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In vitro Evaluation of Different Elicitors against *Fusarium* Wilt of *Musa textilis* Nee (Abaca) caused by *Fusarium oxysporum f. sp. cubense*

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Abstract

***Fusarium* wilt caused by *Fusarium oxysporum f. sp. cubense* (Foc) is one of the most important diseases of abaca. The induction of systemic acquired resistance (SAR) in abaca plants offers a long lasting and sustainable approach in managing this disease. Pure culture of Foc isolates is inoculated into the 3-month old tissue cultured plants for pathogenicity test. Different elicitors are tested in vitro and in vivo against Foc for induction of SAR in abaca. This study was conducted in order to: evaluate the antifungal property of different potential elicitors of resistance against abaca wilt disease in vitro and determine the efficacy of each elicitor for inducing SAR in abaca for the control of abaca wilt disease. The elicitors used such as irradiated chitosan (shrimp), non-irradiated chitosan (shrimp and crabs), Salicylic acid (SA), Silicon dioxide (SiO_2), G-amino-butyric acid (GABA), and Benzothiadiazole (BTH Boost) have no antifungal property against the Foc. Both the phosphonate and benlate treatments have antifungal activity in vitro.The use of these elictors to induce SAR in abaca may be used as an alternative to the use of fungicides in *Fusarium* wilt disease.**

Keywords: anti-fungal property, *Fusarium oxysporum f. sp. cubense*, *Fusarium* **wilt disease, systemic acquired resistance (SAR)**

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1.0 Introduction

Abaca (*Musa textilis* Nee) or Manila hemp, a succulent annual plant similar to banana is an important crop in the Philippines, a country that is recognized as the biggest supplier of Abaca products worldwide (PCAARRD, 2003). Despite the high market potential of Abaca and its adaptability to the climatic condition in the tropics, it has, been attacked by many pests and diseases. *Fusarium* wilt, caused by *Fusarium oxysporum f. sp. cubense* is considered not only a serious disease of Abaca in Central America but also of banana in the Western Hemisphere and in the Philippines between 1900 and 1960 (IDCA, 1977). Infected Abaca plants do not show all the external symptoms which are usually associated with banana wilt disease. Under field conditions, the symptoms considerably are less conspicuous in abaca than in bananas.

The uncontrollable devastation caused by this disease aggravates farmers' abaca production problems. Since 1992, the Fiber Development Authority (FIDA) spent millions of pesos to help farmers rehabilitate their farms. The rehabilitation program covers the Bicol Region, Eastern Visayas and Caraga in Northeastern Mindanao (Bajet and Magnaye, 2002).

Chemical pesticides had brought efficient impact on agricultural pest control program, but several problems have emerged their uncontrolled usage. The development of resistance of the pathogen to the pesticide is just one of its drawbacks. Effective chemical pesticides to control wilt diseases are not available in the market (Boller and Felix, 2009).

Research on new natural antimicrobials is needed to meet a growing consumer demand for food without chemical preservatives (Elmer and Reglinski, 2006; Lichter et al., 2006). For example, Benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH, CGA 245704), a non-toxic, synthetic chemical is identified as a potent inducer of SAR in various crops. A derivative of benzothiadiazole (BTH) has been used to induce resistance in wheat. In tobacco and Arabidopsis, exogenous applications of BTH induced the expression of plant defense genes in a way similar to that found in pathogen-mediated SAR (Gorlach et al., 1996). Acibenzolar-S-methyl (ASM), BTH, is the first commercially available product which is systemic and substitutes for the natural SAR signal molecule salicylic acid (SA).

Systemic Acquired Resistance (SAR) induction in abaca may provide a timely and cost-effective measure against the wilt disease. Resistant varieties may be developed through conventional means in 10 to 20 years and plant genetic transformation in three to five years. However, SAR requires from 2-3 days up to 1-2 weeks to induction time to induce resistance in plants. It may offer a natural, safe, effective, persistent, and durable alternative measure to the use of pesticides in controlling abaca wilt disease caused by *F. oxysporum f. sp. cubense*. SAR induction in abaca against *F. oxysporum f. sp. cubense* has not been documented. Hence, the study aimed to determine the efficacy of different elicitors for inducing SAR in abaca for the control of abaca.

The study was conducted at the Plant Disease Diagnostic Laboratory and Tissue Culture Laboratory of Caraga State University, Ampayon, Butuan City from June 2011 to January 2012. It focused on the evaluation of the antifungal activity

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and the efficacy of different elicitors for the control of abaca wilt disease in the laboratory and screenhouse.

2.0 Research Methodology

Collection, Isolation and Pathogenicity Test of Fusarium Wilt Fungus

Abaca plants showing the characteristic symptoms of wilting were collected, examined for vascular discoloration and isolated into pure culture. The standard procedure for the isolation of fungal pathogens was followed.

A two-week old plated culture of *F. oxysporum f. sp. cubense* was used as inoculum in the pathogenicity test to ensure virulence of the pathogen. The spore suspension was standardized to 10^9 spores/mL. Inoculation of the fungus was done by pouring the spore suspension into the soil and spraying the pseudostem of a three-month old tissue cultured abaca. The treated plants were placed inside the screenhouse and given the necessary cultural care daily thereafter. Plants were observed daily until typical symptoms of *Fusarium* wilt infection appeared.

Procurement of Different Elicitors of SAR

Chemicals that had been reported to induce SAR were obtained from the Department of Pest Management and some were purchased from the chemical suppliers. Irradiated and non-irradiated Chitosan extracted from shrimps and crabs were taken from the Philippine Nuclear Research Institute, Diliman, Quezon, City. Following list of chemicals were used in the experiment: Irradiated chitosan (Ichitosan, shrimp), Salicylic acid (SA), non-irradiated chitosan, (non-ichitosan, shrimp), Silicon dioxide (SiO_2), Gamma-amino butyric acid (GABA), non-irradiated chitosan (non-ichitosan, crabs), Benzothiadiazole (BTH, Boost), Potassium phosphoric acid (Phosphonate) and Benlate (fungicide check).

Bioassay of the Different Elicitors Against Foc

About 10ml of sterile distilled water was poured on a plated culture of a two-week old *Fusarium* wilt isolate. The concentration of the prepared suspension was standardized to 10^9 spores/ml using haemacytometer.

Approximately 15ml of previously sterilized and melted potato dextrose agar (PDA) was poured into sterile petri plates and allowed to solidify. The fungal suspension (0.5ml) was pipetted and spread on the surface of plated PDA medium. Filter paper disc (5.0mm diameter) was immersed in different treatments and placed at the center of the previously inoculated plated PDA medium. The plates were incubated at room temperature. Ten treatments were arranged in Completely Randomized Design (CRD) with five replications per treatment.

Data Collection and Analysis

Data were collected 4, 8 and 12 days after inoculation (DAI). Antifungal activity was evaluated by measuring the zone of inhibition (cm). The distance between the two opposing ends of the fungal colony was measured by passing the ruler through the center of the growth. The data gathered were consolidated and subjected to Analysis of Variance (ANOVA). Treatment means were compared using Duncan Multiple Range Test (DMRT).

3.0 Results and Discussion

Pathogenicity Test of Fusarium oxysporum f. sp. cubense (Foc)

The screenhouse pathogenecity tests revealed that typical symptoms of *Fusarium* wilt caused by *Foc* was first observed in three-month old tissue-cultured plantlets two weeks after inoculation. The infected plants showed yellowing to browning of the leaves starting from the leaf margins to the midrib. Yellowing and drying of leaves started in older leaves (figure 1a-b). Wilting by *Foc* may be due to the disruption of the plants' water conducting vessels, causing yellowing which progressed from older to younger leaves when severe infection occurred. A cross-section of the pseudostem showed blackening of the xylem tissues (figure 1c-d). Distinctive brown, red or yellow lines appeared inside the pseudostem. Moreover, smaller brown streaks or flecks appeared in the corm. When infected plants were about to die, the corm and rhizome turned black and eventually decayed. These observations were similar to the report of Borines et al., (2007) on reaction of abaca accessions and varieties to *Fusarium* wilt caused by *Foc*. At the advanced stage of infection, plants eventually rot and collapse. This was caused by fungal spores which were carried upward from the corm to the pseudostem. These block the xylem tissue which serves as passage of water and nutrient from the root system to the upper parts of the plant causing the plant leaves to turn yellow.

In vitro Evaluation of Chemical Elicitors

The effects of the different elicitors on fungal growth did not vary significantly with each other but varied when compared with Phosphonate and Benlate (Table 1). The same concentration (300 ppm) of Phosphonate and Benlate significantly reduced the growth of *Foc* compared with the elicitors as shown by the clear zone of inhibition from the fourth day until the final measurement which was done on the twelveth day (figure 2). This clearly demonstrated that both chemicals have direct antifungal activity. The application of Phosphonate significantly inhibited the growth of *Foc* as shown in the reduction of the fungal colony diameter of 16.44cm on the 4th day, 17.28cm on 8th day and 16.48cm on the 12th day. The application of Benlate showed a much lower

colony diameter of 13.70cm, 13.4cm and 13.9cm on the 4th, 8th and 12th days, respectively. However, the chemical elicitors have no direct antifungal activity against *Foc* at 300 ppm concentration. In a similar study conducted by Niño (2009) and Piamonte (2011) using Chitosan, SA, SiO_2, GABA and BTH, Boost as chemical elicitors, they found no direct antimicrobial activity against bacterial blight of Abaca and rice blast disease.

In this study, only Phosphonate and Benlate showed direct antifungal activity by inhibiting the *Foc* fungal growth. Phosphonate, the anion of potassium phosphonic acid, is the active ingredient of the fungicide Foli-R-Fos, and is formed as a breakdown product from aluminium ethyl phosphonate (Aliette, Fosetyl-Al) while Benlate (benomyl), is an active ingredient of the fungicide methyl-1-butylcarbamoyl)-2-benzimidazole carbamate. Both fungicides have been proven to be useful in the control of diseases caused by species of the genera *Phytophthora* and *Plasmopara* (Coffey and Ouimette, 1989; Wicks and Hall, 1988; Cohen and Coffey, 1986; Williams et al., 1977) and *Fusarium oxysporum f. sp. melonis* (Davis et al., 1994) by inhibiting mycelial growth. Moreover, Phosphonates are known to inhibit spore formation, both in *Phytophthora* (Coffey and Joseph 1985; Farih et al., 1981) and in other *Oomycete* species (Magarey et al., 1991; Viranyi and Oros, 1991; Jailloux et al., 1987).

Table 1. Fungal growth diameter (cm) of *Foc* at 4, 8 and 12 days as affected by different elicitors at 300 ppm concentration

Treatment	Fungal Colony Diameter (cm) Days After Inoculation (DAI)		
	4	8	12
Distilled water (control)	0.00 c	0.00 c	0.00 c
Irradiated chitosan (shrimp)	0.00 c	0.00 c	0.00 c
Salicylic acid	0.00 c	0.00 c	0.00 c
Non-irradiated chitosan (shrimp)	0.00 c	0.00 c	0.00 c
Silicon dioxide	0.00 c	0.00 c	0.00 c
GABA	0.00 c	0.00 c	0.00 c
BTH, Boost	0.00 c	0.00 c	0.00 c
Non-irrradiated chitosan (crabs)	0.00 c	0.00 c	0.00 c
Phosphonate	16.44 a	17.28 a	16.48 a
Benlate (fungicide check)	13.4 b	13.7 b	13.9 b
A – Days	ns	ns	ns
B – Elicitors	**	**	**
AxB	**	**	**

** Means followed by the same letters are not significantly different at 5% DMRT
ns- No significant difference in means

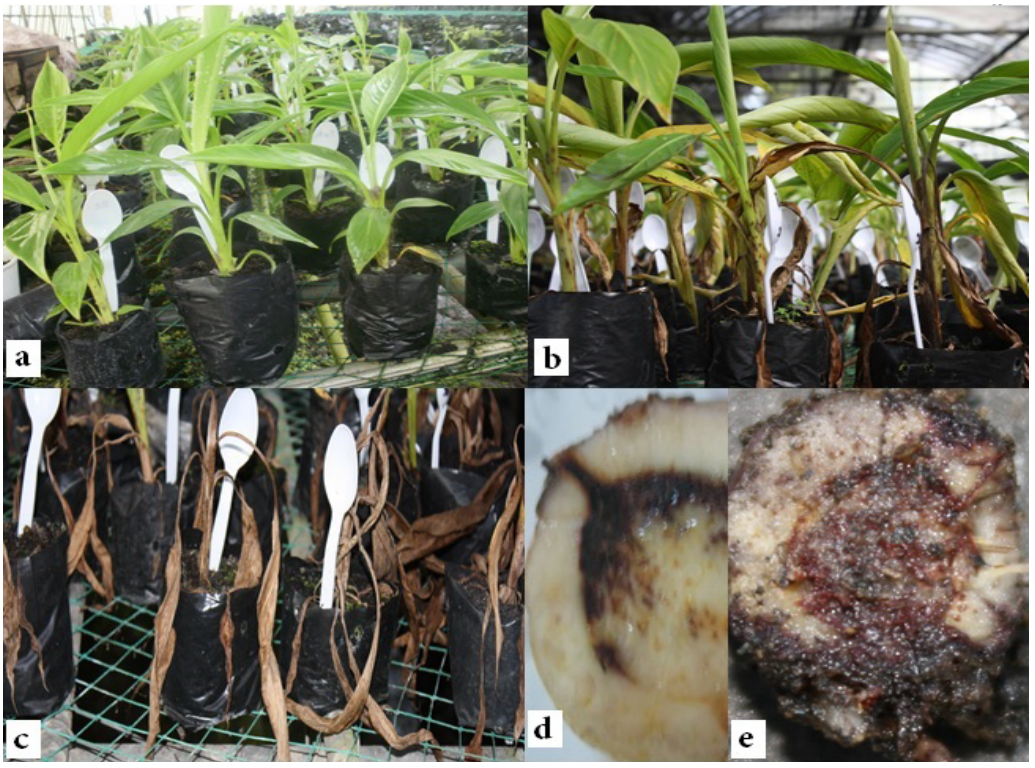

Figure 1. Three-month old tissue-culture derived plants (a) inoculated with *Foc i*solate showing the typical symptom of wilting (b-c) and lesion or browning of pseudostem (d-e)

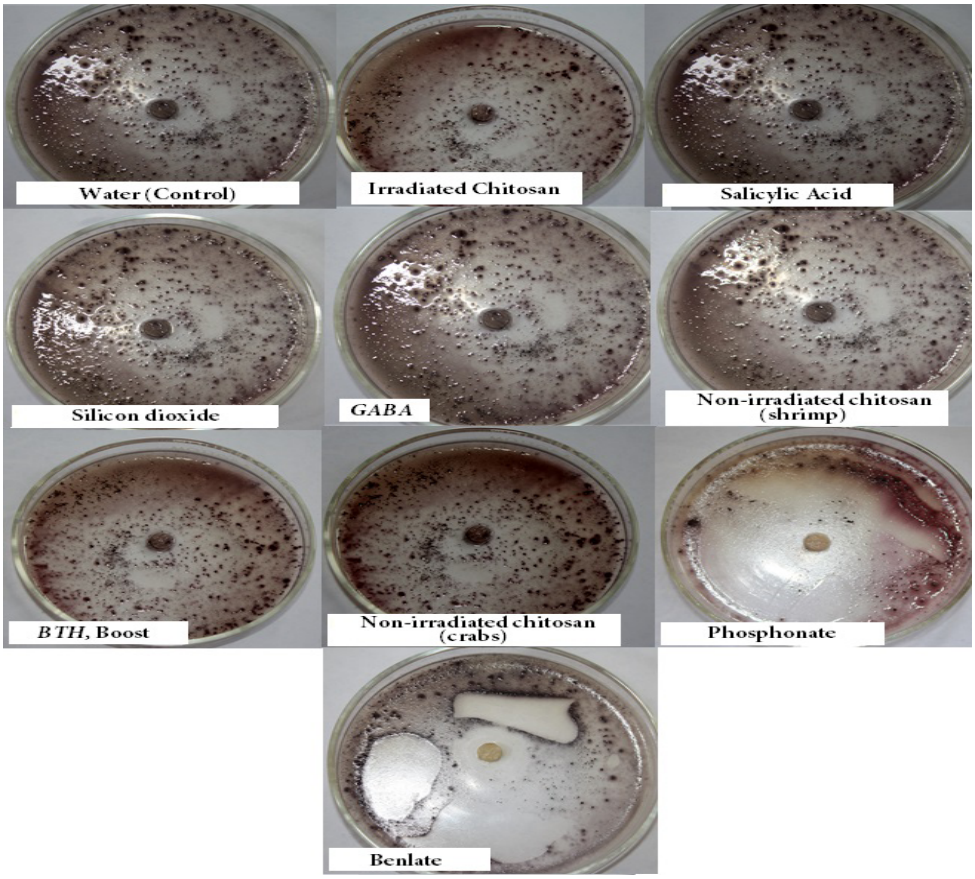

Figure 2. *Foc* isolate grown *in vitro* and applied with different elicitors at 12 days after inoculation (DAI). Note the inhibition zone in plates with Phosphonate and Benlate

4.0 Conclusion

Irradiated (shrimp), non-irradiated chitosan (shrimp and crabs), salicylic acid, silicon dioxide, GABA and BTH Boost showed no direct antifungal activity against *Foc* in vitro. The use of these elictors to induce SAR in abaca may be used as an alternative to the use of fungicides in *Fusarium* wilt disease.

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