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Performance of *Trichoderma koningii* and *Bacillus* sp. as Potential Biocontrol Agents against *Fusarium* Wilt (*Fusarium oxysporum*) of Sweetpotato (*Ipomoea batatas*)

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Abstract

Fusarium wilt (*Fusarium oxysporum*) is now becoming a big concern in Cordillera Administrative Region, Philippines affecting different crops including sweetpotato. In effort to help find solution to this problem, the study documented the biological control ability of *Trichoderma koningii* and *Bacillus* sp. against *Fusarium* wilt of sweetpotato through bioassay and pot experiment. Result of the bioassay test in the laboratory showed significant results. The growth of *F. oxysporum* was inhibited by Benomyl (reference fungicide) and *T. koningii*. The widest inhibition zone of 29 mm after three days was observed in Benomyl followed by *T. koningii* with 20 mm. *Bacillus* sp. gave the least inhibition zone of 3 mm. In the pot experiment, sweetpotato grown in soils amended with *T. koningii* had the lowest percent infection and lowest severity of diseases. At the end of the cropping season, populations of *Bacillus* and *F. oxysporum* decreased while *T. koningii* increased. The highest reduction of *Fusarium* spores from 1.8 x 10^6 to 9.50 x 10^5 was noted in soils amended with *T. koningii*, followed by Benomyl from 1.8 x 10^6 to 1.40 x 10^5 cfu/ml. Results consistently showed better performance of *T. koningii* as sole inoculant in inhibiting *Fusarium* wilt and even better than Benomyl, thus should be encouraged for farmers to adopt.

KEYWORDS

Trichoderma koningii
Bacillus sp.
Fusarium wilt inhibition

Introduction

Globally, sweetpotato is the sixth most important food crop after rice, wheat, potatoes, maize and cassava. In developing countries, it is the fifth most important food crop with 105 million metric tons or more being produced globally each year. The developing countries grow 95% of the total production. Sweetpotato can grow at altitudes ranging from sea level to 2,500 meters. It requires fewer inputs and less labor than other crops such as maize, and tolerates marginal growing conditions (Sweetpotato Facts and Figures, 2015).

In the Philippines, sweetpotato used to be a staple food in the mountainous regions of Northern Philippines (Yen, 1974; Fang-asan et al., 1998). In the Cordillera Administrative Region (CAR), the

province of Ifugao had the highest sweetpotato production of 7,327.75 metric tons (MT) while Benguet had the widest production area of 792 hectares producing 3,744.75 MT. Other provinces in the region have lower productions–Mountain Province with 1,765.78 MT, Abra with 644.84 MT, Kalinga with 172.35 MT and Apayao with 64.15 MT. Among the 13 municipalities of Benguet, Kabayan has the widest production area of sweetpotato with 146 ha (Tenorio & Gonzales, 2013).

Sweetpotato production is usually affected by pests and diseases caused by fungi, bacteria, nematodes and viruses (Mwanga, 2010). In 2013, sweetpotato showing wilt symptoms and browning of stems was brought for diagnosis at the Benguet State University (BSU) Plant Health Clinic from the municipality of Kabayan. The client claimed that majority of sweetpotato in the area was wilting. Further diagnostic test revealed that *Fusarium*, a soil borne fungus, was associated with the wilted sweetpotato plants. Additional sweetpotato specimens showing the same symptoms from other areas such as Ifugao, Buguias and Kapangan were brought for diagnosis at the Plant Health Clinic (Sebiano, 2015). Most of the clients observed that the sweetpotato infected with wilt did not bear roots resulting in economic loss. In most cases, *Fusarium* wilt is usually overlooked until the spread of the disease.

Currently, pesticide application is the main method used to suppress *Fusarium* infection but according to farmers, this somewhat defeat the purpose of planting sweetpotato. According to them, they plant sweetpotato to minimize input such as pesticide since these are expensive and even harmful to their health and the environment. Thus, these farmers are looking at BSU to help solve the problem in a more environment-friendly way. In 2014, farmers and local government agencies sought the assistance of the Northern Philippine Root Crops Research and Training Center (NPRCRTC) in BSU to identify and provide mitigating measures against sweetpotato wilt. In response, clean planting materials and technical assistance are being provided to affected farmers. However, NPRCRTC and the Department of Plant Pathology recognize the need to offer an integrated solution to *Fusarium* wilt and is currently looking at biocontrol agents.

Biocontrol agents act as antagonist by competing for space and nutrients. During this process, the antagonist may suppress the growth of the pathogen population in the rhizosphere and thus reduce disease development. In addition, biological controls are known to be ecologically friendly and do not result in the accumulation of hazardous residues in food commodities. This makes biological control as the banner of Integrated Pest Management local and abroad (Archita, 2018). Disease control agents for sweetpotato are known including *Trichoderm*a, *Pseudomonas* and *Bacillus* species.

There is considerable interest in the exploitation of naturally occurring organisms, such as bacteria, viruses and fungi for the control of crop pests, weeds and diseases (Butt et al., 2001). With the global standing of sweetpotato and the different benefits it provides, there is a big incentive to look into determining and developing biocontrol agents against *Fusarium* wilt. Previous researches have shown that *Trichoderma* and *Bacillus* sp. are potential biological control agents against *Fusarium* wilt (Zaim et al., 2018). However, more studies should be done to further evaluate their capacity in suppressing *Fusarium* wilt when combined with other management strategies or applied alone. This was the primary aim of this study – to determine the biological control ability of *Trichoderma koningii* and *Bacillus* sp. against sweetpotato *Fusarium* wilt through bioassay and through pot experiments either applied alone or in combination. The proponents hope that the results would help advance the search for controlling *Fusarium* wilt and make sweetpotato a viable low input crop again particularly to indigent farmers.

Methodology

Bioassay of *Trichoderma koningii* and *Bacillus* sp. against *Fusarium oxysporum*

Pure culture of *Fusarium oxysporum* with a spore count of $1.8x10^6$ was prepared and used. An amount of 0.1ml was transferred to petri dish. After which, 10ml of Potato Dextrose Agar (PDA) was poured in the petri plates. The plates were carefully rotated to ensure even distribution of the inoculum. After the agar solidified, a 10mm sterile cork borer was used to create a well in the middle of the PDA. After this, 0.1ml of standardized *Bacillus* sp. suspension ($1.7x10^6$) cFu/ml was slowly

dispensed into the well of the PDA until a concave upper rim was achieved. The same procedure was followed in determining the effect of *Trichoderma koningii* and Benomyl against *Fusarium*. The zone of inhibition was measured using a caliper. The zero mark was placed at the center of the well and from that, the inhibition zone was measured from the center to the edge of area with zero growth.

Effectiveness of *Trichoderma, Bacillus* and Benomyl against *Fusarium* Wilt in Pot Experiment

Experimental design. The pot experiment was set-up at the NPRCRTC. Complete Randomized Design (CRD) was used with eight treatments replicated three times. The treatments are presented in Table 1. For each replicate, ten (10) sweetpotato samples were planted. The sweetpotato variety 'Immitlog' was used for the experiment as previous study showed it to be susceptible to *Fusarium* wilt. All recommended cultural management in sweetpotato production such as weeding, irrigation and fertilization were employed uniformly in each treatment as needed.

Table 1

The treatments for the pot experiment in the study

Treatment	Description
T1	No treatment
T2	*Fusarium oxysporum*
T3	Benomyl application
T4	*Trichoderma koningii*
T5	*Bacillus* sp.
T6	*T. koningii* + Benomyl
T7	*Bacillus sp.* + Benomyl
T8	*T. koningii* + *Bacillus sp.* + Benomyl

Preparation of Potting Media and Inocula. The potting media used was made of one part carbonated rice hull ash and one part sterilized forest soil (1:1 ratio). After mixing the media, the 8x14" size polyethylene plastic (PEP) bags were filled with eight (8) kilograms potting media. Agricultural lime at 100 g/pot was applied one week before planting based on the six (6) tons/ha recommended rate and practiced by farmers. In like manner, fully decomposed chicken manure at a rate of 167g/pot was applied one week before planting. This was based on 10 tons/ha recommended rate (Galian & Nagpala, 2006).

Standardized spore count of *Trichoderma koningii* ($1.6x10^6$) and *Fusarium oxysporum* ($1.8x10^6$) was prepared using haemacytometer. For *Bacillus* sp., a suspension of $1.7x10^6$ was prepared using spectrophotometer at 690 nm.

Evaluation of Biocontrol Agents In Vivo. Five (5) ml of *Trichoderma koningii* and *Bacillus* sp. were drenched for one week before planting to allow microorganisms to grow while 5ml of $1.8x10^6$ *Fusarium* suspension was drenched near the root zone 2 weeks after. Benomyl was used as the reference fungicide. For Benomyl application treatment, planting materials were dipped for one minute in a Benomyl suspension following the recommended rate of 20g/16L before planting. Healthy 20-30 cm sweetpotato stem were planted in the PEP bags one week after *T. koningii* and *Bacillus* sp. application.

The number of days from inoculation to symptom appearance, disease incidence, disease severity, final population counts and disease index were determined. Disease incidence is the proportion of infected plants in total plant population while disease severity (sometime called "intensity") refers to the relative or absolute area of plant tissue affected by disease or the degree of infection. It was determined by adopting the scale proposed by Chunsheng et al. (1988).

Data gathered were statistically analyzed with Analysis of Variance (ANOVA). Significant differences between treatments were analyzed using the Tukey's Test at 5% level of significance.

Results and Discussion

Biological Control Ability of *Trichoderma koningii* and *Bacillus* sp. against *Fusarium oxysporum* through Bioassay

Table 2 presents the zone of inhibition observed and recorded after 72 hours of incubation. Results show that Benomyl fungicide (control) had the widest inhibition zone against *Fusarium oxysporum* with 29mm followed by *Trichoderma koningii* with

Table 2

Mean inhibition zone (mm) exhibited by Trichoderma koningii and Bacillus sp. against Fusarium oxysporum after 72 hours

Treatment	Mean inhibition	Remarks
Sterilized distilled water	0[c]	_____
Trichoderma koningii	20[b]	very active
Bacillus sp.	3[c]	_____
Benomyl	29[a]	very active

Means with the same letters in a column are not significantly different at 5% Tukey's test

a mean inhibition zone of 20mm. Both Benomyl and *T. koningii* are categorized as very active in suppressing *Fusarium* in-vitro. Apparently *T. koningii* is comparable to Benomyl in its effect in inhibiting the growth of *Fusarium* in culture. *Bacillus* sp. gave the smallest inhibition zone of 3mm. According to Altinok and Erdogan (2015), *T. harzianum* produces volatile and non-volatile metabolites that inhibites *F. oxysporum* growth on Potato Dextrose Medium and in their in-vitro colonization study also demonstrated the root-colonizing ability of these antagonists. No clear zone was formed between the *Bacillus* sp. and *T. koningii*. After 24 hours of incubation, the growth of *Bacillus* sp. was visible but after three days of incubation *T. koningii* overgrew *Bacillus* sp.. *T. koningii* did not suppress *Bacillus* sp. but competes for nutrition and space.

Efficacy of *Trichoderma koningii*, *Bacillus* sp. and Benomyl against *Fusarium* Wilt of Sweetpotato in pot experiment

Number of days from inoculation to symptom appearance. The number of days from inoculation to symptom appearance significantly differ between treatments (Table 3). Inoculation of *Fusarium* ($1.8x10^6$) through root wounding resulted to symptom development in stem and leaves of sweetpotato. T_2, which has no biocontrol agent amendments, was the first to show symptoms of interveinal yellowing and abscission of leaves after 38.6 days from inoculation, then death of plant as the disease progressed. This was followed by T_5 (*Bacillus* sp. inoculated plants) which showed similar symptoms after 50.2 days of inoculation. This shows that *Bacillus* sp. alone was not able to suppress the *Fusarium* root infection. Benomyl showed suppression of *Fusariumf* root infection at 77.8 days.

On the other hand, T_4 (*T. koningii*) had the longest time of symptom expression at 91.3 days and with minimal interveinal yellowing. Most of the infected plants were able to reach maturity

Table 3

Performances of the treatment in terms of symptom appearance, disease incidence, disease severity and disease index.

Treatment	Number of days before symptom appearance	Disease Incidence (%)	Disease severity (%)	Disease index (%)
T1 (No treatment)	0.00 [d]	0.00 [c]	0.00 [c]	0
T2 (*Fusarium oxyporum*)	38.60[c]	66.70 [a]	51.30[a]	51.3[a]
T3 (Benomyl)	77.80[ab]	30.00[ab]	22.00[bc]	22.0[bc]
T4 (*Trichoderma koningii*)	91.30[a]	16.60[b]	7.30[bc]	7.3[bc]
T5 (*Bacillus* sp.)	50.20[c]	40.00[ab]	32.60[ab]	32.6[ab]
T6 (*T. koningii* + Benomyl)	87.90[ab]	26.60[b]	13.30[bc]	13.3[bc]
T7 (*Bacillus* sp. + Benomyl)	72.70[b]	23.30[b]	14.60[bc]	14.6[bc]
T8 (*T. koningii* + *Bacillus* sp. + Benomyl)	88.50[ab]	17.00[b]	10.00[bc]	10.0[bc]

Means with the same letters in a column are not significantly different at 5% Tukey's test

stage and produced more roots. Treatments with *Trichoderma* combinations such as T_6 (*T. koningii* + Benomyl) and T_8 (*T. koningii* + Benomyl + *Bacillus* sp.) also have lower numbers of days before symptom emergence. Based on these results, it appears that *T. koningii* alone suppressed the infection of *Fusarium* wilt in sweetpotato. This result is similar with findings of other studies where *Trichoderma* species were found effective against *Fusarium* wilt. Thangavelu (2002) reported that application of *T. harzianum* Th-10, as dried banana leaf formulation after planting was most effective against *Fusarium* wilt in banana plantation.

Disease incidence. Significant differences were recorded on the disease incidence as shown in Table 3. Sweetpotato inoculated with *Fusarium* alone (T_2) had the most number of infected plants (66.7 %) from inoculation to harvest, followed by *Bacillus* sp. inoculated pots (T_5) with 40% and fungicide Benomyl treated plants (T_3) with 30%. Pots with *T. koningii (*T_4) treated soil had the lowest disease incidence with 16.6% infection. Combining *T. koningii* with other treatments, such as T_6, T_7, and T_8, also have lower disease incidence at 26.6%, 23.3% and 17%, respectively.

Such result corroborates with the study of Ranasingh et al. (2006) in which the pathogen and the introduced biocontrol agent compete for the availability of space and nutrients. During this process, the antagonist may suppress the growth of the pathogen population in the rhizosphere and thus reduces disease development. In addition, *Trichoderma* strains are known to produce antibiotics and toxins, which are volatile or non-volatile in nature, and have a direct effect on other organisms. Chemical such as trichothecin, sesquiterpine and Trichodermin produced by *Trichoderma* have antimicrobial effect on bacteria and fungi.

Plants treated with *T. koningii* (T_5) were able to reach maturity and produced bigger roots compared to other treatments. The infected plants also reach maturity period and produced more roots but were smaller in size. From this result, *T. koningii* can potentially prevent the progress of infection caused by *Fusarium*. This is consistent with the study of Ubaub and Requina (2016) who reported that different rates of vesicular mycorrhizae and *Trichoderma* delayed symptom appearance and reduced incidence of Panama wilt disease. On the other hand, Benomyl was observed to be more effective when combined with *T. koningii* and *Bacillus* sp.

Disease severity. The severity of *Fusarium* wilt infection in sweetpotato was consistent with disease incidence as seen in Table 3. *Fusarium* inoculated plants (T_2) were the most severely infected at 51.3% disease severity, followed by plants applied with *Bacillus* sp. (T_5) at 32.6%. This result implies that *Bacillus* sp. was not effective enough to suppress the infection. Meanwhile, plants applied with Benomyl (T_3), *T. koningii* + Benomyl (T_6) and *Bacillus* sp. + Benomyl (T_7) decreased disease severity as shown by older leaves turning yellow and by the length of tan vascular system which is 1/3 of total plant length. However, it is inferior compared to infection of plants applied with *T. koningii* only (T_4). T_4 and T_8 (*T. koningii* + *Bacillus* sp. + Benomyl) had the least plant tissue disease severity at 7.3% and 10%, respectively. Also, these treatment had the shortest length of infected tan vascular system at 5cm. These results support the findings of Adeeba et al. (2016) that *Trichoderma* isolates can successfully inhibit the growth of *Fusarium oxysporum f.* sp. *lycopersici* causing tomato wilt. Similar antagonistic effects were also found by Sarker et al. (2013). Likewise, Raghuchander et al. (1997) reported that inoculation of potted abaca plants with *Trichoderma viride* and yeast showed 81.76% and 82.52% reduction of wilt disease severity.

Initial and final population of *Fusarium, Bacillus* sp. and *Trichoderma koningii*. Figure 1 presents the initial and final population count of the microorganism used in the study. *Fusarium* and *Bacillus* sp. had decreased in population after harvest from the initial spore count of $1.8x10^6$ to $1.6x10^6$. Significant difference was noted in the final population of *Fusarium* among the different treatments. The highest colony count after harvest was observed in the T_2 (*Fusarium oxysporum*) with a mean of $2.15x10^5$ CFU. The rest of the treatments had lower colony count ranging from $1.10x10^5$ to $1.47x10^5$. Significantly lowest final population of *Fusarium* was observed in T_4 (*T. koningii* only). From the initial population of $1.8x10^6$, it decreased to $9.50x10^4$ after four months. Other treatments with *T. koningii* component such T_6 and T_8 also have significantly lower final *Fusarium* population but still higher than T_4. This is consistent with other variables observed in this study that show effectivity of *T. koningii* in controlling *Fusarium* wilt singly and it appears that combining it with Benomyl fungicide or with *Bacillus* sp. lower its efficacy.

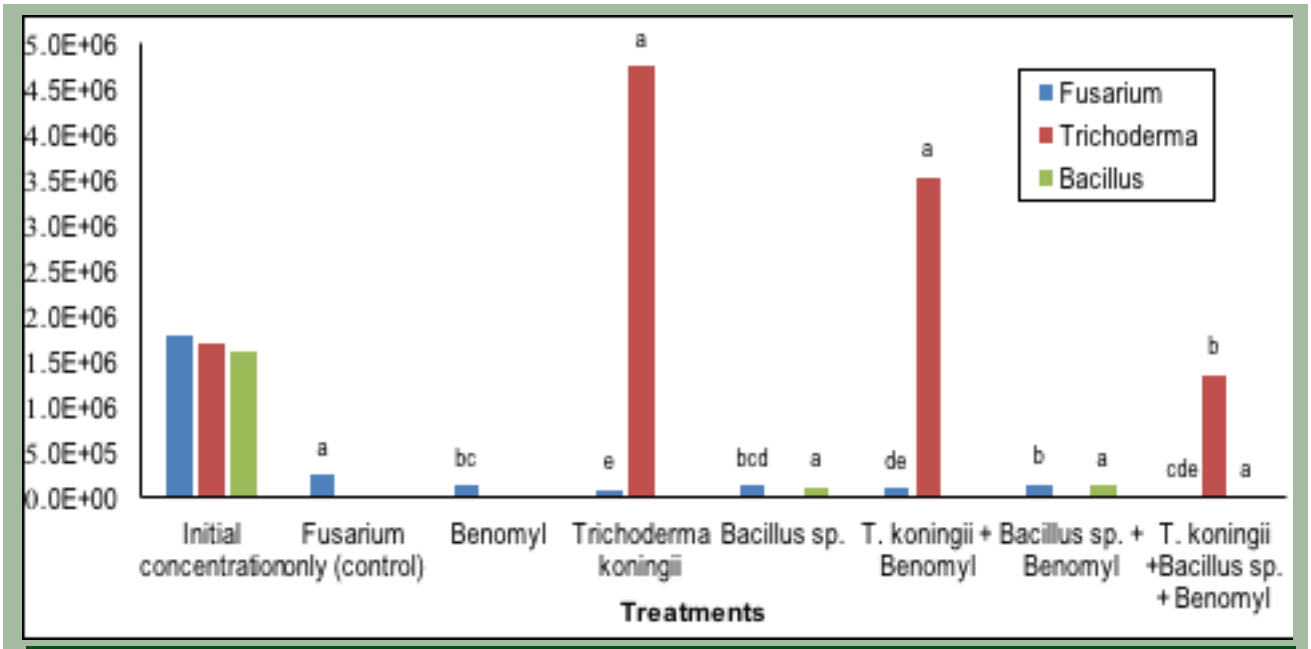

Figure 1. Initial and final populations of *Fusarium oxysporum*, *Trichoderma koningii*, *Bacillus* sp. in the study
(Means with the same letters in same colored bar are not significantly different at 5% Tukey's test)

On the other hand, *T. koningii* population increased in inoculated pots after four months of application particularly in T_4 where it is the sole inoculant with 47.5x 10^5/g soil. However in treatments with other component, *T. koningii* population was lower particularly in T_8 where it has significantly lower population at 13.6x10^5. This shows that *T. koningii* was reduced by both Benomyl and *Bacillus* sp. Lorezco (2004) and Alizadeh et al. (2007) already showed that Benomyl inhibits the growth of *T. koningii* by 11.60-13.00 mm in-vitro. On the other hand, the effect of *Bacillus* sp. could be simply a matter of competition.

Similar with *Fusarium*, the population of *Bacillus* sp. also decreased after four months of application in pots. This corroborates with the study of Weller (1983) which showed that rhizosphere population of introduced microorganisms are initially high but declines as the plant develops. It was presumed that as the plant root system expands, the introduced microorganisms are displaced or preempted by indigenous microorganisms.

Conclusions

Results of the study showed promising biocontrol activity of *Trichoderma koningii* against *Fusarium oxysporum* affecting sweetpotato. In the bioassay test, the reference fungicide Benomyl had the widest inhibition zone followed by *T. koningii* while *Bacillus* sp. has least inhibition zone against *F. oxysporum*. However in the pot experiment, *T. koningii* performed best as sole inoculant in preventing *Fusarium* wilt disease. Sweetpotatoes planted in pots inoculated with *T. koningii* only had the highest number of days before symptom of wilt appearance and lowest disease incidence, severity, and index. The major mechanism for the inhibition of *F. oxysporum* by *T. koningii* is through out competing the pathogen as seen in the final populations of the microorganisms. *Fusarium oxysporum* population was lowest in *T. koningii* inoculated soils while population *T. koningii* increased. Moreover, the result showed that *T. koningii* has better performance and thus, could be better substitute to the fungicide Benomyl.

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