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Efficacy Evaluation of Biopesticides Derived From Entomopathogenic Fungi against Rind Borer and Twig Blight Disease of Citrus

Author's Details:

J.I. Yago[1] K.R. Chung[2] R.A. Bating[3]

[1]Professor VI, Plant Science Department, College of Agriculture, Nueva Vizcaya State University, Bayombong, Nueva Vizcaya (corresponding author: J.I. YAGO; email: jyago2002@yahoo.com).

[2] Research Assistant Professor 7 and Director, National Institute of Molecular Biology and Biotechnology, BIOTECH, UPLB, College, Laguna

[3]University Research Associate 1, Nueva Vizcaya State University, Bayombong, Nueva Vizcaya.

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Abstract

The study focused on citrus rind borer (CRB) and twig blight disease (TBD) which is the dominant cause of low quality and high postharvest losses of citrus production in the Philippines. Egg laying of CRB started during flushing and flowering stage thereby larval stage are ready to infest on flowering stage and fruitlet formation. Almost 80-92% of fruits showed severe lumps due to CRB as a visible symptom which causes poor quality of rind. On the other hand, TBD of citrus imposed serious problems during fruiting season, usually this fungus mainly infect citrus plants during flushing stage.

The two isolates of EPF (Beauveria bassiana and Isaria fumosorosea) were formulated as solid and liquid based biopesticides and tried to test the efficacy under field condition with different level of concentration against disease prevalence of TBD and level of infestation of CRB.

Field trial condition showed that the two entomopathogenic fungi at 100% concentration of two formulated biopesticides induced significant difference than other concentrations (75%, 50%, 25%) in terms of disease incidence and severity of fungal pathogen that caused twig blight disease. In addition, in terms of population dynamics of citrus rind borer on percent damage, number of infested buds, percent damaged flower buds, number of infested fruits, number of fruit fall, and yield performance per kg per tree also showed significant difference at $1x10^{12}$ concentration level compared to treatments $1x10^{6}$, $1x10^{8}$, and $1x10^{10}$.

Verification trials of the two formulated organic-based biopesticides under field condition showed that either B. bassiana or I. fumosorosea has a novel potential as biopesticide at $1x10^{12}$ level of concentrations in Satsuma, Ponkan, Calamansi and Pomelo trees under field condition.

Keywords: *pathogenicity, phytotoxicity, in-vivo trial*

INTRODUCTION

Mainly the use of entomopathogenic fungi (EPF) as bioinsecticides has increased the global attention because of its potent efficacy (Latifian, Rad, Amani, Esmaeil and Rahkhodaei, 2013). Mycoinsecticide components usually derived from *Beauveria bassiana* (Balsamo) Vaillemin (Babu et al., 2001; Sharma, 2004), *Paecilomyces fumosoroseus* or *Isaria fumosoroseus* (Wize) Brown and Smith (Alter and Vandenberg, 2000; Avery et al., 2004) and *Verticillium lecanii* (Zimm.) Viegas (Butt et al., 2001) are the most popular source of compounds against insect pest species. *B. bassiana* according to Tanada and Kaya (1993) considered as fungal contact pathogen and it is microbiologically insecticide which disrupt the insect cuticle through its enzymes followed by infecting its hemocoel and eventually kill the insect. No research was conducted against citrus rind borer using entomopathogenic fungi, hence this study was conceptualized.

Preliminary studies (*in-vitro* and *in-vivo*) was conducted using two types of formulation derived from EPF; a.) mycelial-free extracts (3 litres of crude extracts per 16 litres (1 knapsack sprayer) and b.) combined formulation of ascospores (1x 10^6) and crude extracts containing cell wall degrading enzymes. Spraying of EPF against CRB and TBD resulted to 75 to 82% and 80-92% respectively. Using EPF dramatically reduced number of infested buds, percent damaged flower buds, number of infested fruits, and number of fruit fall. Likewise, disease severity due to TBD during flushing stage, flowering stage, fruitlet formation was reduced on the average by almost 86% (Yago et. al. 20016, unpublished). However, there is a need to verify this data for different fruiting season of different citrus cultivars. It was hypothesized that using EPF would also be effective against citrus rind borer.

The above results is proven as effective mycoinsecticide however, few researches are conducted and mycofungicide. It is commonly used as mycoinsecticide however the production of active compounds makes EPF as a potential candidate against fungal pathogens. Demain (1999) cited that *B. bassiana* produced wide range of secondary metabolites such as antibiotics, cytotoxic substances, insecticides, compounds that promote or inhibit growth, attractor as well as repellent. To be consider as potential myco-fungicides.

The presence of low molecular weight compounds such as Oosporein, Beauvericin, Enniatins, Isarolides and Bassianolide have been found as effective compounds against fungal pathogens (Weiser, 1993; Montlor and Hwang, 1993; Castlebury, 1999). Kodaira (1961) shown that antimicrobial against fungi has been observed.

The proposed study utilize *Beauveria bassiana* and *Isaria fumosoroseus* as a source of potential biopesticides against citrus rind borer and twig blight disease of citrus.

Mass Production Method for EPF. Blastospores production in submerged liquid fermentation are good in mass production of spores (Romback, 1989) and production of aerial conidia in solid state condition (Rousson et al., 1983). According to Kumar and Mukerji (1996) uses several nutrition as substrate for sporulation of filamentous fungi such as *B. bassiana*, *M. anisopliae* and *I. fumosorosea*. Cooked rice is good for solid state production of blastospores and its spores are used for field spray applications (Posada 1993; Bustillo and Posada 1996) and harvesting spores with with a 1 % oil-water suspension (Antía et al. 1992). However, shelf life of blastospores are more viable using fungal inoculum in liquid culture (Burges and Hussey, 1981).

MATERIALS AND METHODS

Site Selection. The study will be conducted in three geographic location in Cagayan Valley covering mandarin (Satsuma and Ponkan), calamansi and pummelo. For Satsuma and Ponkan, this will be conducted in three barangays of Kasibu, Nueva Vizcaya: a). Barangay Tadji, b). Barangay Binogawan and c). Barangay Malabing. For Calamansi, the trial will be conducted at barangay Dimantina, Aurora, Isabela and at Abulug, Cagayan (Abulug Central Experiment Station) for pummelo. Separate set up will be prepared for CRB and TBD respectively.

Experimental Mandarin cultivars and Testing Season. Two mandarin cultivars will be used in the experiment. A 5 year old trees of Ponkan and Satsuma chosen from the farmer co-operator. Two sites will be selected as testing sites of using the two formulations derived from two isolates of entomopathogenic fungi (EPF). Testing sites will be located at Barangay Tadjie, Barangay Binogawan and barangay Malabing Valley, Kasibu, Nueva Vizcaya. Efficacy trials will be conducted in four cropping seasons covering two years duration of the study.

Pure Culture Preparation. The two isolates of entomopathogenic fungi (EPF) will be prepared and properly coded in pure culture. Two methods of mass culture will be followed, liquid culture and solid culture. Liquid culture media will be extracted following protocol made by Bidochka, Pfeifer and Khachatourians (1987) to come up with mycelial free extracts. Mycelial-free extracts will be collected using filter paper and vacuum pump machine and kept under refrigerator. After seven days of incubation under room temperature, mycelia and spores will be collected from solid media by flooding with distilled water. Harvested mycelia and spores will be standardized based on the required number of spores per ml applied to a treatment.

Crude Extract and Spores Standardization. Crude extracts and spores per ml will be standardized according to the assigned treatment. Crude extracts will be prepared from 25%, 50%, 75% and 100% while spores per ml will be prepared using haemocytometer from $1x10^6$, $1x10^8$, $1x10^{10}$ and $1x10^{12}$.

Methods of Application, Treatment Assignment, Statistical Design and Analysis. Two methods of application will be applied in the trials, using two formulated products derived from the two EPF, mycelial-free extracts and spores. Crude extract will be applied first prior to application of spores. The assigned treatments on the number of spores per ml (Table 1) and the dosage of crude extracts prepared (Table 2). Factorial experiment using Randomized Completely Block Design (RCBD) will be used in the experiment. Three blocks will be assigned in each treatment. An RCBD factorial, 2 x 2 x 6 x 3 with a total of 72 treatment combination. Factor A will be the isolates, factor B- cultivars used in the study and Factor C- Treatment

Phytotoxicity Test Using Two Formulation of Biopesticides. Mode of application will be complied with good agricultural practices. Details on the spray equipment, type of nozzle and water volume used at different stages of crop growth will be used. Addition of sticker, to improve the uptake of active ingredient, if any will be specified. Sticker will be tried in the check plot with water spray. The type of application will be using foliar spray and time of application will be specified by the required treatment. The time and frequency will be normally depend on critical growth stage of the crop & local environment conditions. The number of applications and the date of each should be recorded. The product will be tested at the proposed dose(s) specified in the treatment. The dosage will be expressed in g a.i/ha and formulation ml/ha.

Observation and assessment will be conducted by gathering data on growth parameters such as plant height, number of primary branches per plant at 15 or 30 days interval after application. Yield and yield attributes will also be recorded such as number of fruits, fruit length, fruit weight and yield. The standard phytoxicity rating scale (PRS) will be followed. Rating shall be recorded individually for yellowing, stunting, necrosis, epinasty hyponasty etc. (Henderson and Tilton 2014).

Phytoxicity Rating Scale (PRS)

Crop response/ Crop injury	Rating
0-00	0
1-10%	1
11-20%	2
21-30%	3
31-40%	4
41-50%	5
51-60%	6
61-70%	7
71-80%	8
81-90%	9
91-100%	10

Table 1. Spore concentrations applied in each treatment

Factor A EPF1	Factor B Cultivar	Factor B Treatment	Treatment Description
	Mandarin	T1	Negative control (no application)

Factor A	Factor B	Factor B	Treatment Description
		T2	Positive control (farmers practice, active ingredient (a.i. copper oxychloride)
		T3	$1x10^6$
		T4	$1x10^8$
		T5	$1x10^{10}$
		T6	$1x10^{12}$
	Satsuma	T1	Negative control (no application)
		T2	Positive control (farmers practice, active ingredient (a.i. copper oxychloride)
		T3	$1x10^6$
		T4	$1x10^8$
		T5	$1x10^{10}$
		T6	$1x10^{12}$

Table 2. Crude extracts dosage applied in each treatment

Factor A - EPF1	Factor B - Cultivar	Factor B - Treatment	Treatment Description
	Mandarin	T1	Negative control (no application)
		T2	Positive control (farmers practice, active ingredient (a.i. copper oxychloride)
		T3	25%
		T4	50%
		T5	75%
		T6	100%
	Satsuma	T1	Negative control (no application)
		T2	Positive control (farmers practice, active ingredient (a.i. copper oxychloride)
		T3	25%
		T4	50%
		T5	75%
		T6	100%
Factor A - EPF2	**Factor B - Cultivar**	**Factor B - Treatment**	**Treatment Description**
	Mandarin	T1	Negative control (no application)
		T2	Positive control (farmers practice, active ingredient (a.i. copper oxychloride)
		T3	25%
		T4	50%
		T5	75%
		T6	100%
	Satsuma	T1	Negative control (no application)
		T2	Positive control (farmers practice, active ingredient (a.i. copper oxychloride)
		T3	25%
		T4	50%
		T5	75%
		T6	100%

Statistical Analysis were conducted by gathering data and will be subjected to appropriate transformations and analyzed using standard experimental designs. Enhancement of the crop has to be brought out using appropriate statistical and other tools so that there is no confusion statement in drawing the inference.

Effect on Natural Enemies. The natural enemy spectrum in the test area will be gathered recorded and identified. The investigator (evaluator) will be looking for possible occurrence of any new pests (insects / mites / nematodes / diseases) in each of the evaluation plots. The help of relevant specialists for each of these will be taken and need to be recorded throughout the experiment period.

Randomization and Lay-out. Treatment combinations of the different factors will be laid-out using Assistat.

Frequency of Application. Application of formulated biopesticides should be applied during flushing stage for TBD timing of application in controlling CRB will be on pre-blooming stage. Mycelial free extracts will be applied every two weeks while concentration of spores will be applied once a month. Application will be started on the onset of flushing stage, flower bud initiation, flowering stage, fruitlet formation and two weeks before harvesting.

Number and Distribution of CRB Larvae in Citrus Fruits. The study will be conducted at Kasibu, Nueva Vizcaya (satsuma and ponkan) and Aurora, Isabela (calamansi) from March 2018 to March 2020. The trees intended for field observation will be randomly tagged and selected. A total of 15 inflorescences will be tagged five to six days to flower opening. The flower buds per inflorescences will be counted, labelled and observed using hand lens twice daily early in the morning & late in the afternoon for CRB oviposition. Damage of CRB larva starting from flower buds to mature fruits ready to harvest will also be monitored, observed & described. Damaged fruits will be gathered and examined in the laboratory to measure and characterize.

The pattern of tunnels formed by the borer, sample flower buds and fruit will be measured and cut into sections to determine the extent of damage inside the bud or rind. Damaged buds and fruits will be characterized by exit holes in the petals or/and lumps and exit holes in the fruit rind. This will be counted and percent damage will be completed using the formula:

$$\%\ \text{damage} = \frac{\text{total number of damaged buds/fruits}}{\text{Total number of sample buds/fruits}} \times 100\%$$

To get the number and distribution or larvae in a fruit, five trees will be used. Newly formed flowers will be tagged, allowed to develop for use later as sample fruits. Five damaged fruits per tree will be collected using sample random sampling and done weekly starting at 1 week from fruit set until harvest, covering 25 weeks observation period. A total of 20 fruits will be collected and examined for presence of CRB larva every sampling period. For infected young fruits pin prick entry holes will be examined using hand lens while for developed and bigger fruits slight tumor-like swelling of the afflicted portions of the rind surface will be indicator for infected fruits to be collected. Destructive sampling will be done to confirm the presence of larvae inside by dissecting abnormally protruding portion of the rind surface of the fruits. The larvae and head capsule present will be counted and recorded per gall per fruit. The tunnels formed by the larvae will also be examined and counted, especially in cases where the mature larvae will be exited from the rind. Each tunnel will be represented by the larva. The phenology of the trees and size of the sample fruits will be recorded every sampling period. The corresponding weather data during day prior to each sampling period will be obtained from FMS of NVSU situated in the site. The physical variables will be considered where the percent relative humidity, ambient temperature (C), and amount of rainfall (mm).

The distribution and number of larvae in a fruit follows Poisson distribution where in the variance is equal to the mean; that is S^2=X (Gomez et.al 1984). To determine whether the data conform to Poisson distribution. Anderson Darling will be used to test the goodness if fit and the descriptive data will be analyzed using Easy Fit Software Professional. The probability of each fruit present in a particular sampling period to have corresponding number of larvae will be estimated.

The relationship between the number of larvae per fruit and time will be analyzed as well as the correlation of temperature, rainfall and relative humidity in the population density of CRB by simple correlation analysis using pearson's correlation coefficient in Assistat Software.

Care of the Citrus Plants. Proper care of the citrus plants will be employed in the experiment. A recommended rate of application of herbicides, fertilizers, pesticides and water management will be followed. Cultural management such as pruning and other cultural practice will also be followed. Proper care and management that will be employed in the experiment will be followed using the techno guide in citrus planting.

Conduct of Farmer's Field Day. Group of citrus farmers will be invited to see the effect of the treatments as affected by application of using two formulation of EPF. This endeavor will be coordinated through the MLGU of each town where the study will be conducted. Three farmer's field day will be planned to showcase the effect of the treatments. Three (1 each for calamansi, pummelo and Satsuma and Ponkan respectively) farmer's field day will be conducted during the duration of the study.

RESULTS AND DISCUSSION

Two Formulated Organic-Based Biopesticides under Field Condition of Satsuma and Ponkan Trees in Kasibu Nueva Vizcaya:

Disease Incidence and Severity of Satsuma and Ponkan Trees caused by Twig Blight Disease (TBD)

Table 1 shows the disease incidence and severity of satsuma and ponkan trees as affected with crude extract of two different entomopathogenic fungi (EPF) *Beauveria bassiana* and *Isaria fumosoroseae* against twig blight disease (TBD). The disease incidence of the two mandarin (satsuma and ponkan) varied with according to the assigned treatment and the crude extract concentrations used. Under field condition, the highest disease incidence was observed in satsuma of *B. bassiana* and *I. fumosoroseae* treatment which had means DI of 81.33 and 80.00, respectively. Lower twig blight disease incidence observed in ponkan of *B. bassiana* and *I. fumosoroseae* treatment with means DI of 53.33 and 51.00, respectively. Based on the statistical analysis, for the satsuma and ponkan trees grown in open fields (Kasibu, Nueva Vizcaya) had a significant effect on the highest concentration (100%) of both *B. bassiana* and *I. fumosoroseae* which had means of 12.00, 9.33, 10.33, and 9.33, respectively. However, no significant differences were observed in other concentrations of 75%, 50%, and 25%.

In disease severity, the two mandarin (satsuma and ponkan) also varied according to the assigned treatment and concentrations of the crude extract that was applied. The highest mean DS observed in satsuma treated with *B. bassiana* and *I. fumosoroseae* where both had mean 70.33. Lower mean DS observed in ponkan where *B. bassiana* and *I. fumosoroseae* had 50.33 and 48.33, respectively. Statistically, the highest concentration (100%) showed significant effect both in *B. bassiana* and *I. fumosoroseae* on satsuma and ponkan which had 5.67, 8.67, 6.33, and 9.33, respectively. No significant differences observed on 75%, 50%, and 25% concentrations.

Studies from Kang B.R. *et al* stated that certain entomopathogenic fungi are also known to directly inhibit plant microbial pathogens, induce systemic resistance, and enhance plant growth. Furthermore, certain strains of *B. bassiana* produce a broad-spectrum antimicrobial compound, beauvericin, which also produced by other entomopathogens including certain strains of *Isaria*. Hence, it can be noted that both *B. bassiana* and *I. fumosoroseae* can be used as a novel biocontrol agents for twig blight disease under field condition.

Table 1. Disease incidence and severity of satsuma and ponkan with crude extract of the two different entomopathogenic fungi (EPF) against twig blight disease (TBD)

Treatment	Disease incidence* (%)				Disease severity* (%)			
	B. bassiana		*I. fumosoroseae*		*B. bassiana*		*I. fumosoroseae*	
	Satsuma	Ponkan	Satsuma	Ponkan	Satsuma	Ponkan	Satsuma	Ponkan
Negative	81.33^{a}	53.33^{a}	80.00^{a}	51.00^{a}	70.33^{a}	50.33^{a}	70.33^{a}	48.33^{a}
Positive	11.00^{e}	11.00^{e}	10.00^{e}	11.33^{e}	7.00^{e}	13.00^{d}	7.33^{e}	14.67^{d}
25%	74.33^{b}	41.00^{b}	73.33^{b}	43.33^{b}	41.00^{b}	34.67^{b}	42.67^{b}	33.67^{b}
50%	35.00^{c}	28.00^{c}	33.67^{c}	25.67^{c}	30.00^{c}	23.33^{c}	29.67^{c}	22.33c
75%	25.67^{d}	18.33^{d}	23.67^{d}	18.67^{d}	21.33^{d}	13.00^{d}	22.67^{d}	12.33^{d}
100%	12.00^{e}	9.33^{e}	10.33^{e}	9.33^{e}	5.67^{e}	8.67^{e}	6.33^{e}	9.33^{e}
C.V. (%)	**6.85**	**3.66**	**4.16**	**5.27**	**5.39**	**8.52**	**6.06**	**6.21**

*Means followed by the same letter do not differ statistically using DMRT at 0.05 level

Table 2 shows the percent damage of citrus rind borer (CRB) using two formulated bio-pesticide *Beauveria bassiana* and *Isaria fumosorosea*. Based on the data gathered, it was observed that on the negative treatment, there was a high percent damage of CRB, while low percent damage was observed on the positive treatment (Betacyflutrin). This signifies that CRB can cause a lot of damage to citrus plant by feeding on the buds and fruits without the application of any fungicides. Both liquid and solid substrates can be used for mass production and application of the two EPF in the field since there was no significant difference between them. The suppression of the two EPF against CRB is noticeable since they have this high level of persistence in the host population and in the environment (Hamlen, 1979).

Table 2. Percent damage of CRB using two formulated biopesticide at different concentrations of the two different entomopathogenic fungi (EPF)

Treatment	*B. bassiana*				*I. fumosorosea*			
	Satsuma		Ponkan		Satsuma		Ponkan	
	Liquid	Solid	Liquid	Solid	Liquid	Solid	Liquid	Solid
Negative	19.00^{a}	19.67^{a}	21.00^{a}	21.00^{a}	20.33^{a}	19.00^{a}	20.33^{a}	20.33^{a}
Betacyflutrin	11.33^{d}	10.67^{d}	13.00^{c}	10.67^{de}	11.67^{c}	11.67^{c}	11.67^{c}	11.67^{c}
1x10^{6}	14.33^{bc}	12.67^{c}	14.33^{b}	12.67^{c}	15.00^{b}	15.00^{b}	15.00^{b}	15.00^{b}
1x10^{8}	14.67^{b}	14.67^{b}	14.67^{b}	14.67^{b}	15.00^{b}	15.00^{b}	15.00^{b}	15.00^{b}

For the other treatments, lower percent damage was observed using the two different EPF at different concentrations. At 1 x 10^{12}, the two EPF tested caused the lowest percent damage of CRB which is significantly different from the negative treatment. Hence, making it very effective at 1 x 10^{12} spore concentration level against CRB. And also, the higher the spore concentration caused lower percent damage of CRB due to increased number of spores which suggests that CRB is a good host to EPF.

Table 3 shows the number of infested buds of Satsuma and Ponkan with the solid and liquid substrates using *B. bassiana* and *I. fumosorosea* at different levels of spore concentrations. Based on the data gathered, it was observed that the negative treatment was significantly different with the rest of the treatments used. The reduction of the number of infested buds of Satsuma and Ponkan can be due to decreased infestation of CRB because of the release of certain metabolites of the two EPF. The EPF uses a set of enzymes to breach the cuticle (Butt, 2002) and also release secondary metabolites, to suppress the immune system of CRB during colonization. For the comparison of the efficacy between the two EPF, it revealed that *B. bassiana* is more effective with increasing spore concentration level compared to *I. fumosorosea.* Lower number of infested buds were observed at 1 x 10^{12} spore concentration level of *B. bassiana* in both Satsuma and Ponkan. And, the higher the spore concentration level of the two EPF caused lower number of infested buds of Satsuma and Ponkan. Thus, *B. bassiana* and *I. fumosorosea* can be very effective in controlling CRB infestation at high levels of spore concentrations in the field.

Table 3. Number of infested buds of satsuma and ponkan caused by citrus rind borer (CRB) with the solid and liquid substrates using the two different entomopathogenic fungi (EPF)

Treatment	***Beauveria bassiana***				***Isaria fumosoroseae***			
	Satsuma		Ponkan		Satsuma		Ponkan	
	Liquid	Solid	Liquid	Solid	Liquid	Solid	Liquid	Solid
Negative	271.00^{a}	284.33^{a}	272.33^{a}	270.67^{a}	284.00^{a}	271.33^{a}	291.33^{a}	270.67^{a}
Betacyflutrin	201.33^{b}	140.33^{c}	202.00^{b}	144.00^{cd}	140.67^{d}	203.00^{b}	161.33^{d}	144.00^{cd}
1x10^{6}	183.33^{c}	207.33^{b}	185.00^{c}	207.00^{b}	204.33^{b}	185.67^{c}	200.33^{b}	207.00^{b}
1x10^{8}	156.33^{d}	144.33^{c}	155.00^{d}	187.33^{bc}	149.67^{c}	155.33^{d}	169.33^{c}	187.33^{bc}
1x10^{10}	140.33^{e}	134.00^{c}	141.00^{e}	154.00^{bc}	134.00^{d}	142.33^{e}	140.00^{e}	154.00^{bc}
1x10^{12}	85.67^{f}	93.00^{d}	94.33^{f}	99.67^{d}	95.00^{e}	87.00^{f}	101.00^{f}	99.67^{d}
C.V. (%)	**1.54**	**3.81**	**2.08**	**16.06**	**2.41**	**2.24**	**2.11**	**16.06**

*Means followed by the same letter do not differ statistically using DMRT at 0.05 level

Table 4 shows the percent damage of flower buds of Satsuma and Ponkan caused by CRB using solid and liquid formulation of *B. bassiana* and *I fumosorosea* at different levels of spore concentrations. It was seen that there is a significant effect on the different level of spore concentrations on the damage exhibited by CRB. As the spore concentration increases, there is a decrease in the percent damage of flower buds caused by CRB.

At 1 x 10^{10}, percentage damage is not significantly different from that of Betacyflutrin. This means that in this level of spore concentration, the two EPF tested are as effective as the said synthetic insecticide in reducing the damage of flower buds caused by RCB. Percentage damage of flower buds is noticeably low at 1 x 10^{12} level of spore concentration. This result collaborates with the work of Alves et al. (2005) on the efficacy of entomopathogenic fungi as biological control agents against *Phyllocoptruta oleivvora*. The result showed that there was a significant effect of the different concentrations of EPF on the mortality of *P. oleivora*. The greatest mortality was observed in the *B. bassiana* while all concentrations of *I. fumosorosea* caused moderate mortality in *P. oleivora*. While based on the table, both of the two EPF were seen to be effective since lower percent damage of flower buds was achieved at 1 x 10^{12} which means that there was a high mortality rate of RCB. Therefore, commercial development of these two EPF for biological control should be the primary target against foliar feeding pest like RCB.

Table 5 shows the number of infected fruits of the two mandarin (satsuma and ponkan) using the two formulated organic-based biopesticide at different concentration level ($1x10^{6}$, $1x10^{8}$, $1x10^{10}$, $1x10^{12}$) which revealed significant differences among treatment means on both *Beauveria bassiana* and *Isaria fumosoroseae*. Based on the statistical analyses using DMRT that satsuma and ponkan treated at different concentration level observed that the number of fruits infected is significantly lessen as the concentration level increases. Furthermore, there is no significant difference of the two formulated organic-based biopesticides at $1x10^{12}$ concentration treatment that showed relative trend.

Table 4. Percent damage of flower buds caused by citrus rind borer (CRB) using the solid and liquid based formulation of the two different entomopathogenic fungi (EPF)

Treatment	***Beauveria bassiana***				***Isaria fumosorosea***			
	Satsuma		Ponkan		Satsuma		Ponkan	
	Liquid	Solid	Liquid	Solid	Liquid	Solid	Liquid	Solid
Negative	22.67^{a}	23.00^{a}	22.00^{a}	19.00^{a}	284.00^{a}	22.33^{a}	21.00^{a}	22.67^{a}
Betacyflutrin	9.67^{c}	8.00^{d}	11.33^{cd}	10.33^{cd}	140.67^{d}	11.00^{d}	9.67^{dd}	10.67^{d}
$1x10^{6}$	16.33^{b}	15.33^{b}	14.67^{b}	14.67^{b}	204.33^{b}	17.33^{b}	14.33^{b}	17.67^{b}
$1x10^{8}$	15.00^{b}	15.33^{b}	13.33^{bc}	14.00^{bc}	149.67^{c}	14.00^{c}	14.33^{b}	14.33^{c}
$1x10^{10}$	10.00^{c}	9.67^{c}	9.67^{de}	10.00^{d}	134.00^{d}	10.00^{d}	10.67^{c}	10.67^{d}
$1x10^{12}$	7.33^{d}	7.67^{d}	7.67^{e}	8.33^{d}	95.00^{e}	8.33^{e}	7.33^{d}	8.67^{e}
C.V. (%)	**7.89**	**6.04**	**11.45**	**16.09**	**2.41**	**6.47**	**10.38**	**5.44**

*Means followed by the same letter do not differ statistically using DMRT at 0.05 level

Table 5. Number of CRB infected fruits of satsuma and ponkan affected with two formulated organic-based biopesticide of the two different EPF

Treatment	***Beauveria bassiana***				***Isaria fumosoroseae***			
	Satsuma		Ponkan		Satsuma		Ponkan	
	Liquid	Solid	Liquid	Solid	Liquid	Solid	Liquid	Solid

Table 6 shows the number of fruit fall of the two mandarin (satsuma and ponkan) treated with the two formulated organic-based biopesticide at different concentration level ($1x10^6$, $1x10^8$, $1x10^{10}$, $1x10^{12}$) observed significant differences among treatment means on both *B. bassiana* and *I. fumosoroseae*. Statistically, the satsuma and ponkan treated with different concentration showed that the number of fruit fall is significantly reduced as the concentration level of biopesticide increases in both formulation. The highest concentration ($1x10^{12}$) of satsuma and ponkan in two formulated organic-based biopesticide had no significant difference.

Table 6. Number of fruit fall of satsuma and ponkan as affected with the two formulated organic-based biopesticide of the two different entomopathogenic fungi (EPF) against citrus rind borer (CRB)

Treatment	***Beauveria bassiana***				***Isaria fumosoroseae***			
	Satsuma		Ponkan		Satsuma		Ponkan	
	Liquid	Solid	Liquid	Solid	Liquid	Solid	Liquid	Solid
Negative	206.33^b	204.00^b	202.33^b	202.33^b	203.67^b	205.67^b	208.67^b	205.00^b
Positive	110.67^f	113.00^e	112.33^f	112.67^e	111.00^f	111.67^e	112.00^f	111.67^f
$1x10^6$	215.67^a	218.33^a	218.00^a	217.00^a	$215.\ 00^a$	217.00^a	217.67^a	217.33^a
$1x10^8$	175.00^c	174.67^c	177.33^c	178.00^c	176.00^c	175.33^c	176.33^c	176.67^c
$1x10^{10}$	160.33^d	160.00^d	160.00^d	162.00^d	162.00^d	163.33^d	159.33^d	161.00^d
$1x10^{12}$	116.00^e	116.67^e	118.33^e	116.67^e	116.67^e	116.00^e	118.67^e	117.67^e
C.V. (%)	**1.5**	**1.39**	**1.68**	**1.73**	**1.31**	**1.99**	**1.92**	**1.61**

*Means followed by the same letter do not differ statistically using DMRT at 0.05 level

Figure 1 shows the satsuma yield per kg per tree treated with two formulated biopesticide at different concentration level ($1x10^6$, $1x10^8$, $1x10^{10}$, and $1x10^{12}$). The statistical analyses revealed that under field condition, the highest yield was observed in both $1x10^{12}$ concentration and positive control which relatively no significant difference. However, lower yield observed on untreated which no significant difference of the two formulated biopesticide.

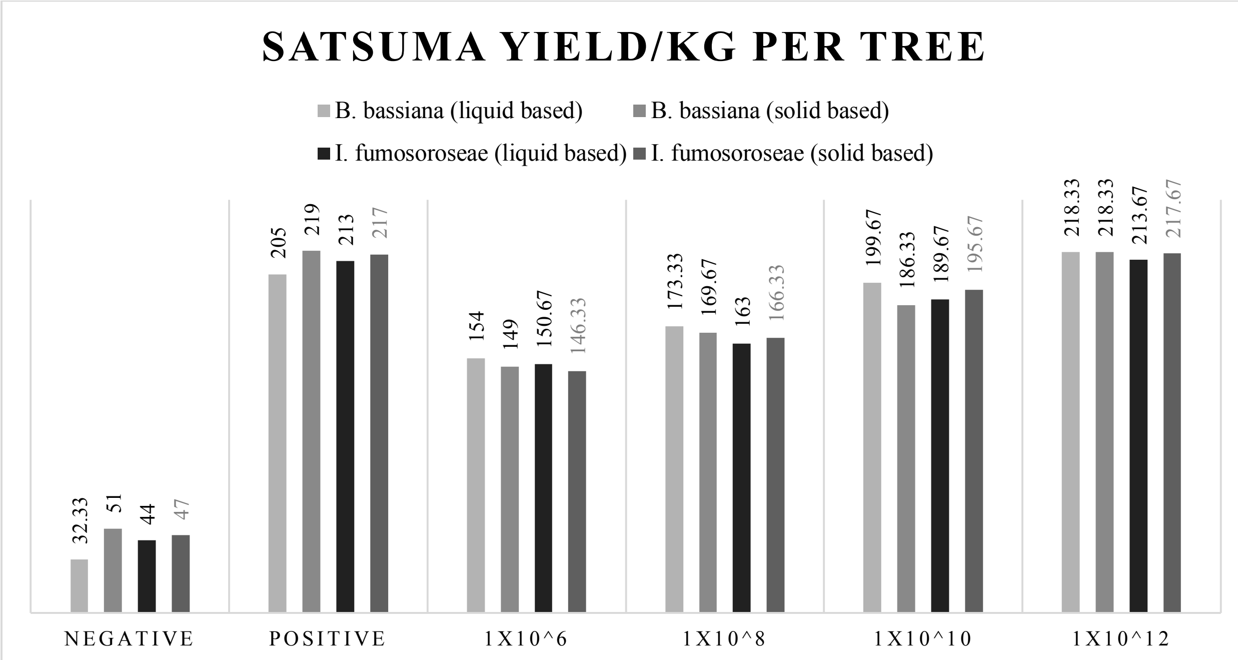

Figure 1. Satsuma yield/kg per tree as affected with two formulated biopesticide at different concentration level

Figure 2 shows the yield of ponkan per kg per tree treated with two formulated biopesticide at different concentration level ($1x10^6$, $1x10^8$, $1x10^{10}$, and $1x10^{12}$). Under field condition, the highest yield of ponkan observed in $1x10^{12}$ concentration and has no significant difference of other formulated biopesticide as well as in the positive control of both *B. bassiana* and *I. fumosoroseae*.

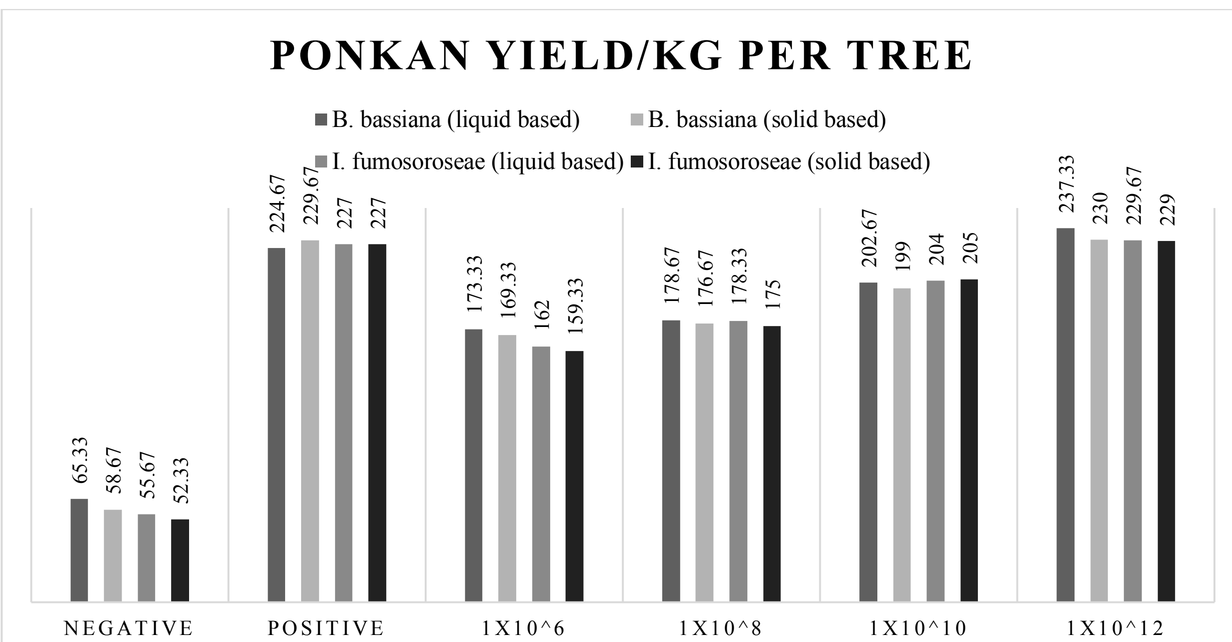

Figure 2. Ponkan yield/kg per tree as affected with two formulated biopesticide at different concentration level

Two Formulated Organic-Based Biopesticides under Field Condition of Calamansi Tree in Aurora, Isabela

Disease Incidence and Severity of Calamansi Tree caused by Twig Blight Disease (TBD)

Table 7 shows the disease incidence and severity of calamansi tree treated with two formulated organic-based biopesticide Beauveria bassiana and Isaria fumosoroseae caused by twig blight disease (TBD).

The disease incidence of the calamansi tree varied according to the assigned treatment and the formulation used. Under field condition, the highest disease incidence was observed in untreated which had mean DI of 25.00. Lower twig blight disease incidence observed in *B.* bassiana (solid based) with mean DI of 8.33 as per 100% concentration. Based on the statistical analysis, calamansi trees grown in open fields (Kasibu, Nueva Vizcaya) had a significant effect on the highest concentration (100%) of both *B. bassiana* (liquid and solid based) and *I. fumosoroseae* (liquid and solid based) which had 9.33, 8.33, 9.00, and 9.33, respectively. However, no significant difference were observed in other concentrations of 75%, 50%, and 25%.

Table 7. Disease incidence and severity of calamansi as affected with two formulated organic-based biopesticide of the two different entomopathogenic fungi (EPF) against twig blight disease (TBD)

Treatment	**Disease incidence* (%)**				**Disease severity* (%)**			
	B. bassiana		***I. fumosoroseae***		***B. bassiana***		***I. fumosoroseae***	
	Liquid	Solid	Liquid	Solid	Liquid	Solid	Liquid	Solid
Negative	25.00^{a}	24.00^{a}	23.00^{a}	23.33^{a}	15.67^{a}	16.33^{a}	16.00^{a}	16.33^{a}
Positive	11.67^{d}	12.00^{cd}	12.67^{d}	11.67^{de}	7.33^{cd}	7.67^{d}	7.33^{d}	7.67^{d}
25%	19.00^{b}	18.67^{b}	18.67^{b}	17.67^{b}	12.00^{b}	12.67^{b}	12.33^{b}	13.00^{b}
50%	17.00^{c}	14.00^{c}	15.00^{c}	15.67^{bc}	8.67^{c}	10.33^{c}	10.33^{c}	10.00^{c}
75%	12.33^{d}	11.67^{d}	12.67^{d}	14.00^{cd}	8.67^{c}	10.00^{c}	9.33^{c}	12.00^{bc}
100%	9.33^{e}	8.33^{e}	9.00^{e}	9.33^{e}	6.00^{d}	7.33^{d}	7.00^{d}	7.00^{d}
C.V. (%)	**6.74**	**7.97**	**3.99**	**8.75**	**9.14**	**8.28**	**7.17**	**11.50**

*Means followed by the same letter do not differ statistically using DMRT at 0.05 level

In disease severity, it also varied according to the assigned treatment and concentrations of the two formulations applied. The highest mean DS observed in untreated which had mean 16.33. Lower mean DS observed in *B. bassiana* (liquid based) had mean 6.00. Statistically, the highest concentration (100%) showed significant difference both in *B. bassiana* (liquid and solid based) and *I. fumosoroseae* (liquid and solid based) which had mean of 6.00, 7.33, 7.00, and 7.00, respectively.

Hence, both *B. bassiana* and *I. fumosoroseae* has a novel potential in terms of reducing the fungal pathogen that caused twig blight disease of citrus either solid or liquid formulation. Furthermore, the biopesticide is best used as 100% concentration to significantly reduced the fungal pathogen disease incidence and severity under field condition.

In table 8, it shows the percent damaged of citrus rind borer (CRB) using the solid and liquid based formulation of the two different EPF. The statistical analysis revealed significant differences in both *B. bassiana* and *I. fumosoroseae* according to the formulation and concentration level. The highest concentration ($1x10^{12}$) in both *B. bassiana* (solid-based) *I. fumosoroseae* (liquid-based) had mean 6.00. However, $1x10^{6}$, $1x10^{8}$, $1x10^{10}$ revealed almost no significant difference from the untreated regardless of the formulation. It was observed that as the level of concentration increases, the percent damage of CRB decreases.

Table 8. Percent damaged of citrus rind borer (CRB) as affected with two formulated organic-based biopesticide of two different entomopathogenic fungi (EPF)

Treatment	*Beauveria bassiana*		*Isaria fumosoroseae*	
	Liquid	Solid	Liquid	Solid
Negative	17.33[a]	18.33[a]	17.00[a]	16.33[a]
Positive	6.33[d]	8.00[c]	6.67[cd]	6.00[c]
$1x10^6$	13.33[b]	13.33[b]	12.00[b]	13.00[b]
$1x10^8$	10.67[c]	12.00[b]	11.33[b]	11.00[b]
$1x10^{10}$	8.67[cd]	8.33[c]	8.33[c]	8.00[c]
$1x10^{12}$	6.33[d]	6.00[d]	6.00[cd]	6.67[c]
CV%	**12.81**	**8.94**	**11.25**	**12.44**

*Means followed by the same letter do not differ statistically using DMRT at 0.05 level

In table 9, it shows the number of CRB infested calamansi buds treated with solid and liquid based formulation of the two different EPF at different level of concentration ($1x10^6$, $1x10^8$, $1x10^{10}$, $1x10^{12}$). Statistically, among the treatments regardless of the formulation used the lowest number of infested calamansi buds is observed in *B. bassiana* (liquid-based) at $1x10^{12}$ concentration level which had mean of 102.33, and the same concentration level of the other EPF and formulation observed relatively no significant difference. As the treatment concentration level increases in both formulation, the number of CRB infested calamansi buds decreases. Hence, both formulation is a potential biopesticide and the concentration level can be increases more than $1x10^{12}$.

Table 9. Number of CRB infested calamansi buds as affected with solid and liquid based formulation of the two different EPF

Treatment	*Beauveria bassiana*		*Isaria fumosoroseae*	
	Liquid	Solid	Liquid	Solid
Negative	199.67[a]	199.00[a]	197.00[a]	193.00[a]
Positive	118.00[e]	117.00[e]	115.67[e]	115.00[e]
$1x10^6$	157.67[b]	159.00[b]	157.67[b]	158.00[b]
$1x10^8$	134.67[c]	134.00[c]	135.33[c]	134.67[c]
$1x10^{10}$	122.00[d]	120.67[d]	119.33[d]	119.00[d]
$1x10^{12}$	102.33[f]	106.33[f]	106.00[f]	103.00[f]
CV%	**1.12**	**1.12**	**1.12**	**1.14**

*Means followed by the same letter do not differ statistically using DMRT at 0.05 level

Table 10 shows the damaged flower buds of calamansi treated with solid and liquid based formulation of the two different EPF Beauveria bassiana and Isaria fumosoroseae towards citrus rind borer at different concentration level ($1x10^6$, $1x10^8$, $1x10^{10}$, $1x10^{12}$).

Table 10. Damaged flower buds of calamansi as affected with two formulated organic-based biopesticide of the two different EPF against CRB

Treatment	*B. bassiana*		*I. fumosoroseae*	
	Liquid	Solid	Liquid	Solid

Negative	21.67[a]	21.67[a]	22.67[a]	21.00[a]
Positive	11.00[c]	11.00[d]	10.33[d]	10.00[d]
$1x10^6$	19.67[ab]	19.00[b]	17.33[b]	18.33[b]
$1x10^8$	18.67[b]	19.00[b]	18.67[b]	18.00[b]
$1x10^{10}$	13.00[d]	14.33[c]	14.67[c]	14.00[c]
$1x10^{12}$	8.33[d]	8.00[e]	8.00[d]	9.00[d]
CV%	**7.75**	**6.77**	**8.92**	**8.2**

*Means followed by the same letter do not differ statistically using DMRT at 0.05 level

Based on the statistical analyses, among the treatments the highest damaged flower buds of calamansi observed in untreated under *I. fumosoroseae* which had mean of 22.67. The lowest damaged flower buds of calamansi mean observed at $1x10^{12}$ concentration both in *B. bassiana* (solid-based) and *I. fumosoroseae* (liquid-based) which had 8.00, the same concentration level of both treatments and formulations had relatively no significant difference. Furthermore, the level of concentration had significant effect because it reduces the damaged of calamansi flower buds as the concentration level increases.

Table 11 shows the number of infested calamansi fruits treated at different level of concentration ($1x10^6$, $1x10^8$, $1x10^{10}$, $1x10^{12}$) with solid and liquid based formulation of the two different EPF which statically revealed that the highest number of infested calamansi fruit is 258.33 as per untreated mean under *B. bassiana* (solid-based) and has no significant difference of the rest untreated. However, at the concentration level $1x10^{12}$ *B. bassiana* (liquid-based) had the lowest mean of 101.33, on the same concentration level of the other the other formulation and the two EPF had relatively no significant difference. It was observed that as the concentration level increases on both EPF, the number of infested calamansi fruit decreases.

Table 12 shows the number of calamansi fruit fall treated at different concentration level ($1x10^6$, $1x10^8$, $1x10^{10}$, $1x10^{12}$) with solid and liquid based formulation of the two different EPF. Statistically, it was observed that the highest number of calamansi fruit fall as per untreated had mean of 196.33 under *B. bassiana* (liquid-based) which is not significantly different of the other untreated mean. In the concentration level of $1x10^{12}$ observed to have the lowest calamansi fruit fall mean under *B. bassiana* (solid-based) of 101.33 and not significantly differ on the rest EPF and formulation. This result revealed that as the concentration level increases the number of calamansi fruit fall reduced. Hence, the level of concentration of both EPF plays important role in decreasing the fruit fall caused by CRB and either solid and liquid based formulation has potential as biopesticicde since it shows no significant difference in terms of its performance.

Table 11. Number of infested calamansi fruits as affected with solid and liquid based formulation of the two different EPF against CRB

Treatment	***B. bassiana***		***I. fumosoroseae***	
	Liquid	Solid	Liquid	Solid
Negative	255.67[a]	258.33[a]	256.33[a]	255.67[a]
Positive	105.33[e]	106.67[e]	107.00[e]	106.00[e]
$1x10^6$	193.67[b]	194.67[b]	193.67[b]	192.00[b]
$1x10^8$	175.67[c]	175.67[c]	177.67[c]	177.33[c]
$1x10^{10}$	152.00[d]	153.33[d]	153.33[d]	154.67[d]
$1x10^{12}$	101.33[e]	102.67[e]	102.00[f]	104.00[e]
CV%	**1.52**	**2.02**	**1.29**	**2.29**

*Means followed by the same letter do not differ statistically using DMRT at 0.05 level

Table 12. Number of calamansi fruit fall as affected with solid and liquid based formulation of the two different EPF against CRB

Treatment	*B. bassiana*		*I. fumosoroseae*	
	Liquid	Solid	Liquid	Solid
Negative	196.33[a]	191.00[a]	193.33[a]	195.33[a]
Positive	110.67[e]	109.67[e]	110.00[e]	111.00[e]
$1x10^6$	174.67[b]	172.33[b]	173.33[b]	174.00[b]
$1x10^8$	167.33[c]	165.67[c]	164.00[c]	165.67[c]
$1x10^{10}$	155.33[d]	155.00[d]	153.67[d]	154.33[d]
$1x10^{12}$	101.67[f]	101.33[f]	103.00[f]	108.00[e]
CV%	**1.89**	**1.21**	**1.48**	**1.5**

*Means followed by the same letter do not differ statistically using DMRT at 0.05 level

Below (Figure 1) represents the calamansi fruit yield per kg per tree treated with different concentration level ($1x10^6$, $1x10^8$, $1x10^{10}$, and $1x10^{12}$) of two formulated biopesticide of the two different EPF. Based on the statistical analyses using DMRT, under field condition the highest yield per kg per tree observed at the highest concentration level ($1x10^{12}$) of the two formulated biopesticide and has relatively no significant difference of both *B. bassiana* and *I. fumosoroseae* formulation. Lower yield observed in untreated. Hence, the concentration level has significant effect in reducing the fungal pathogen and pests that possibly affect the yield.

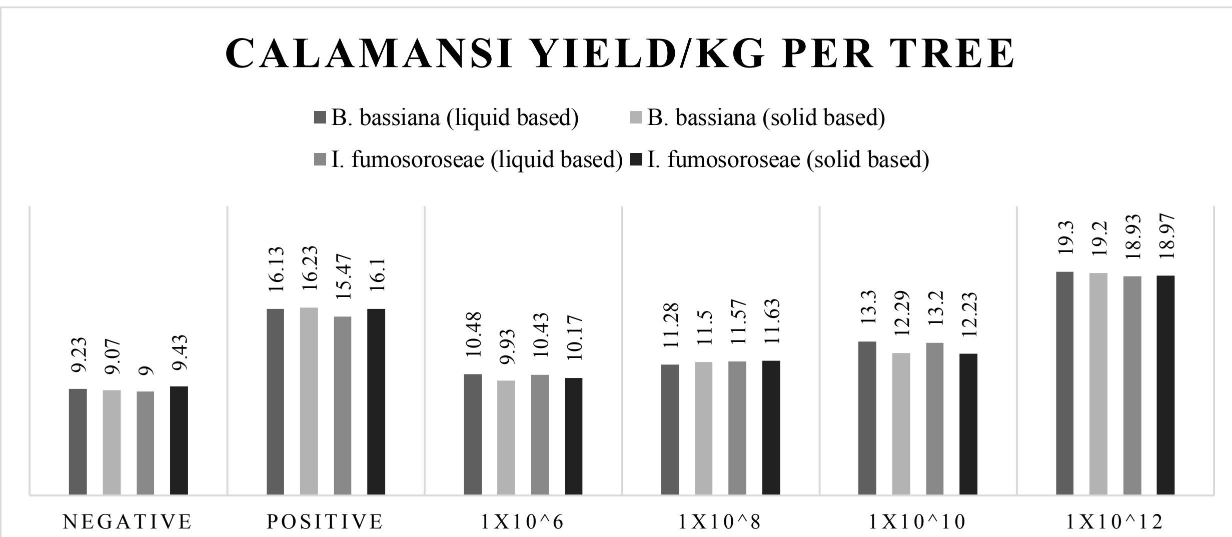

Figure 3. Calamansi yield/kg per tree as affected with two formulated biopesticide at different concentration level

Two Formulated Organic-Based Biopesticides under Field Condition of Pomelo Tree in Abulog, Cagayan

Disease Incidence and Severity of Pomelo Caused by Twig Blight Disease (TBD) in Citrus

Table 13 shows the disease incidence and severity of twig blight disease in pomelo plant with two formulated organic-based biopesticide *Beauveria bassiana* and *Isaria fumosorosea* at different concentrations ($1x10^6$, $1x10^8$, $1x10^{10}$, $1x10^{12}$).

Table 13. Disease incidence and severity of twig blight disease in pomelo with the application of two different formulated biopesticides at different concentrations

Treatment	Disease Incidence				Disease Severity			
	Beauveria bassiana		*Isaria fumosorosea*		*Beauveria bassiana*		*Isaria fumosorosea*	
	Liquid	Solid	Liquid	Solid	Liquid	Solid	Liquid	Solid
Negative	39.00[a]	38.67[a]	41.00[a]	40.33[a]	36.67[a]	36.33[a]	40.67[a]	43.33[a]
Betacyflutrin	11.00[d]	9.33[de]	11.00[d]	10.33[de]	11.67[d]	11.33[d]	11.67[c]	12.00[d]
25%	21.33[b]	21.00[b]	21.33[b]	22.00[b]	22.67[b]	23.33[b]	20.33[b]	22.67[b]
50%	18.00[c]	18.00[c]	17.00[c]	17.67[c]	20.33[c]	17.67[c]	19.00[b]	18.33[c]
75%	10.67[de]	11.00[d]	10.33[d]	10.67[d]	12.00[d]	11.00[d]	12.00[c]	11.00[de]
100%	8.33[e]	7.00[e]	8.00[e]	7.67[e]	8.67[e]	7.33[e]	9.33[c]	9.00[e]
C.V. (%)	**7.13**	**9.04**	**5.94**	**8.23**	**6.11**	**7.31**	**7.45**	**5.93**

*Means followed by the same letter do not differ statistically using DMRT at 0.05 level

It was observed that there was no significant difference between the two EPF in terms of the incidence of twig blight disease in Pomelo. The lowest percentage of infected plants were observed at 100% level of spore concentration while highest percentage was observed on the negative treatment. The two EPF were observed to be effective at 75% similar to Betacyflutrin and there was a significant difference on the negative treatment with the rest of the treatments.

On the other hand, similar result was observed with the disease severity of twig blight disease in Pomelo. Low percentage of disease severity was observed at 100% level of spore concentration. This means that there were lesser damage caused by the disease in Pomelo plant. The severity of the disease was observed to be high in the negative treatment. This would only mean that with the application of the two EPF, the severity of the disease has been reduced. Thus, the higher the spore concentration level caused lower incidence and severity of the twig blight disease in Pomelo. This result strongly supports the possibility of utilizing the two EPF as effective biological control agent against twig blight disease.

Table 14 shows the percent damage of CRB in Pomelo using the two formulated biopesticides. It was observed that there was a significant difference on the negative treatment compared to other treatments used. The two EPF can be seen to be as effective as the synthetic insecticide (Betacyflutrin) at 1 x 10^{12} spore concentration level. The lowest percentage damage of CRB was observed at 1 x 10^{12} and this signifies that both *B. bassiana* and *I. fomosorosea* are very effective at this level of spore concentration. Hence, it is recommended to apply these two EPF with the level of spore concentration starting at 1x 10^{12} in the field.

On the other hand, table 15 shows the number of fruitlets formed of Pomelo as affected by the used of two different biopesticide formulations. It was observed that there was a low number of fruitlets of Pomelo on the negative treatment, and seemed to be significantly different with the rest of the treatments used. This means that there was a significant effect of the two EPF against RCB in all of the levels of spore concentrations tested. Although there was a higher number of fruitlets observed in the use of Betacyflutrin, the result still showed that EPF can be used as a better alternative control against CRB, since using EPF is cost- effective and more sustainable than the said synthetic chemical.

Table 14. Percent damage of citrus rind borer (CRB) in Pomelo as affected by the application of entompathogenic fungi (EPF) at different concentrations

Treatment	*Beauveria bassiana*		*Isaria fumosorosea*	
	Liquid	Solid	Liquid	Solid
Negative	73.33[a]	72.67[a]	75.00[a]	82.33[a]
Betacyflutrin	22.00[d]	20.00[d]	20.67[d]	21.67[d]
$1x10^6$	60.67[b]	59.33[b]	60.33[b]	63.33[b]
$1x10^8$	33.00[c]	32.00[c]	31.67[c]	34.00[c]
$1x10^{10}$	22.00[d]	21.67[d]	20.33[d]	22.67[d]
$1x10^{12}$	10.33[e]	9.67[e]	11.33[e]	9.00[e]
C.V. (%)	**2.87**	**3.2**	**4.42**	**6.38**

*Means followed by the same letter do not differ statistically using DMRT at 0.05 level

Table 15. Number of fruitlets formed in Pomelo as affected by CRB with the application of entomopathogenic fungi (EPF) at different concentrations

Treatment	*Beauveria bassiana*		*Isaria fumosorosea*	
	Liquid	Solid	Liquid	Solid
Negative	87.00[a]	87.00[e]	83.33[d]	84.00[e]
Betacyflutrin	87.67[a]	123.00[a]	121.00[a]	123.00[a]
$1x10^6$	104.00[a]	104.00[d]	100.67[c]	101.33[d]
$1x10^8$	111.33[a]	111.33[c]	111.00[b]	110.00[c]
$1x10^{10}$	118.33[a]	118.33[ab]	118.00[ab]	117.33[b]
$1x10^{12}$	116.33[a]	116.33[bc]	117.33[ab]	115.67[b]
C.V. (%)	**24.03**	**2.99**	**3.43**	**2.27**

*Means followed by the same letter do not differ statistically using DMRT at 0.05 level

Table 16 shows the number of fruits of Pomelo as affected by CRB with the application of two different formulated biopesticides. Based on the data gathered, it was observed that there was no significant difference between the use of negative control (Betacyflutrin) and EPF at 1 x 10^{12} level of spore concentration in controlling CRB. Both treatments were also significantly different from the negative treatment which means that there was a significant effect of the two EPF against CRB. Given that Betacyflutrin is a synthetic chemical, this suggests that there is likelihood for utilizing EPF as a biological control agent against CRB.

Table 16. Number of fruits of Pomelo as affected by CRB with the application of entomopathogenic fungi (EPF) at different concentrations

Treatment	*Beauveria bassiana*		*Isaria fumosorosea*	
	Liquid	Solid	Liquid	Solid
Negative	12.67[d]	13.33[c]	10.00[d]	11.33[c]
Betacyflutrin	41.33[b]	44.00[a]	45.00[a]	42.33[a]
$1x10^6$	13.67[d]	11.67[c]	14.67[c]	11.67[c]
$1x10^8$	16.33[d]	14.00[c]	17.00[c]	15.00[c]
$1x10^{10}$	24.33[c]	24.00[b]	23.00[b]	23.00[b]
$1x10^{12}$	45.67[a]	45.00[a]	43.67[a]	39.67[a]
C.V. (%)	**8.26**	**7.21**	**5.79**	**9.72**

*Means followed by the same letter do not differ statistically using DMRT at 0.05 level

Table 17 shows the number of fruits with lumps of Pomelo as affected by the use of two formulated biopesticides at different concentrations. It was observed that all the treatments used was significantly different from the negative treatment. This indicates that with the use of EPF, there was a reduction in the number of fruits with lumps of Pomelo. Low number of fruits with lumps of Pomelo was observed at 1 x 10^{12} spore concentration level, while there was no significant difference among the treatments at 1 x 10^6, 1 x 10^8 and 1 x 10^{10}. For the number of fruits without lumps of Pomelo, it is presented in Table 18. It was observed that, with an increasing level of spore concentrations of EPF, there was an increase in the number of fruits without lumps of Pomelo that were harvested. This signifies that *B. bassiana* and *I. fumosorosea* are both effective biopesticides against CRB and can be useful to farmers since it can reduce the infestation of the said pest in the field.

Table 17. Number of fruits with lumps of Pomelo caused by CRB as affected by the application of entomopathogenic fungi (EPF) at different concentrations.

Treatment	*Beauveria bassiana*		*Isaria fumosorosea*	
	Liquid	Solid	Liquid	Solid
Negative	10.33^a	9.67^a	12.00^a	12.00^a
Betacyflutrin	6.33^b	6.00^b	2.67^b	4.67^b
$1x10^6$	3.33^b	3.33^c	1.67^b	2.33^b
$1x10^8$	3.33^b	3.67^c	1.33^b	2.33^b
$1x10^{10}$	5.33^b	2.33^c	3.00^b	2.67^b
$1x10^{12}$	4.67^b	2.67^c	1.67^b	3.67^b
C.V. (%)	**28.59**	**26.76**	**35.93**	**37.07**

*Means followed by the same letter do not differ statistically using DMRT at 0.05 level

Table 18. Number of fruits without lumps of Pomelo caused by CRB as affected by the application of entomopathogenic fungi (EPF).

Treatment	*Beauveria bassiana*		*Isaria fomosorosea*	
	Liquid	Solid	Liquid	Solid
Negative	0.67^e	1.33^d	0.33^a	1.00^d
Betacyflutrin	36.67^b	41.00^a	39.00^a	35.00^a
$1x10^6$	11.33^d	10.00^c	11.33^c	8.33^c
$1x10^8$	14.00^d	12.67^c	13.00^c	11.67^c
$1x10^{10}$	21.67^c	21.00^b	20.67^b	17.67^b
$1x10^{12}$	41.67^a	43.33^a	41.00^a	35.00^a
C.V. (%)	**9.4**	**7.01**	**7.19**	**12.86**

*Means followed by the same letter do not differ statistically using DMRT at 0.05 level

Figure 4 shows the yield of Pomelo in kilogram per tree with lumps on its fruits. Based on the data gathered, it was observed that in the negative treatment, high number of Pomelo fruits with lumps were harvested in the field. For the other treatments, low number of harvested fruits with lumps were harvested on the four levels of spore concentrations of EPF. This means that the two EPF tested were effective in controlling infestation of CRB. However, this result can be more justified in Figure 5 showing the yield of Pomelo in kilogram per tree without lumps of its fruits. There was a significant effect of the EPF of all the concentration used against CRB since more fruits without lumps were harvested in these treatments compared to the negative treatment. The highest yield was observed at 1 x 10^{12} level of spore concentration, while it is noticeable that the negative treatment had the lowest yield of Pomelo fruits without lumps in it. The infestation of CRB decreases when the application of spore concentrations of EPF is increased. For this reason, the two EPF tested in this research is said to be really effective biopesticides against CRB.

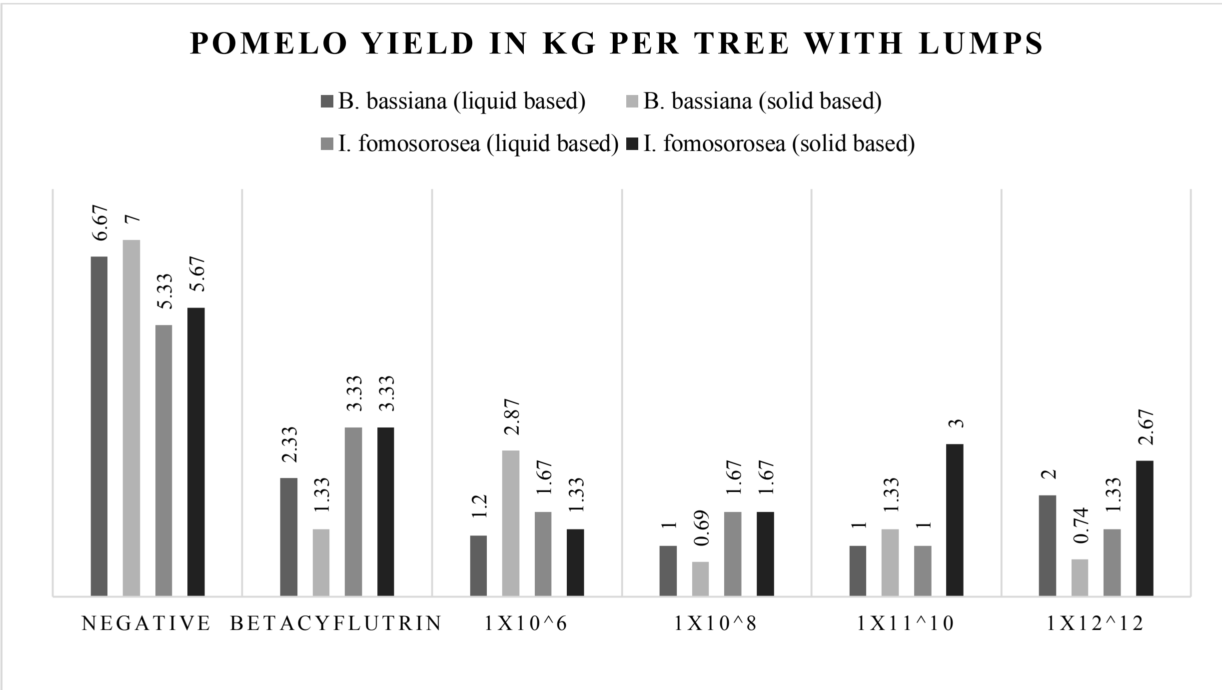

Figure 4. Yield of Pomelo in kg/tree with lumps on the fruits as affected by two different formulated biopesticides at different concentrations

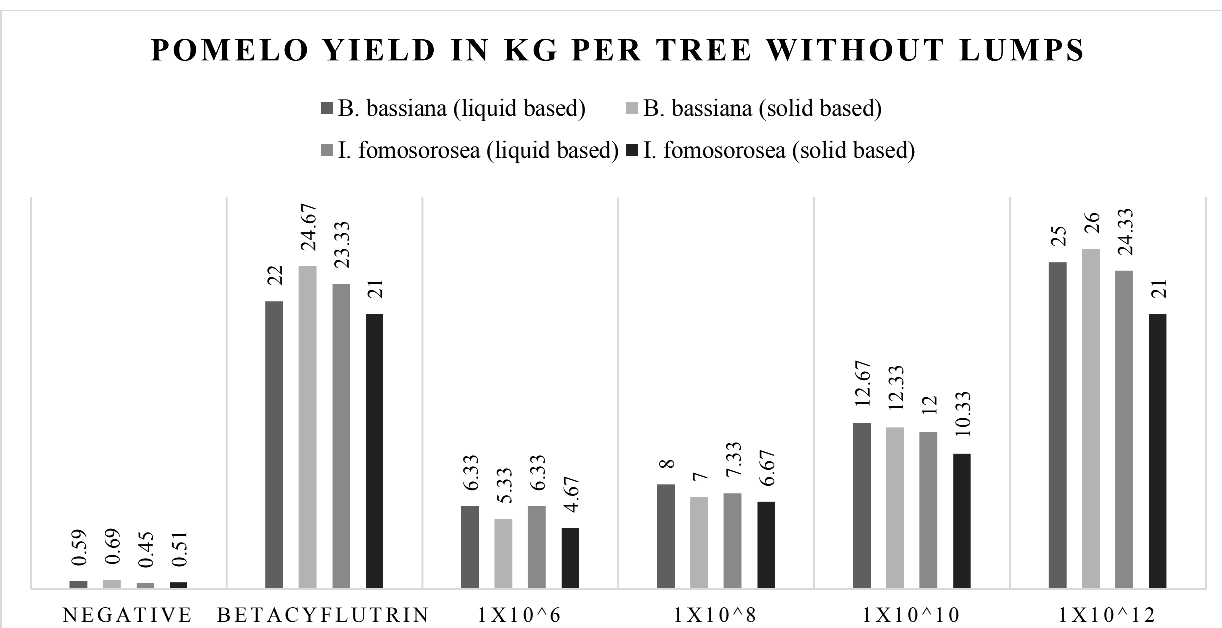

Figure 5. Yield of Pomelo in kg/tree without lumps on the fruits as affected by two different formulated biopesticides at different concentrations.

SUMMARY AND CONCLUSION

This study verified the two formulated organic biopesticides *Beauveria bassiana* and *Isaria fumosoroseae* at different level of concentrations ($1x10^6$, $1x10^8$, $1x10^{10}$, $1x10^{12}$) under field condition of the two mandarin (Satsuma and Ponkan) in Kasibu Nueva Vizcaya, Calamansi tree in Aurora Isabela, and Pomelo in Abulog Cagayan.

The two mandarin (Satsuma and Ponkan) under field condition revealed that the best concentration was observed at $1x10^{12}$. Both *B. bassiana* and *I. fumosoroseae* showed its best performance at the highest concentration ($1x10^{12}$) level. Statistically using DMRT at 0.05 level, the concentrations was observed to have significantly differ. However, either liquid or solid based formulation of both *B. bassiana* and *I. fumosoroseae* has no significant difference in terms of disease incidence and severity of fungal pathogen that caused twig blight disease (TBD), population dynamics of citrus rind borer on percent damage, number of infested buds, percent damaged flower buds, number of infested fruits, and number of fruit fall. Furthermore, Satsuma and Ponkan in terms of yield performance per kg per tree observed that $1x10^{12}$ concentration has no significant difference in positive treatment and significantly differ of the other treatment.

In Calamansi tree, the concentration of the two formulated organic-based biopesticide significantly varied and revealed that the best concentration was observed at $1x10^{12}$. Both *B. bassiana* and *I. fumosoroseae* either using liquid or solid based formulation observed to have no significant difference in terms of its performance under field condition on the disease incidence and severity caused by twig blight disease (TBD), percent damage, number of infested buds, percent damaged flower buds, number of infested fruits, and number of fruit fall caused by citrus rind borer. Additionally, the yield performance per kg per tree observed that $1x10^{12}$ significantly differ of the rest treatment.

In Pomelo tree, the best concentration of both *B. bassiana* and *I. fumosoroseae* observed at $1x10^{12}$ under field condition. The $1x10^{12}$ concentration treatment significantly varied of the other treatments in terms of its performance in reducing the disease incidence and severity caused by twig blight disease (TBD), percent damage, number of infested buds, number of fruits with lumps and without lumps, number of fruitlets and fruits formed. Furthermore, the yield per kg per tree of Pomelo in $1x10^{12}$ concentration observed significantly difference of the other treatments.

Either *Beauveria bassiana* or *Isaria fumosoroseae* has a novel potential as biopesticide at $1x10^{12}$ level of concentrations in Satsuma, Ponkan, Calamansi, and Pomelo trees under field condition.

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