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RESEARCH ARTICLE OPEN ACCESS

Interaction of Antagonistic Bacteria that Effective to Control Rice Bacterial Leaf Blight Disease with Agricultural Chemicals and Bio-products

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Abstract

This research aimed to study the compatibility of antagonistic bacteria that effective to control rice bacterial leaf blight disease, including *Bacillus pumilus* FDKF5 and *Bacillus* sp. IKM1 with agricultural chemicals and bio-products. Compatibility test of antagonistic bacteria with pesticides by using poison plate method showed that *B. pumilus* FDKF5 and *Bacillus* sp. IKM1 were able grow together with eight pesticides including 5 types of insecticide such as Conbalis (pyridaben 20% WP), Centaur (imidachloprid 70% WG), Posch (carbosulfan 20% W/V EC), Microthiol sulfur (sulfur 80% WG) and Abamectin (abamectin 1.8% W/V EC) and 3 types of fungicide including Terraclor Super-X (etridiazole 6% + quintozene 4% W/V EC), Fundasol 50 (benomyl 50% WP) and Octave (prochloraz 50% WP). However, streptomycin sulfate bactericide was inhibited their growth. Co-culture test of antagonistic bacteria with bio-products by using dual culture technique showed that *Trichoderma* and *Beauveria* bio-products could overgrowth on colonies of antagonistic bacteria but did not inhibit their growth. While, *Metarhizium* bio-products could inhibit the growth of both antagonistic bacteria. Dual culture of antagonistic bacteria with *Bacillus* bio-product showed that *Bacillus* sp. IKM1 could grow very well while *B. pumilus* FDKF5 could grow moderately.

Keyword: *Bacillus*, bio-products, fungicides, insecticides.

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INTRODUCTION

The crucial main food crop is rice (*Oryza sativa* L.). The crop is widespread all over the world due to its wider adaptability under different environmental conditions. The primary limitations rice production is plant disease and insect pests.[1,2] *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is bacterial leaf blight causal agent, its widely prevalent destroyed disease of rice.[3] Hence, endophytic bacteria isolates were test for their efficiency against *X. oryza* pv. *oryzae* (*Xoo*). *In vitro* test, higher inhibition of *Xoo* was *Bacillus subtilis* var. *amyloliquefaciens* (FZB 24), EPB 9, EPB10, EPCO 29 and EPCO 78.[4] While, testing activities of eleven chemicals including blasticidin, celdion, tricyclazole, streptomycin, sumithione, saturn, mipcine, stem F-34, hinosan, kasumin, phytomycin against *Xanthomonas campestris* pv. *oryzae*, a cause of paddy bacterial blight. *In vitro* studies, six chemicals such as phytomycin, streptomycin, blasticidin, kasumin, tricyclazole and sumithione inhibited bacterial growth. There are three chemicals *viz* blasticidin, kasumin and streptomycin which confident control disease *in vivo*.[5] Moreover, investigation of the anti - activity of different broad spectrums antibiotics against *X. oryza* pv. *oryzae* (*Xoo*) isolates *in vitro* assays. The results show that five virulent *Xoo* isolates was checked again six antibiotics including benzyl-penicillin, ampicillin, kanamycin, streptomycin, chloramphenicol and sinobionic.[6] The challenge of pest management is to use natural substances to be more effective in controlling pests. Most farmers use expensive insecticides, that making the problem of worse pests and it can cause damage to the environment and may be related to a crisis that cannot be solved for farmers that need to use insecticides.[7] Combinations between different crop protection and pests monitor and natural enemies was defined to Integrated Pest Management (IPM).[8] The objective of IPM is reduced chemical pesticides damage to health and ecosystem, its incorporate various strategy including biological, chemical, and cultural practice and resistance varieties, for pest population management to be at economic ratio.[9] In plant protection, *Bacillus thuringiensis, Trichoderma viridae, Metarhizium anisopliae*, *Beauveria bassiana*, nuclear polyhedrosis virus (NPV) and neem are favorite biopesticides.[10] Strategies for controlling plant disease may be achieved by using different biocontrol agents. Therefore, the study of the compatibility of chemicals and bio-products to be used in the IPM program such as studies compatibility of *Metarhizium anisopliae* with neem derivatives. The most compatible was 0.5% (w/v) neem soap with least growth inhibition of *M. anisopliae*.[11] *Streptomyces* sp. SAI-25, *M. anisopliae* and neem seed powder are the biopesticides that effective to control *Helicoverpa armigera*. In pod borer complex management, compatibility between *Streptomyces* sp. SAI-25, *M. anisopliae*, and neem seed powder were tested. The results indicated that all of biopesticides were compatible with each other, therefore can mixed to manage *H. armigera*. Evaluation of compatibility between commercial product of neem oil emulsion (*Azadirachta indica*) and neem seeds and leaves extract with *B. bassiana in vitro.* Seed and leaf extracts were less injurious to *B. bassiana* than oil emulsion. The oil is incompatible with *B. bassiana*. At high concentrations oil can inhibiting conidia germination, growth and decrease conidia production and viability. In all concentrations of neem seed and leaf extracts were compatible with the entomopathogenic fungi. Reduction of conidia germination and production was affected of seed extract but did not affect to spore viability.[12]

Moreover, institute to evaluation of compatibility between *B. bassiana*, fungicides and botanicals. The compatibility of *Beauveria* isolate BP1.1 was evaluated with different fungicides that are carbendazim (50 WP) and copper oxychloride (50 WP) and botanicals for example aqueous neem leaf extract (1% w/v), aqueous garlic extract (1% w/v) and neem seed kernel extract (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.[13] While, poisoned plate method was use for compatibility studies of eight fungicides including propineb, carbendazim, tebuconazole, nativo, fosetyl aluminium, tricyclazole, metalaxyl, kresoxim methyl and *Bacillus amyloliquefaciens*

B15. The results show that, all concentration tests of kresoxim methyl and carbendazim compatible with *B. amyloliquefaciens* B15.[14]

Thus, evaluation compatibility of *Bacillus* spp. and *Trichoderma* spp. and antagonistic activity to *S. cepivorum*. Dual cultures method show 42-50% and 100% reduced mycelial growth of *S. cepivorum* by eight isolates of *Bacillus* spp. and five isolates of *Trichoderma* spp. Collaboration of *Bacillus* spp. and *Trichoderma* spp. can reduce *S. cepivorum* mycelium growth. Therefore, it can be used together to inhibit *S. cepivorum*.[15]

Hence, the present study aimed to assess the interaction of chemical pesticides, bio-products and antagonistic bacteria. Compatibility tests can be used as a guideline to enhance future integrated pest management.

MATERIALS AND METHODS

Antagonistic bacteria

Two isolates of antagonistic bacteria including *Bacillus pumilus* FDKF5 and *Bacillus* sp. IKM1 obtained from Microbiology Laboratory, Department of Biology Faculty of Science, Mahasarakham University.

Compatibility test of antagonistic bacteria and pesticides

Study on compatibility between antagonistic bacteria and pesticides such as insecticides, fungicides and bactericide were tested by poison plate technique. Antagonistic bacteria including *B. pumilus* FDKF5 and *Bacillus* sp. IKM1 were culture on nutrient broth (NB) shaking at 150 rpm for 24 - 48 hr. After that bacterial suspension were prepared adjust concentration to $1x10^6$ cfu/ml. Bacterial suspension was swab on NA petri plates. Whatman no.1 paper disc with 0.5 cm diameter was sterilized and then drop 30 ul of each pesticides with the concentrations according to company recommend (Table 1). Paper disc with pesticides were place on NA petri plates with antagonistic bacteria 4 pieces per plate in the cross direction. Incubated at 28°C and observe the clear zone for 5 days. Three replicates were maintained along with the control plate with dH_2O instead of pesticides.

Table 1. Pesticides used to study compatibility with *B.pumilus* FDKF5 and *Bacillus* sp. IKM1

Chemicals	Trade name/ Company	Active ingredient	Concentration
Insecticides			
abamectin	Abamectin Asia Agrotech Co., Ltd., Thailand	1.8% W/V EC	20 ml/20l
carbosulfan	Posch Pitsulin Co., Ltd. Thailand	20% W/V EC	50 ml/20l
imidachloprid	Centaur	70% WG	10 ml/20 l
pyridaben	Conbalis Contact Group Co., Ltd., Thailand	20% WP	15 g/20 l
sulfur	Microthiol sulfur Sotus International Co., Ltd., Thailand	80% WG	60 g/20 l
Fungicides			
etridiazole + quintozene	Terraclor Super-X Sotus International Co., Ltd., Thailand	6% + 4% W/V EC	40 ml/20l
benomyl	Fundasol 50 Angro Thai Chemical Supplies Ltd., Thailand	50% WP	30 g/20 l
prochloraz	Octave FMC Chemical (Thailand) Ltd	50% WP	20 g/20 l
Bactericide			
streptomycin sulfate	Streptomycin sulfate Face Agro Co., Ltd., Thailand		10 g/20 l

Compatibility test of antagonistic bacteria and fungal bio-products

Trichoderma sp. was isolates from *Trichoderma* bio-product retrieve from Biological Control Laboratory, Department of Plant Pathology, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University. Compatibility between *Trichoderma* bio-product and *B. pumilus* FDKF5 or *Bacillus* sp. IKM1 using dual culture technique. First, place 0.5 cm diameter mycelium disc of *Trichoderma* sp. on PDA surface, 2 cm distance from the edge plate. After that *B. pumilus* FDKF5 or *Bacillus* sp. IKM1 was streak with an inoculation loop paired with 4.5 cm distance from *Trichoderma* sp. Observation the growth of both microorganisms for 5 days and then bacteria colony were re-streak on NA petri dish for test viability.

Beauveria bassiana and *Metarhizium anisopliae* were isolated from *Beauveria* bio-product and *Metarhizium* bio-product obtained from TAB Innovation Co., Ltd. Thailand. Dual culture technique was used by streak *B. bassiana* or *M. anisopliae* in a half of petri dish and incubated at 28°C for 3 days. After that *B. pumilus* FDKF5 or *Bacillus* sp. IKM1 were streak on the remaining half of petri dish. Incubation at 28°C for 5 days, observe microorganisms growth.

Compatibility test of antagonistic bacteria and bacterial bio-products

Bacillus sp. was isolates from *Bacillus* bio-product retrieve from Biological Control Laboratory, Department of Plant Pathology, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University. Dual culture test by streak *Bacillus* sp. bio-product and *B. pumilus* FDKF5 or *Bacillus* sp. IKM1 in parallel with 0.5 cm distance. Incubation at 37°C and observe growth and rating with - not growth, + slightly growth, ++ moderate growth and +++ considerably growth.

RESULTS

Compatibility test of antagonistic bacteria and pesticides

Compatibility test of antagonistic bacteria *B. pumilus* FDKF5 and *Bacillus* sp. IKM1 and pesticides the results show that two antagonistic bacteria can growth associated with all pesticides test including insecticides (abamectin 1.8% W/V EC, carbosulfan 20% W/V EC, imidachloprid 70% WG, pyridaben 20% WP and sulfur 80% WG) and fungicides (etridiazole 6% + quintozene 4% W/V EC, benomyl 50% WP and prochloraz 50% WP), except bactericide streptomycin sulfate shown zone of inhibition (clear zone) (Fig. 1, 2).

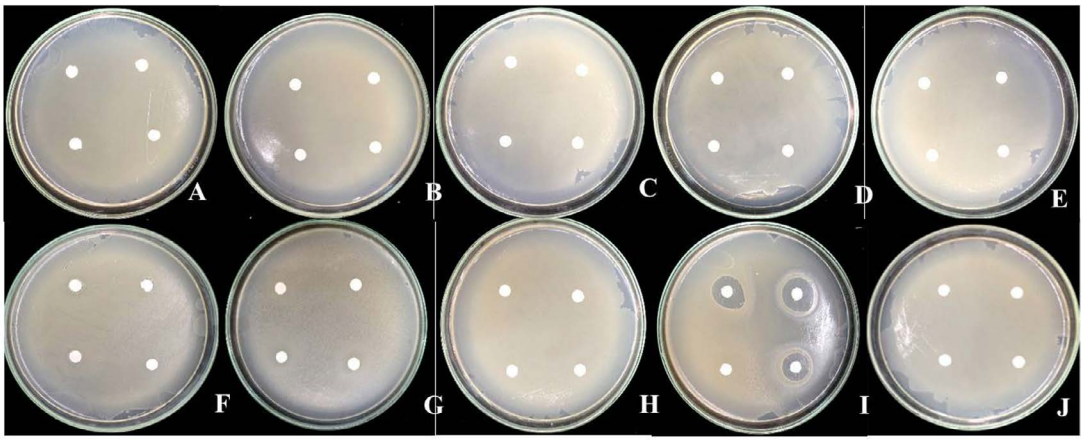

Fig. 1. Compatibility test of antagonistic bacteria *Bacillus pumilus* FDKF5 and pesticides
A: pyridaben 20% WP, B: imidachloprid 70% WG, C: carbosulfan 20% W/V EC,
D: sulfur 80% WG, E: abamectin 1.8% W/V EC,
F: etridiazole 6% + quintozene 4% W/V EC, G: benomyl 50% WP,
H: prochloraz 50% WP, I: streptomycin, J: dH_2O

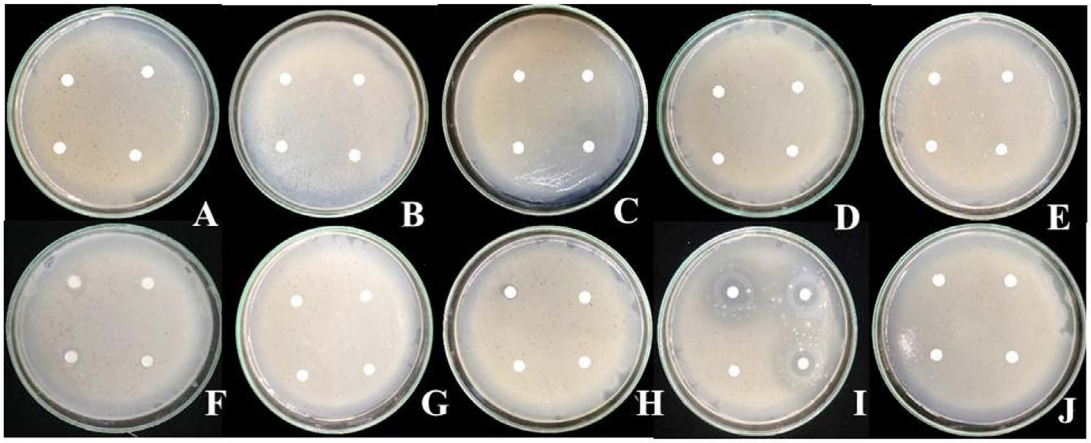

Fig. 2. Compatibility test of antagonistic bacteria *Bacillus* sp. IKM1 and pesticides
A: pyridaben 20% WP, B: imidachloprid 70% WG, C: carbosulfan 20% W/V EC,
D: sulfur 80% WG, E: abamectin 1.8% W/V EC,
F: etridiazole 6% + quintozene 4% W/V
EC, G: benomyl 50% WP, H: prochloraz 50% WP, I: streptomycin, J: dH_2O

Compatibility test of antagonistic bacteria and fungal bio-products

Compatibility test of antagonistic bacteria *B. pumilus* FDKF5 and *Bacillus* sp. IKM1 and *Trichoderma* bio-product the results show that *Trichoderma* sp. over growth on *B. pumilus* FDKF5 or *Bacillus* sp. IKM1 but not inhibit their growth**.** When re-streak *B. pumilus* FDKF5 or *Bacillus* sp. IKM1 on NA found normal growth and normal colony (Fig. 3).

Compatibility test of antagonistic bacteria *B. pumilus* FDKF5 and *Bacillus* sp. IKM1 and

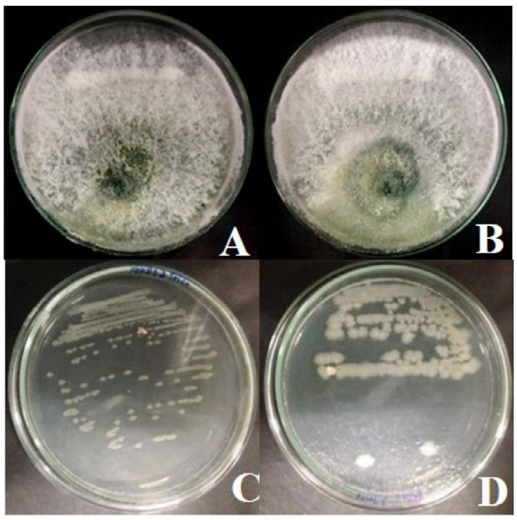

Fig. 3. Compatibility test of antagonistic bacteria *B. pumilus* FDKF5 or *Bacillus* sp. IKM1 and *Trichoderma* bio-product
A: *Trichoderma* bio-products and *Bacillus pumilus* FDKF5
B: *Trichoderma* bio-products and *Bacillus* sp. IKM1
C: Re -streak of *Bacillus pumilus* FDKF5
D: Re -streak of *Bacillus* sp. IKM1

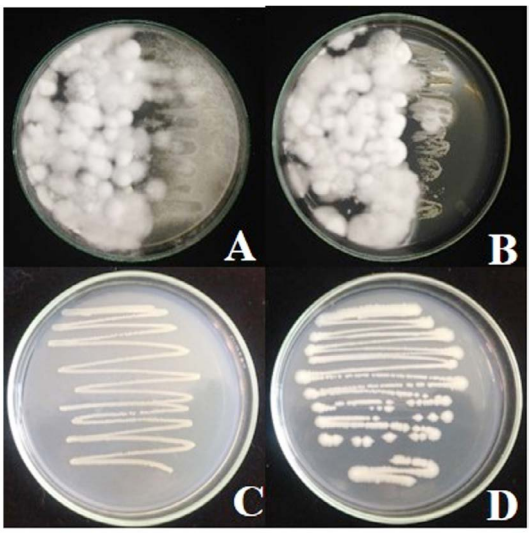

Fig. 4. Compatibility test of antagonistic bacteria *B. pumilus* FDKF5 or *Bacillus* sp. IKM1
and *Beauveria* bio-product
A: *Beauveria* bio-products and *Bacillus pumilus* FDKF5
B: *Beauveria* bio-products and *Bacillus* sp. IKM1
C: Re -streak of *Bacillus pumilus* FDKF5
D: Re -streak of *Bacillus* sp. IKM1

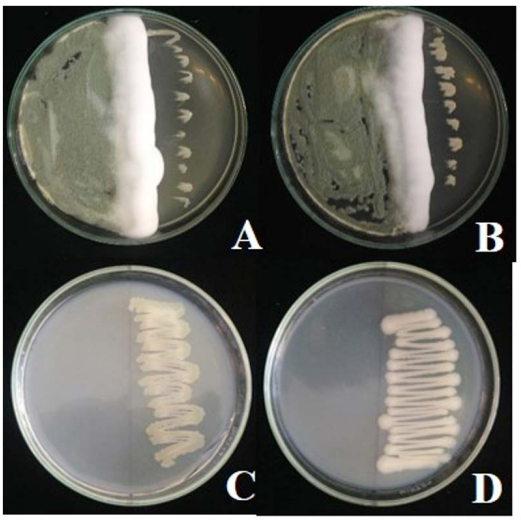

Fig. 5. Compatibility test of antagonistic bacteria *B. pumilus* FDKF5 or *Bacillus* sp. IKM1 and *Metarhizium* bio-product
A: *Metarhizium* bio-product and *Bacillus pumilus* FDKF5
B: *Metarhizium* bio-product and *Bacillus* sp. IKM1
C: *Bacillus pumilus* FDKF5
D: *Bacillus* sp. IKM1

Beauveria bio-product the results show that *Beauveria bassiana* over growth on *B. pumilus* FDKF5 or *Bacillus* sp. IKM1 but not inhibit their growth. When re-streak *B. pumilus* FDKF5 or *Bacillus* sp. IKM1 on NA found normal growth and normal colony (Fig. 4).

Compatibility test of antagonistic bacteria *B. pumilus* FDKF5 or *Bacillus* sp. IKM1 and *Metarhizium* bio-product found that *Metarhizium anisopliae* growth normally but *B. pumilus* FDKF5 or *Bacillus* sp. IKM1 show slightly growth (Fig. 5).

Compatibility test of antagonistic bacteria and bacterial bio-products

Compatibility test of antagonistic bacteria *B. pumilus* FDKF5 and *Bacillus* sp. IKM1 and *Bacillus* bio-product the results show that both isolates of antagonistic bacteria can growth associated with *Bacillus* bio-product. *Bacillus* sp. IKM1 show considerably growth (+++), while *B. pumilus* FDKF5 show moderate growth (++) (Fig. 6).

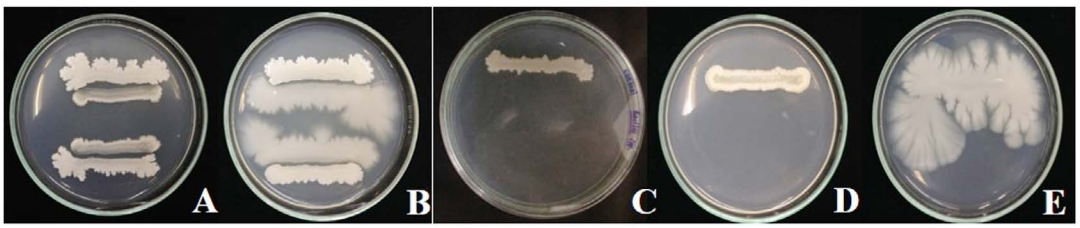

Fig. 6. Compatibility test of antagonistic bacteria *B. pumilus* FDKF5 or *Bacillus* IKM1 and *Bacillus* bio-product
A: *Bacillus* bio-product and *Bacillus pumilus* FDKF5
B: *Bacillus* bio-product and *Bacillus* sp. IKM1
C: *Bacillus* bio-product
D: *Bacillus pumilus* FDKF5
E: *Bacillus* sp. IKM1

DISCUSSION

In South and Southeast Asia, the major essential food crop is rice. It is attacked by insect, disease, weed and vertebrate pests. Integrated pest management (IPM) is combination of many strategies for pest management for economic values, consist of a biological control, chemical control, cultural practices and resistance plant varieties. In this experiment, compatibility between antagonistic bacteria that effective to control rice bacteria leaf blight and insecticides, fungicides, bactericides and bio-pesticides were evaluated.

Two antagonistic bacteria including *B. pumilus* FDKF5 and *Bacillus* sp. IKM1 can growth associated with all tested pesticides including insecticides (abamectin, carbosulfan, imidachloprid, pyridaben and sulfur) and fungicides (etridiazole + quintozene, benomyl and prochloraz), except bactericide streptomycin sulfate. Similarity with that studies compatibility of bacterial biocontrol agent *Bacillus subtilis* with commonly used chemical fungicides such as carbendazim, mancozeb, metalaxyl, wettable sulpher, hexaconazole, difenconazole, tebuconazole and kresoxim methyl. The

compatibility tests revealed that among the solid formulation fungicides, the *B. subtilis* showed more tolerance to carbendazim and among the liquid formulation fungicides hexaconazole and kresoxim methyl showed maximum compatibility up to 3000µl/ l concentration.[16]

Trichoderma bio-product and *Beauveria* bio-product show over growth on *B. pumilus* FDKF5 and *Bacillus* sp. IKM1 but not inhibit their growth**.** Moreover, compatibility among the most efficient isolates of *Bacillus* spp. and *Trichoderma* spp. was evaluated. Dual culture demonstration of *Bacillus* spp. and *Trichoderma* spp., found that eight and five isolates can reduce mycelium growth of *S. cepivorum*. Combination between *Bacillus* spp. and *Trichoderma* spp., there are 12 were incompatible but combination of *Bacillus* spp. (SF45, SF32 and SF311) and *Trichoderma* spp. can reduce mycelial growth of *S. cepivorum*. Thus, shows that both microorganisms can be used together to control white rot disease.[15] *In vitro*, studied compatibility of biocontrol agent including *Trichoderma harzianum*, *Bacillus subtilis* and *Pseudomonas fluorescens*. Absence of inhibition zone indicated that the biocontrol agents were compatible with each other.[17] Study of effect of pesticides comprise of imidaclopride, flufenoxuron, teflubenzuron+ phuzalon, endosulfane and amitraz on conidia germination, vegetative growth and sporulation of *B. bassiana* and test their compatibility too.The research found that *B. bassiana* is incompatible with flufenoxuron. Flufenoxuron was causal of complete inhibition in *B. bassiana* growing. *B. bassiana* DEBI008 was compatible with imidacloprid, thus could use at the same time in IPM program.[18]

Dual culture of antagonistic bacteria and *Metarhizium* bio-product, the results show that *Metarhizium* bio-product growth normally but *B. pumilus* FDKF5 and *Bacillus* sp. IKM1 show slightly growth. While, *Streptomyces* sp. SAI-25, *M. anisopliae*, and neem seed powder were evaluated their compatible for using to controlling pod borer complex. The result show that, there were compatibility and can be combined for *Helicoverpa armigera* management.[10]

Compatibility test of antagonistic bacteria *B. pumilus* FDKF5 or *Bacillus* sp. IKM1 and *Bacillus* bio-product the results show that both isolates of antagonistic bacteria can growth associated with *Bacillus* bio-product. Bio-control agents could be used as a way not harmful to the environment and effective to control disease and may be advised to the farmers for profitable organic farming. Compatibility study of antagonistic bacteria with agricultural chemicals and bio-products to increase the efficacy of biocontrol agents to controlling rice bacterial leaf blight diseases. Moreover, the pesticides tolerance ability broadened the use as these bio-pesticides in combination with pesticides can be applied under integrated disease management for the management of rice pest.

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CONFLICT OF INTEREST

The authors declares that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Author listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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