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Antibacterial Ability of *Beauveria bassiana* (Balsamo) Vuillemin against *Xanthomonas oryzae* pv. *oryzae* causing Bacterial leaf Blight of Rice

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ABSTRACT: *Beauveria bassiana* (Balsamo) Vuillemin, a white muscardine fungus has drawn attention worldwide as potential biocontrol agent against insect-pests since decades. In the rice ecosystem, *B. bassiana* has been established as potential mycoinsecticide against major insect-pests of rice *viz.*, *Cnaphalocrocis medunalis, Nilaparvata lugens, Dicladispa armigera, Scirpophaga incertulas* etc., but its efficacy against phytopathogens of rice in simultaneous application is least studied. Among major phytopathogens of rice, bacterial leaf blight incurs a yield loss of up to 12-75%, affecting crop at seedling, tillering and vegetative stages. Therefore, recent studies have inclined towards unravelling antimicrobial potential of *B. bassiana* as effective plant disease antagonist, thereby, opening a newer dimension in dual-purpose crop protection strategies. In the present study, antagonistic abilities of fifteen (15) native isolates of *B. bassiana* were evaluated against *Xanthomonas oryzae* pv. *oryzae* causing bacterial leaf blight of rice by conducting agar-plug diffusion and inverted plate assays. The results showed that, majority of *B. bassiana* isolates *viz.*, Bb31, Bb45, Bb48, Bb25, Bb53 were able to inhibit bacterial growth, with maximum per cent inhibition was recorded in the range of 60-84% through development of inhibition zone. Further, antibacterial ability of *B. bassiana* was also attributed to release of non-volatile inhibitory metabolites responsible for the formation of inhibition zone on the bacterial lawn. These findings provide substantial evidences on antibacterial abilities of *B. bassiana* against *X. oryzae* pv. *oryzae* and further provides a scope to test the efficacy of *B. bassiana* against bacterial leaf blight of rice in the glasshouse and field condition in addition to their potential entomogenous behaviour against insect-pests of rice.

Keywords: Antibacterial, *B. bassiana*, entomopathogen, *X. oryzae* pv. *oryzae*, rice.

INTRODUCTION

Rice (*Oryza sativa* Linn.) is an important staple food crop for more than half of world's population, of which, Asian countries accounts for 90% of the world's rice production. Among several biotic and abiotic factors affecting production of rice, bacterial leaf blight paves devastating effect in rice cultivation occurring at seedling, vegetative and reproductive stages but severe at tillering stage, causing a yield loss up to 10-75 % depending on weather, location and cultivars used in rice production (Shivalingaiah and Umesha, 2011). Symptomatically, bacterial leaf blight (BLB) in rice is characterized by greenish water-soaked translucent spots of 5-10 mm from tip that enlarge in length forming yellow lesions with wavy margin along the leaf edges, whereas, kresek or wilting symptom appears one or two weeks after transplanting (Mason and Mathew, 1996). *Xanthomonas oryzae* pv. *oryzae* Dawson (Proteobacteria: Gammaproteobacteria: Xanthomonadales: Xanthomonadaceae), a gram negative, rod-shaped, aerobic bacterium measuring 0.5-0.8 × 1-2 µm in size with single polar flagella is the incitant of bacterial leaf blight of rice. The bacterium when grown on nutrient agar (NA) medium is identified as yellow, convex, mucoid colonies with shiny texture (Samanta *et al.*, 2014). Management of bacterial leaf blight disease conventionally involves soaking seeds in 0.025% streptocycline solution, hot water treatment at 52 ºC for 30 minutes, spraying of Vitavex at 0.15-3.0% and biological control with *Trichoderma asperellum* T42 and *Bacillus amyloliquefaciens* FZB42 (Wu *et al.* 2015; Singh *et al.* 2019). But, in the recent times, use of microbial inoculants have emerged as potential management strategy with an aim to minimize degradative effects posed by increased application of synthetic chemicals on environment and human health. However, studies on *B. bassiana*, an entomopathogenic fungus as potential plant disease antagonist by Ownley *et al.*, (2008); Vega *et al.*, (2009), has introduced a new concept in plant disease management.

B. bassiana (Balsamo) Vuillemin (Ascomycota: Hypocreales: Cordycipitaceae), soil-dwelling necrotrophic fungus, is widely known as potential biocontrol agent against insects-pests of rice belonging

to the order Lepidoptera, Coleoptera, Hymenoptera, Hemiptera and Orthoptera (Zimmermann, 2007). In addition to entomopathogenic behaviour, recent studies also showed other beneficial roles of *B. bassiana* as plant disease antagonist, rhizosphere colonizer, endophyte and plant growth promoter (Vega *et al.*, 2009). In several studies, *B. bassiana* was reported as successful plant disease antagonist against *Phytophthora infestans*, *Pythium* sp., *Botrytis* sp., *Phoma* sp., *Rhizoctonia solani*, *Curvularia lunata*, *Fusarium* sp., *Colletotrichum* sp., etc. (Griffin, 2007; Ownley *et al.*, 2008; Vega *et al.*, 2009; Deb and Dutta, 2021; Deb *et al.*, 2021). Possible mechanisms of plant disease suppression were attributed to mycoparasitism, competition, antibiosis through production of lytic enzymes, release of volatile and non-volatile biologically active compounds and stimulation of induced systemic resistance (ISR) (Jaber and Enkerli, 2017; Jaber and Ownley, 2018). From earlier studies by Griffin *et al.*, (2006), pre-treatment of cotton seedlings with *B. bassiana* has resulted in significant reduction of *Xanthomonas* bacterial blight due to induction of systemic resistance (ISR) in treated plants as compared to control. *B. bassiana* Bb 11-98 was also found capable of inducing ISR in cotton seedlings, according to the study conducted by Ownley *et al.* (2008) against *X. axonopodis* p.v. *malvacearum*, when conidia were applied as root drench at the rate of 10^7 CFU/seedling root resulted in significant reduction in bacterial blight in cotton. It was observed that over six days of disease assay, the disease severity indices were consistently lower for Bb 11-98 treatments based on disease progress curve.

Though several literatures reported effective utilization of *B. bassiana* as mycoinsecticide against major insect-pests of rice *viz.*, leaf folder *Cnaphalocrocis medunalis*, brown plant hopper *Nilaparvata lugens*, rice hispa *Dicladispa armigera*, green leaf hopper *Nephotettix virescens* but their biocontrol potential against major rice phytopathogen, BLB following simultaneous application is least studied. With this background, the present study was conducted to study the antibacterial potential of *B. bassiana* against *X. oryzae* pv. *oryzae* and the possible underlying mechanisms to devise effective management strategy against bacterial blight disease of rice.

MATERIALS AND METHODS

B. bassiana isolates

Fifteen (15) native isolates of *B. bassiana* with NCBI GenBank accession numbers *viz.*, Bb4 (MW628496), Bb5 (MW633007), Bb11 (MW633208), Bb13 (MW633013), Bb16 (MW628497), Bb17 (MW633014), Bb18 (MW633015), Bb25 (MW628498), Bb28 (MW633020), Bb31 (MW622068), Bb44 (MW628499), Bb45 (MW633217), Bb48 (MW633219), Bb50 (MW633221) and Bb53 (MW627305) isolated from mulberry silkworm (*Bombyx mori)* from different locations of Meghalaya during June, 2018- July, 2019 were used in the study. All isolates of *B. bassiana* were sub-cultured on Sabouraud Dextrose Agar (SDA- dextrose 40g, peptone 10g and agar agar 15g in 1000 ml distilled water) following 7-15 days incubation at 28±1ºC under dark condition (Senthamizhlselvan *et al.*, 2010). Morphologically, *B. bassiana* was identified as disperse to dense growth, white coloured colony on front and reverse side, circular, smooth texture and powdery in appearance (Fig. 1.i). Under light microscope (Leica), hyphae of *B. bassiana* was observed as septate, hyaline, 1-2 µm in size hyphae grouped into sympodial branched conidiophores consisting of 3-6 µm sized bottle-shaped phialides, bearing single-celled, hyaline, round to oval shaped conidia (1.5-4) × (1.5-3) µm in size and were arranged either singly or in group on geniculate rachis (Fig. 1.ii).

Isolation of *Xanthomonas oryzae* pv. *oryzae*. Naturally infected blighted rice leaves with typical bacterial blight symptoms *viz.*, straw yellow-coloured lesions with wavy margin were collected from lowland rice field located in Umiam, Meghalaya (25º41′21″N, 91º55′25″E) were used for isolation of *X. oryzae* pv. *oryzae* by following tissue segment method given by Rangaswami (1972). Leaf sections (1 × 1 cm^2) were prepared by serial washing technique *via.*, disinfecting with 0.05% sodium hypochlorite, followed by thrice rinsing with double distilled water and blot dried with sterile filter paper (Whatmann no. 1). Leaf sections were inoculated on nutrient agar (NA- peptone 5g, meat extract 3g and agar agar 15g in 1000 ml distilled water) plates (90 mm) and incubated at 28±1ºC, while, constant observation was made till bacteria oozes out from inoculated sections (Srivastava and Rao, 1964). Putative bacterial culture was aseptically picked with the help of inoculation needle (nichrome) and purified by quadrate streaking method (Dhingra and Sinclair, 1995) to obtain single colony and maintained by periodic streaking on fresh NA slants and stored in refrigerator at 4ºC. Identification of *X. oryzae* pv. *oryzae* was made based of biochemical tests as follows:

Gram staining and KOH test. Thin smear of 24 h old bacterial suspension was heat fixed on glass slide, treated with hacker's ammonium oxalate crystal violet stain followed by gram's iodine solution for one minute each and rinsed with tap water. Specimen was decolourized with 95% ethanol and counter-stained with safranine for 30 seconds, blot dried and examined under microscope at 100X oil immersion. Observation of red colour indicated gram negative bacteria (Ghasemine *et al.*, 2008). To confirm gram staining test, a loopful of 24 h old bacterial suspension was mixed with 3 % KOH solution for 10 seconds on glass slide. Development of viscid strands confirmed KOH test (Ryu, 1940).

Catalase, oxidase and indole test. Loopful of 24 h old bacterial suspension was mixed with 3% H_2O_2 solution for 20 seconds on glass slide. Observation of bubble formation indicates positive catalase activity (Lelliott and Stead, 1987). Similarly, loopful of freshly grown (24 h old) bacterial culture was rubbed on oxidase discs and DMACA indole discs (Himedia) and development of blue-purple colour within 10-30 seconds indicated positive reaction (Kovac, 1956).

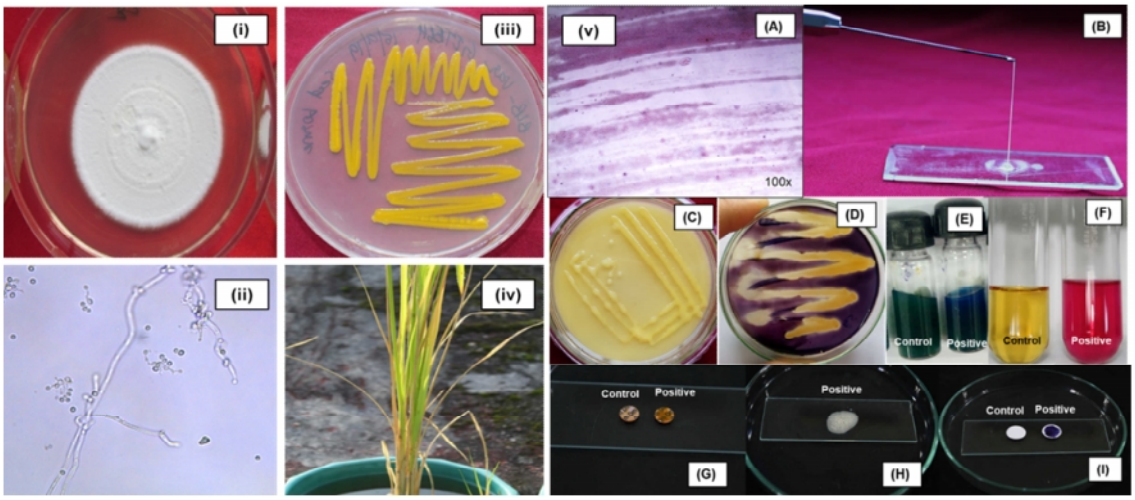

Fig. 1 (i-v): (i) Pure culture of *B. bassiana*, (ii) micro-image of *B. bassiana*, (iii) pure culture of *Xanthomonas oryzae* pv. *oryzae*, (iv) pathogenicity test proving association of *X. oryzae* pv. *oryzae* with bacterial leaf blight of rice and (v) biochemical test for *X. oryzae* pv. *oryzae* viz., (A) gram staining test, (B) KOH test, (C) YCDA test, (D) starch test, (E) citrate utilization test, (F) urease test, (G) indole test, (H) catalase test and (I) oxidase test

Urease test. Ability of bacteria to hydrolyze urea was evaluated by inoculating loopful of 24 h old bacterial suspension in christensen's urea broth (10 ml) in screw capped vials (Brink, 2010). Inoculated vials were incubated at 28±1ºC for 48 hours and development of pink colour indicated positive urease activity.

Citrate reduction test. Ability of bacteria to utilize citrate was evaluated by stab inoculation of freshly grown bacteria (24 h old) on simmon's citrate agar dispensed in screw capped vials (20 ml). inoculated vials were incubated at 28±1ºC for 48 hours and change in colour from green to blue indicated positive citrate utilization ability (Simmon, 1926).

Starch hydrolysis (Amylase test). Freshly grown bacterial culture (24 h old) was streaked onto petri plates (90 mm) containing starch agar media (20 ml) and incubated at 28±1ºC for 48 hours (Covan, 1974). After incubation, plates were flooded with gram's iodine solution and formation of yellow coloured halo surrounding the culture indicated positive starch hydrolysis activity.

Growth on YDCA medium. Freshly grown bacterial culture (24 h old) was streaked onto petri plates (90 mm) containing yeast dextrose carbonate agar (YDCA) media (20 ml) and incubated at 28±1ºC for 48 hours (Schaad, 1988). Production of mucoid, convex and shiny colony confirmed identification of *X. oryzae* pv. *oryzae* colonies.

Pathogenicity test: Twenty-one (21) days-old healthy rice seedlings were grown in 25 cm diameter plastic pots containing 5 kg sterilized soil. Pathogenicity test for *X. oryzae* pv. *oryzae* was proved by adopting leaf clipping technique by inoculating freshly clipped-off leaf tips with 24 h old bacterial suspension (Yinggen *et al.*, 2017). Inoculated plants were covered with perforated polypropylene bag for incubation, while, optimum temperature and humidity was maintained by spraying sterile distilled water (SDW) (Nazari *et al.*, 2015). Development of characteristic symptoms were observed and the pathogen was re-isolated from infected leaves and compared with the original culture to prove the pathogenicity test (Fig. 4).

Antibacterial potential of *B. bassiana* against *Xanthomonas oryzae* pv. *oryzae*. The antagonistic potential of *B. bassiana* isolates were evaluated against *Xanthomonas oryzae* pv. *oryzae*, a bacterial pathogen causing bacterial leaf blight of rice by performing agar plug diffusion assay (Heatley, 1944). Twenty-four (24) h old bacterial suspension of *X. oryzae* pv. *oryzae* was prepared by growing loopful of bacterial culture in 10 ml nutrient broth (NB) incubated at 28±1ºC in rotary shaker (ROTEK LES, 150 rpm) at for 24 hrs. Nutrient agar (NA) plates were streaked with loopful of bacterial suspension (24 h old) and allowed to dry for 30 min. Agar wells of 7 mm diam. were made at centre of Petri plate with the help of sterile cork borer (7 mm diam.) and mycelial discs (7 mm diam.) of actively growing (7 days old) *B. bassiana* isolates were plugged. Inoculated plates were incubated at 28±1ºC under dark condition for 48 hours and inhibition zone was calculated by formula:

$$\text{Per cent inhibition (\%)} = \left\{\frac{\text{Zone of inhibition (mm)}}{\text{Diameter of petri plate (mm)}}\right\} \times 100$$

Three replications were maintained and zone of inhibition was compared with chloramphenicol (Himedia susceptibility test disc, 30 µg/ disc).

Effect of volatile inhibitory substances released by *B. bassiana* against *Xanthomonas oryzae* pv. *oryzae*. Ability of *B. bassiana* isolates to produce volatile inhibitory substances was evaluated under *in vitro* condition against *X. oryzae* pv. *oryzae* by following inverted plate technique (Dennis and Webster, 1971). Petri plates (90 mm) containing SDA were inoculated with actively growing (7 days old) mycelial disc (7 mm diam.) of *B. bassiana* at centre, sealed with parafilm and incubated at 28±1ºC for 3 days under dark condition. Separately, test pathogens in active growing stage (48 h old for bacteria) were streaked on NA plates (90 mm), sealed with parafilm and incubated at 28±1ºC for 5 hours. After incubation, the lid of Petri plates

containing *B. bassiana* and test pathogens were aseptically removed and both bottoms were inverted over each other, double sealed with parafilm and further incubated at 28±1ºC for 48 hrs under dark condition. Three replications were maintained and Petri plates without antagonist served as control. Bacterial growth was observed after 48 hrs post incubation and inhibition over control was recorded as strong inhibitory (+++), medium inhibitory (++), least inhibitory (+) and no inhibition (-) respectively.

Statistical analysis. The statistical design used in the present study was completely randomized design (CRD) and was analysed by using software ICAR Web Agricultural Statistical Package 2.0. The significant difference, if any, among the treatment means were compared by using critical difference (CD) at $P=0.05$ (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

Identification and pathogenicity test for *Xanthomonas oryzae* pv. *oryzae*. The bacterial culture in NA medium was identified as light yellow to straw-coloured, shiny-opaque, mucoid, circular colonies with slimy, convex and smooth in appearance on 24 hrs post incubation (Fig. 1.iii). A series of biochemical test *viz.*, on citrate reduction, catalase, urease, starch production, oxidase, indole test etc were conducted. Under Gram's staining test, appearance of red-coloured, rod-shaped bacterium with monotrichous, polar flagella indicated gram negative nature, which was further confirmed by viscous thread formation in KOH test. On YDCA medium, bacterial colonies appeared as yellow, circular, smooth, convex and viscous nature. The bacterium showed positive reaction under starch hydrolysis, urease test (pink colour development), citrate reduction (blue colour development), catalase (formation of air bubbles), oxidase (blue colouration of discs) and negative reaction in indole test (No change in colour) (Fig. 1.v). The results of biochemical characterization were in accordance with Samanta *et al.* (2014), who reported *X. oryzae* pv. *oryzae* as gram negative bacterium with positive catalase, negative starch hydrolysis and oxidase properties. Further in the present study, pathogenicity test was characterized by appearance of symptoms like water soaked, translucent lesions starting from leaf margins that enlarged with wavy margin (Fig. 1.iv). As disease progresses, lesions turned straw-yellow and covered entire plant ultimately killing whole plant. The results were in conformity with Mew and Gonzales (2002), who have observed similar symptoms i.e., greenish water-soaked translucent spots initiating from wavy margin along the leaf edges followed by wilting one or two weeks after transplanting by using leaf clipping as artificial inoculation method.

Antibacterial ability of *B. bassiana* against *X. oryzae* pv. *oryzae*. The antibacterial activity of *B. bassiana* isolates against *X. oryzae* pv. *oryzae* was studied *in vitro* by following agar well diffusion assay through the formation of inhibition zone surrounding agar well on nutrient agar (NA) plates. Development of inhibition zone surrounding agar well was observed 48 hours post incubation by the interaction of inhibitory substances released by *B. bassiana* isolates and the test pathogen (Fig. 2). The inhibition percentage (%) against *X. oryzae* pv. *oryzae* was found significantly higher with isolate Bb31 (84%) followed by Bb45 (73.40%), Bb48 (73.33), Bb25 (70.33%) and Bb53 (70.33%) by developing an inhibition zone of 42 mm, 36.70 mm, 36.67 mm, 35.17 mm and 35.17 mm respectively (Table 1). Apart from that, other potential *B. bassiana* isolates (8) *viz.*, Bb4, Bb13, Bb25, Bb31, Bb44, Bb45, Bb50 and Bb53 showed efficient antibacterial abilities ranging from 60-84% against the test pathogen. Among which, nine (9) *B. bassiana* isolates showed higher inhibition percentage (%) as compared to recommended antibiotic check i.e., chloramphenicol (@ 30 µg/ disc) with (63.00%) with 31.50 mm inhibition zone. Whereas, only one isolates *viz.*, Bb16 showed the inhibition percentage ranging from 50-60%, while, five (5) isolates Bb5, Bb11, Bb17, Bb18 and Bb28 were found inefficient in their antibacterial abilities showed no/ least inhibition zone surrounding the *B. bassiana* plugged agar well on NA plate.

Previous literatures of Vega *et al.* (2009); Ownley *et al.* (2010) showed that *B. bassiana* operated *via.*, more than one mechanism for antagonistic interaction as well as suppression of plant diseases either through direct mechanisms *viz.*, mycoparasitism, competition and antibiosis or complex indirect interaction by stimulating induced systemic resistance as well as promotion of plant growth. Further, several toxins and secondary metabolites released by *B. bassiana* possess insecticidal, antimicrobial and antioxidant properties as studied by Jeffs and Khachatourians (1997) may be the reason behind efficient antibacterial ability of *B. bassiana* observed in the present study. Further in the present study, formation of an inhibition on the bacterial lawn impregnated with *B. bassiana* agar-disc provides significant evidence of antagonistic interaction of *B. bassiana* against *X. oryzae* pv. *oryzae*, probably due to the phenomenon of antibiosis. Whereas, according to Fravel (1988), the phenomenon of antibiosis is usually mediated by the production of certain specific or non-specific metabolites such as antibiotics, bioactive volatile organic compounds (VOCs), lytic agents, enzymes and toxic substances by one microorganism, which are deleterious to the growth or metabolic activities of other organisms. Also, in earlier studies by Jeffs and Khachatourians (1997); Alurappa *et al.* (2015), first description on antibiotic and cytotoxic activity of *B. bassiana* was reported through the inhibition of erythrocyte membrane ATPase activity (Ca^{2+}-ATPase than Na^{+}/K^{+}ATPase) causing membrane disruption and cell lysis by yellow and red pigments produced by *B. bassiana viz.*, tennelin, bassianin and oosporein.

Several literatures by Griffin, (2007); Vega *et al.* (2009); Jaber and Enkerli (2017) also reported extracellular enzymes produced by *B. bassiana viz.*, chitinases, glucanases, proteases, caesinases, lipases and cellulases, responsible for governing various physiological processes such as morphogenesis, parasitism, growth regulation, infection and virulence, therefore, facilitating pathogenesis against wide range

of phytopathogens. Such enzyme production ability of *B. bassiana* may be the reason behind lysis and dissolution of bacterial cell wall components of *X. oryzae* pv. *oryzae* as observed in the present study in the form of an inhibition zone on bacterial lawn surrounding the fungal disc. In similar studies, antibacterial ability of other entomopathogenic fungi *viz., B. bassiana, Metarrhizium anisopliae, Paecilomyces lilacinus* against *Xanthomonas* reported by Griffin (2007); Parine *et al.,* (2010); Ravindran *et al.,* (2014) further supported the findings of the present study. In addition to bacteria, several reports also highlighted antibiotic effects of *B. bassiana* against several soil-borne and foliar phytopathogens *viz., Gaeumannomyces graminis* var. *tritici, Armallaria mellea, Rossellinia necatrix, Fusarium oxysporum, Botrytis cinerea, Phoma* sp., *Pythium ultimum* and *Rhizoctonia solani,* further supports the present findings.

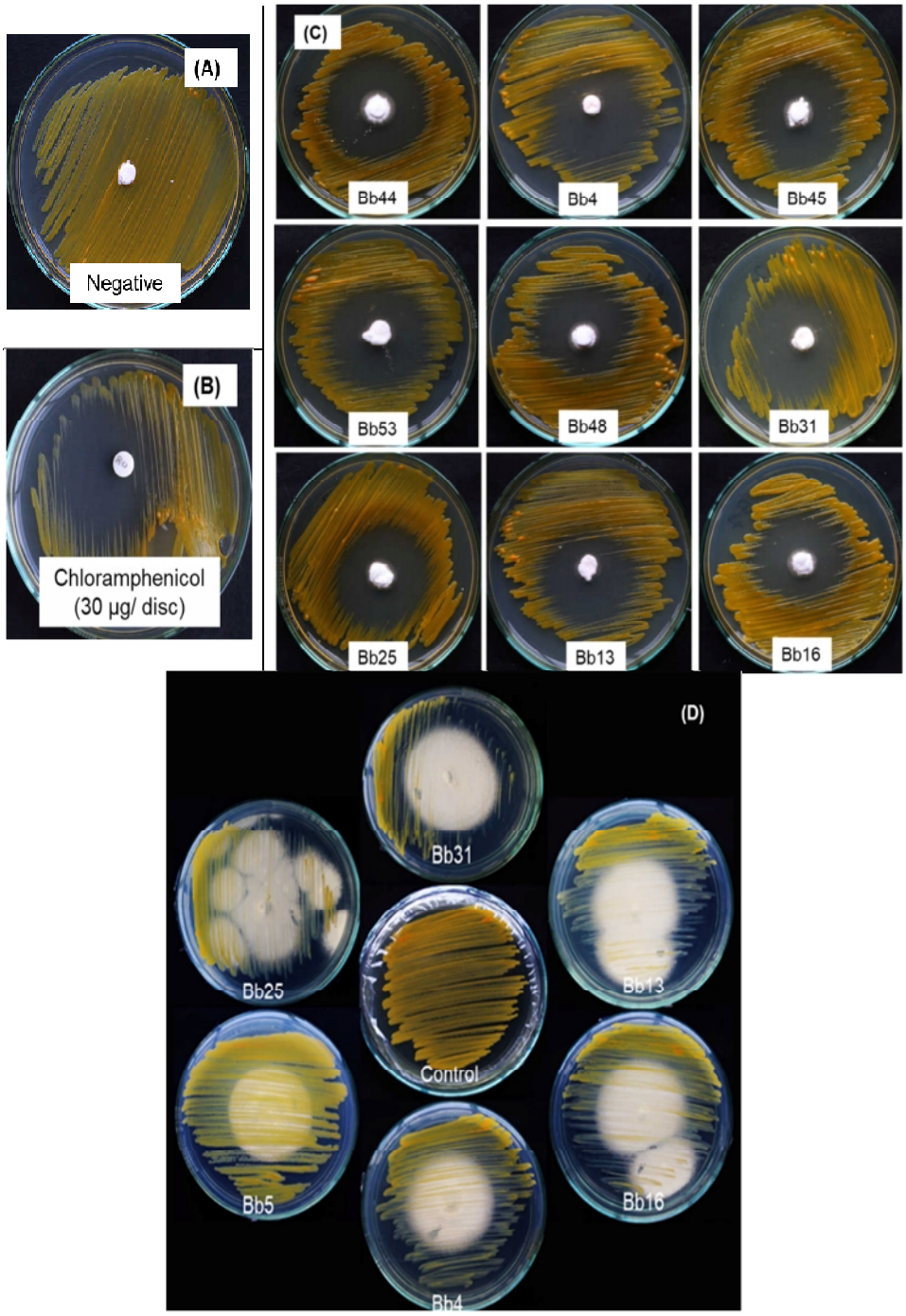

Fig. 2 (A-D): Formation of inhibition zone as antagonistic effect exhibited by *B. bassiana* isolates against *X. oryzae* pv. *oryzae* in (A) negative, (B) antibiotic check, (C) positive plates and (D) inhibitory effect of volatile organic compounds (VOCs) released by *B. bassiana* isolates in inverted plate assay against growth of *Xanthomonas oryzae* pv. *oryzae.*

Production of volatile inhibitory substances by *B. bassiana.* The ability of potential *B. bassiana* isolates to produce volatile inhibitory substances under *in vitro* condition was evaluated against *X. oryzae* pv. *oryzae* of rice by following inverted plate technique. Volatile diffusible substances derived from *B. bassiana* isolates exhibited growth inhibition of *X. oryzae* pv. *oryzae* at 48 hours, when control plate attained luxurious growth (90 mm). In case of *X. oryzae* pv. *oryzae*, majority of the *B. bassiana* isolates showed inhibitory effect against growth of the test pathogen through release of diffusible, toxic volatile compounds (Fig. 2.D). The volatile metabolites produced by *B. bassiana* isolates *viz.,* Bb31 showed strongest inhibitory (+++) activities against *X. oryzae* pv. *oryzae* followed by Bb48, Bb25, Bb53 and Bb45 showed medium inhibition (++), while, Bb4, Bb13, Bb16, Bb44 and Bb50 showed lowest inhibition (+) efficiency (Table 1).

Table 1: Per cent inhibition of *X. oryzae* pv. *oryzae* through release of non-volatile and volatile inhibitory substances by *B. bassiana* isolates.

Isolate code	Inhibition zone (mm)	*Per cent Inhibition (%)	**Inhibition
Bb4	33.33^{d}	66.66 (54.73)^{d}	+
Bb5	0.00^{h}	0.00 (0.28)^{h}	-
Bb11	0.00^{h}	0.00 (0.28)^{h}	-
Bb13	34.50^{cd}	69.00 (56.17)^{cd}	+
Bb16	26.16^{f}	52.33 (46.33)^{f}	+
Bb17	0.00^{h}	0.00 (0.28)^{h}	-
Bb18	5.00^{g}	10.00 (18.43)^{g}	-
Bb25	35.16^{c}	70.33 (56.99)^{c}	++
Bb28	0.00^{h}	0.00 (0.28)^{h}	-
Bb31	42.00^{a}	84.00 (66.42)^{a}	+++
Bb44	34.00^{cd}	68.00 (55.55)^{cd}	+
Bb45	36.70^{b}	73.40 (58.95)^{b}	++
Bb48	36.66^{b}	73.33 (58.91)^{b}	++
Bb50	34.50^{cd}	69.00 (56.16)^{cd}	+
Bb53	35.16^{c}	70.33 (57.01)^{c}	++
Control	31.50^{e}	63.00 (52.55)^{e}	
SEm(±)	0.43	0.53	
$CD_{0.05}$	1.26	1.56	

Figures in data are mean of three (3) replications; *Data within parentheses are angular transformed values; **Inhibition over control: strong inhibitory (+++), medium inhibitory (++), least inhibitory (+), No inhibition (-)

In the support of present findings, several literatures *viz.,* Crespo *et al.* (2008) and Xu *et al.* (2009), reported that *B. bassiana* produces wide array of biologically active metabolites mainly volatile organic compounds (VOCs) *viz.,* diisopropyl napthalenes, ethanol, sesquiterpenes etc., alkaloids (tennelin, bassianin, pyridovericin, pyridomacrolidin), non-peptide pigment (oosporein), non-ribosomally synthesized cyclodepsipeptides (beauvericin, allobeauvericins) and cyclopeptides (beauveriolides). Earlier studies by Blomquist *et al.* (1987) and Jaurez (1994) confirmed the involvement of VOCs released by *B. bassiana* in facilitating penetration and invasion of insect by *B. bassiana* through disruption of cuticle layer by breakdown of very-long-chain hydrocarbons with fatty alcohols and fatty acids. Also, Champlin and Grula, (1979) reported that several cyclopeptides and cyclodepsipeptides *viz.,* beauvericin, albo-beauvericin, bassianoloides and beauveriolides produced by *B. bassiana* cause cytotoxic activity due to their potential ionophore antibiotic properties. Later, Valencia *et al.* (2011) also reported that beauvericin produced by *B. bassiana* exhibit broad-spectrum strong anti-bacterial activity through interfering biosynthesis of cell organelles and enzyme production but not by affecting peptidoglycan cell wall biosynthesis.

Therefore, the antibacterial activity of *B. bassiana* against *X. oryzae* pv. *oryzae* through the release of diffusible, non-volatile and volatile compounds, further claim *B. bassiana* as potential plant disease antagonist in addition to its inbuild entomopathogenic properties. Further, the present study provides a scope for investigating unexplored potential of *B. bassiana* as plant disease antagonist and unravelling the hidden mechanisms responsible for such antagonistic interaction. Therefore, present study can act as catalytic in the direction of upcoming researches on studying dual-purpose biocontrol potential of *B. bassiana* against both insect-pests and phytopathogens.

CONCLUSION

The native isolates of *B. bassiana viz.,* Bb31, Bb45, Bb48, Bb25 and Bb53 were found to exhibit antagonistic activities against *X. oryzae* pv. *oryzae* causing bacterial leaf blight disease in rice in addition to their entomopathogenic behaviour. Biocontrol potential of native *B. bassiana* isolates as plant disease antagonist was attributed to production of volatile and non-volatile metabolites. However, the efficacy of the potential isolate must be further evaluated in field condition for their role in disease management, plant growth promotion and enhancement of crop yield. *B. bassiana* can also be established as dual purpose biocontrol agent due to their antagonistic potential against both insect pests and phytopathogens, though further studies need to be done in this respect.

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Conflict of Interest. None.

REFERENCES

Alurappa, R., Venkataramana, M., Nirmaladevi, D., Gupta, V. K., Chandranayka, S., & Srinivas, C. (2015). Cytotoxic effects of oosporein isolated from endophytic fungus *Cochliobolus kusanoi. Frontiers of Microbiology*, *6*: 870.

Blomquist, G. J., Nelson, D. R., & De Renobales, M. (1987). Chemistry, biochemistry and physiology of insect cuticular lipids. *Archives of Insect Biochemistry and Physiology, 6*: 227-265.

Brink, B. (2010). Urease test protocol. American Society for Microbiology.

Champlin, E. R., & Grula, E. A. (1979). Non-involvement of beauvericin in the entomopathogenecity of *Beauveria bassiana*. *Applied Environmental Microbiology, 37*(6): 1122-1126.

Covan, S. T. (1974). Manual for the identification medical bacteria. Cambridge University Press. Great Britain. Pp. 238.

Crespo, R., Pedrini, N., Juarez, M. P., & Dal Bello, G. M. (2008). Volatile organic compounds released by the entomopathogenic fungus *Beauveria bassiana. Microbiological Research, 163*(2): 148-151.

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

Deb, L., Devi, R. K. T., Majumder, D., Dutta, P., Thakuria, D., Rajesh, T., & Ningthoujam, K. (2021). Antifungal ability of *Beauveria bassiana* (Balsamo) Vuillemin against *Curvularia lunata* causing leaf spot of rice. *The Pharma Innovational, 10*(4): 298-303.

Dennis, C., & Webster, J. (1971). Antagonistic properties of species groups of *Trichoderma* I. Production of volatile antibiotics. *Transactions of British Mycological Society, 57*: 41-48.

Dhingra, O.D. and Sinclair, J.B. (1995). Basic plant pathology methods (2nd Edition) CRC Press, Boca Raton.

Fravel, D. R. (1988). Role of antibiosis in the biocontrol of plant diseases. *Annual Review of Phytopathology, 26*: 75–91.

Ghasemine, E., Kazempour, M. N., Ferydon, P. (2008). Isolation and identification of *Xanthomonas oryzae* pv. *oryzae* the causal agent of bacterial blight of rice in Iran. *Plant Protection Research, 48*(1): 53-62.

Gomez, K. A., & Gomez, A. A. (1984). Statistical procedures for agricultural research (2nd ed.). John Wiley and Sons, New York, Pp. 680.

Griffin, M. R. (2007). *Beauveria bassiana*, a cotton endophyte with biocontrol activity against seedling disease. Ph.D. Thesis, Submitted to The University of Tennessee, Knoxville, USA.

Griffin, M. R., Ownley, B. H., Klingeman, W. E., & Pereira, R. M. (2006). Evidence of induced systemic resistance with *Beauveria bassiana* against Xanthomonas in cotton. *Phytopathology, 96*: 42.

Heatley, N. G. (1944). A method for the assay of penicillin. *Biochemical Journal, 38*: 61-65.

Jaber, L. R., & Enkerli, J. (2017). Fungal entomopathohgens as endophytes: Can they promote plant growth? *Biocontrol Science Technology, 27*: 28-41.

Jaber, L. R., & Ownley, B. H. (2018). Can we use entomopathogenic fungi as endophytes for dual biological control of insects pests and plant pathogens. *Biological Control, 116*: 36-45.

Jaurez, M. P. (1994). Inhibition of insect surface lipid synthesis, and insect survival. *Archives of Insect Biochemistry and Physiology, 25*: 177-191.

Jeffs, L. B., & Khachatourians, G. G. (1997). Toxic properties of *Beauveria* pigments on erythrocyte membranes. *Toxicon, 35*(8): 1351-1356.

Kovac, N. (1956). Identification of *Pseudomonas pyocyanea* by oxidase reaction. *Nature, 4*: 535-703.

Lelliott, R. A., & Stead, D. E. (1987). Methods for the diagnosis of bacterial disease of plants. British Society of Plant Pathology, Blackwell Scientific Publications.

Mason. J. R., & Mathew, D. N. (1996). Evaluation of neem as a bird repellent chemical. *International Journal of Pest Management, 42*: 47-49.

Mew, T. W., & Gonzales, P. (2002). A handbook of rice seed-borne fungi. Los Banos, Phillipines: International Rice Research Institute (IRRI) and Enfield, N. H., USA: Science Publishers. Pp. 83.

Nazari, S., Nikkah, M. J., Fotouhifar, K. B., Khosravi, V., & Alizadeh, A. (2015). *Bipolaris* species associated with rice plant: pathogenicity and genetic diversity of *Bipolaris oryzae* using rep-PCR in Mazandaran province of Iran. *Journal of Crop Protection, 4*(4): 497-508.

Ownley, B. H., Griffin, M. R., Klingeman, W. E., Gwinn, K. D., Moulton, J. K., & Pereira, R. M. (2008). *Beauveria bassiana*: Endophytic colonization and plant disease control. *Journal of Invertebrate Pathology, 98*: 267 –270.

Ownley, B., Gwinn, K. D., & Vega, F. E. (2010). Endophytic fungal entomopathogens with activity against plant pathogens: Ecology and evolution. *Biocontrol, 55*: 113–128.

Parine, N. R., Pathan, A. K., Sarayu, B., Nishanth, V. S., & Bobbarala, V. (2010). Antibacterial efficacy of secondary metabolites from entomopathogenic fungal *Beauveria bassiana*. *International Journal of Chemical and Analytical Science, 1*(5): 94-96.

Rangaswami, G. (1972). Diseases of crop plants in India. Prentice Hall of India Pvt. Ltd., New Delhi, pp. 520.

Ravindran, K. Satishkumar, G., Rajkuberan, C., & Sivaramakrishnan, S. (2014). Antibacterial activity of indigeneous entomopathogenic fungus *Metarhizium anisopliae* against clinically isolated human pathogens. *International Journal of Pharmacy and Pharmaceutical Sciences, 6*(7): 179-182.

Ryu, E. (1940). A simple method of differentiation between Gram positive and gram-negative organisms without staining, Kitasato. *Archives of Experimental Medicine, 17*: 58-63.

Samanta, T. T., Samanta, P., & Das, A. (2014). Isolation and characterization of *Xanthomonas oryzae* isolates from different regions of Midnapore district of West Bengal and their eco-friendly management by some medicinal plant extracts. *International Journal of Phytomedicine, 6*: 29-42.

Schaad, N. W. (1988). Laboratory guide for the identification of plant pathogenic bacteria. The American Phytopathological Society, St. Paul, MN. Pp. 81-82.

Senthamizhlselvan, P., Alice, J., Sujeetha, R. P., & Jeyalakshmi, C. (2010). Growth, sporulation and biomass production of native entomopathogenic fungal isolates on a suitable medium. *Journal of Biopesticide, 3*(2): 466-469.

Shivalingaiah & Umesha, S. (2013). *Psuedomonas fluorescens* inhibits the *Xanthomonas oryzae* pv.

oryzae, the bacterial leaf blight pathogen in rice. *Canadian Journal of Plant Protection, 1*(5): 147-153.
Simmon (1926). Journal of Infectious Diseases, *39*: 209.
Singh, B. N., Dwivedi, P., Sarma, B. K., & Singh, H. B. (2019). *Trichoderma asperellum* T42 induces local defense against *Xanthomonas oryzae* pv. *oryzae* under nitrate and ammonium nutrients in tobacco. *RSC Advances, 9*: 39793-39810.
Srivastava, D. N., & Rao, Y. P. (1964). A single technique for detecting *Xanthomonas oryzae* in rice seeds. *Seed Science and Technology, 5*: 123-127.
Valencia, J. W., Gaiten, B. A. L., Jimenez, A. V., & Grossi-de-Sa, M. F. (2011). Cytotoxic activity of fungal matabolites from the pathogenic fungus *Beauveria bassiana:* an intraspecific evaluation of beauvericin production. *Current Microbiology, 63*(3): 306-312.
Vega, F. E., Goettel, M. S., & Blackwell, M. (2009). Fungal entomopathogens: new insights on their ecology. *Fungal Ecology, 2*: 149–159.
Wu, L., Wu, H., Chen, L., Yu, X., Borriss, R., & Gao, X. (2015). Difficidin and bacilysin from *Bacillus amyloliquefaciens* FZB42 have antibacterial activity against *Xanthomonas oryzae* rice pathogens. *Scientific Reports, 5*: 12975.
Xu, Y., Orozco, R., & Kithsiri, W. E. M. (2009). Biosynthesis of the cyclooligomer depsipeptide bassianolide, an insecticidal virulence factor of *B. bassiana*. *Fungal Genetics and Biology, 46*(5): 353-364.
Yinggen, K., Shugang, H., & Yuan, M. (2017). *Xanthomonas oryzae* pv. *oryzae* inoculation and growth rate on rice by leaf clipping method. *Bio-protocol, 7*(19). Doi:10.21769/BioProtoc.2568.
Zimmermann, G. (2007). Review on safety of the entomopathogenic fungus *Beauveria bassiana* and *Beauveria brongniartii*. *Biocontrol Science and Technology, 17*: 553-596.

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