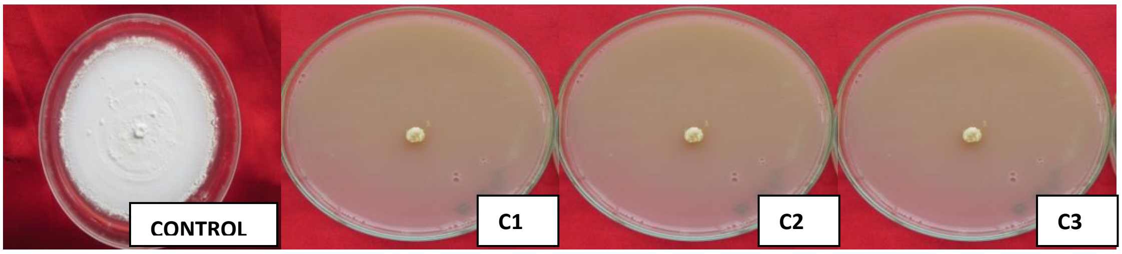

Fig 2: Compatibility of *B. bassiana* BP1.1 with carbendazim 50WP

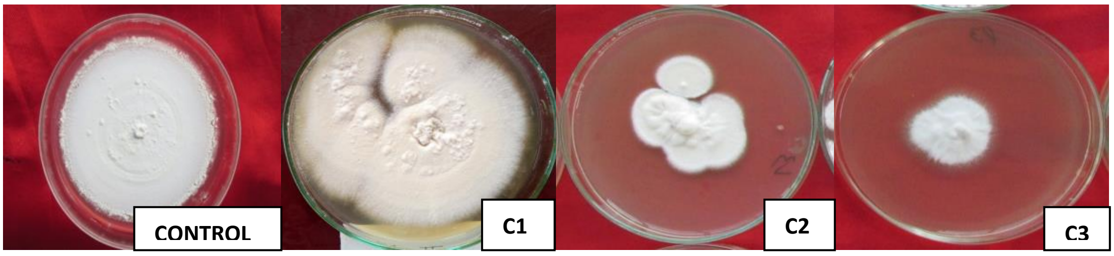

Fig 3: Compatibility of *B. bassiana* BP1.1 with copper oxychloride 50 WP

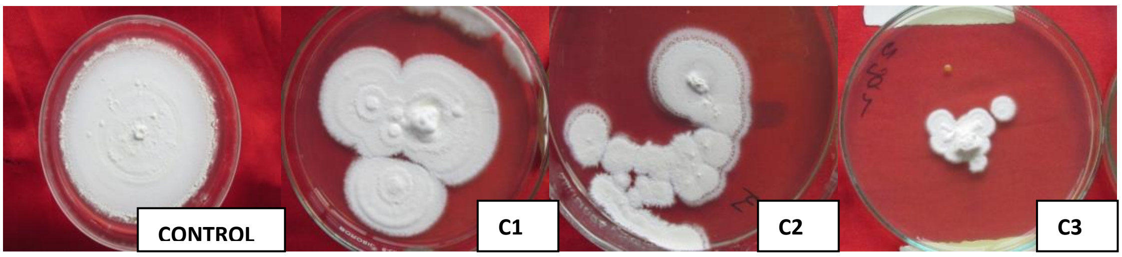

Fig 4: Compatibility of *B. bassiana* BP1.1 with aqueous neem leaf extract

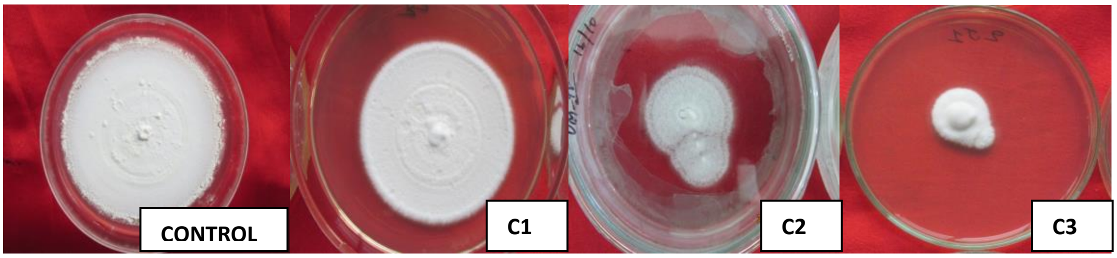

Fig 5: Compatibility of *B. bassiana* BP1.1 with neem seed kernel extract

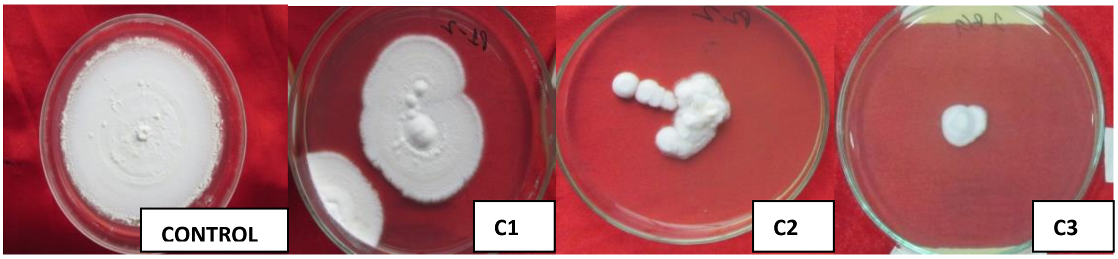

Fig 6: Compatibility of *B. bassiana* BP1.1 with aqueous garlic extract

Aqueous neem leaf extract (ANLE) consists of high amount of azadiracthin, which is a complex of triterpenoid and tetranortriterpenoid compounds. It effects directly on cells and tissues by inhibiting cell division and protein synthesis and indirectly by affecting endocrine systems. Castiglioni *et al.* (2003) reported significant inhibition effects on vegetative growth, conidiogenesis and germination of Beauveria spores caused by commercial formulation of neem leaves, in concentrations that are equal or greater than 5% active ingredient. Diepieri *et al*. (2005) reported the compatibility of aqueous extracts of neem leaves and seeds with *B. bassiana* at 1.5% and 15% concentrations. The difference in inhibition rates of mycelial growth of *B. bassiana* by aqueous neem leaf extract and neem seed kernel extract (NSKE) was due to the variability in the amount of terpenoids, phytoalexins, sulfurade compounds and triterpenoids (Bandopadhyaya, 2002; Sidhu *et al.*, 2004). Ambethgar *et al.* (2009) has also reported that NSKE (5%) exhibited minimum inhibition of *B. bassiana*. Similarly, the compatibility of *B. bassiana* with ANLE and NSKE was also observed in present study at all the concentrations.

In the present study, *B. bassiana* was also found compatible with aqueous garlic extract (AGE) at all three concentrations. AGE consists of high concentration of sulphur (aliin, allicin *etc.*), amino acids, enzymes (flavonoids, saponins), phenol content of 3.57 ± 0.49 mg/g and antioxidants. The antimicrobial activity of AGE is due to the interaction of allicin with cysteine undergoin biosynthetic pathway and inhibition of RNA synthesis. Compatibility of AGE with biocontrol agents *e.g.*, *Trichoderma* spp. was also studied by Maheshwari (2014). Usha *et al.* (2014) also studied the compatibility of entomopathogenic fungus *B. bassiana* with pesticides, fungicides and botanicals at 0.1X, 0.5X and 1.0X concentrations and reported that copper oxychloride was compatible with *B. bassiana* at lower concentrations but showed slight toxicity at higher levels. It was also reported that copper oxychloride was found to promote the mycelial growth in many isolates and concluded copper oxychloride as best fungicide formulation in combination with entomopathogenic fungi to be used in IPM programme.

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