SDSSU Multidisiciplinary Research Journal (SMRJ), Vol 6, 2018
ISSN: 2244-6990 (Print) 2408-3577 (Online)

Induction of Resistance in Abaca (*Musa textilis* Nee) Against Fusarium Wilt (*Fusarium oxysporum* f sp. *cubense)* using Elicitors

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Abstract

Different elicitors were tested in vitro and in vivo against Fusarium wilt for induction of systemic acquired resistance (SAR) in abaca. The study aimed to determine the efficacy of each elicitor for inducing SAR for the control of abaca wilt. 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. Among the elicitors evaluated for SAR induction in vivo, the irradiated chitosan (shrimp), non-irradiated chitosan (shrimp), G-amino-butyric acid (GABA), Benzothiadiazole (BTH, Boost) applied at 1 week or 3 weeks interval consistently delayed the onset of the disease, lower percent infection, reduced leaf symptom index (LSI) and rhizome discoloration index (RDI) as compared to control plants. These effects were comparable to Phosphonate and Benlate-treated plants. The results showed the potential of these elicitors as promising inducers of resistance in abaca for Fusarium wilt control. The use of irradiated and non-irradiated chitosan (shrimp) to induce SAR in abaca is cost-effective and, thus, maybe used as an alternative to the use of fungicides in Fusarium wilt disease.

Keywords*: Fusarium wilt, Fusarium oxysporum, systemic acquired resistance, elicitor*

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1.0 Introduction

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* Foc (E.F. Smith) Snyd. and Hans (*Foc*) is one of the most important diseases of abaca worldwide. Infected abaca plants do not show all the external symptoms which are usually associated with banana wilt disease (Borines et.al., 2007). 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.(PCARRD, 2007). The use of chemical pesticides had brought efficient impact on agricultural pest control program, but several problems have emerged due to 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 (Staples & Toenniessen, 1981). Research on new natural antimicrobials is needed to meet a growing consumer demand for food without chemical preservatives (Elmer & Reglinski, 2006; Litchter, 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 systemic acquired resistance (SAR) in various crops. 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), a derivative of benzothiadiazole (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 (Ortiz & Vuylsteke, 1996) and plant genetic transformation in three to five years (Aquino et.al., 2005). SAR, however, requires only an induction time from 2-3 days up to 1-2 weeks to induce resistance in plants (Percival, 2001). It may offer a natural, safe, effective and lasting alternative to the use of pesticides in controlling abaca wilt disease caused by *F. oxysporum* f. sp. c*ubense.* SAR induction in abaca against *F. oxysporum* f. sp. c*ubense* has not been documented. Hence, the study aimed to determine the efficacy of different elicitors for inducing SAR in abaca for the control of Fusarium wilt.

2.0 Materials and Methods

2.1 Bioassay of the Different Elicitors Against Foc

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 Benomyl (fungicide check) were used as treatments.

Sterile distilled water (10 ml) was poured on a plated culture of a two-week old Foc isolate. The concentration of the prepared suspension was standardized to 10^9 spores/ml. The melted potato dextrose agar (15 ml) was poured into sterile Petri plates and allowed to solidify. The fungal suspension (0.5 ml) was pipetted and spread on the surface of plated PDA medium. A 5 mm diameter sterile filter paper disc 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 was done at 4, 8 and 12 days after inoculation. Antifungal activity was evaluated by measuring the zone of inhibition (cm) at 4, 8 and 12 days after treatment. The distance between the two opposing ends of the fungal colony was measured by passing the ruler through the center of the growth.

2.2 Application of SAR Elicitors

Each elicitor (100 ml) at different concentrations was sprayed in each of the three-month old tissue cultured abaca in the screenhouse. The elicitors were applied at 1 week and 3 weeks interval. The experiment was set-up in a 3-factor factorial in Randomized Complete Block Design (RCBD) with elicitors, different concentrations and spray intervals as factors. The treatments were replicated 5 times.

2.3 Preparation of the Inoculum and Inoculation of the Pathogen

Two-week old culture of the pathogen was added with sterile water, scraped with glass rod and the fungal suspension was standardized to 10^9 spores/ml. Inoculation of the fungus was done by spraying the pseudostem as well as pouring into the soil 10 days after application of the elicitors with 10mL of 10^9 spores/mL of fungal inoculum. The plants were observed for manifestation of symptoms. The data gathered were as follow: incubation period, percent infection, disease severity index and plant height.

Disease severity index was gathered when the abaca plants were seven months old. The internal and external symptoms was rated based on the Leaf Symptom Index (LSI) and Rhizome Discoloration Index (RDI) as described by Mohammed et. al., 1999. The plant height was measured every 15 days , starting 10 days after application of each treatment.

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2.4 Data Analysis

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

3.1 *Evaluation of Antifungal Property of Different Elicitors Against the Fusarium Wilt Pathogen (Fusarium oxysporum f. sp. cubense)*

Concentration (300ppm) of Phosphonate and Benomyl reduced the growth of *Foc* compared to 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 (Fig.1). This clearly demonstrated that both chemicals have direct antifungal activity. The application of Phosphonate inhibited the growth of *Foc* as shown in the reduction of the fungal colony diameter of 16.44 cm, 17.28 cmand 16.48 cm on the 4th, 8th and 12th day, respectively.

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
Benomyl (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

3.2 Effect of Concentrations and Spray Intervals

Regardless of concentration and spray interval, incubation period which ranged from 16.40-16.60 days was observed for the non-treated plants and 26.20-34.20 days for the elicitor-treated plants (Figs.1 & 2). Elicitors like irradiated chitosan (shrimp), GABA, non-irradiated chitosan (shrimp), BTH, Boost and Phosphonate delayed the onset of the disease which were comparable with the fungicide, Benomyl.

At 1- and 3-week spray intervals, irradiated (shrimp) and non-irradiated chitosan (shrimp) consistently delayed the onset of *Foc* symptoms from 32.00-34.20 days incubation period for non-irradiated chitosan (shrimp) and 32.00-32.80 days for irradiated chitosan. The effect of both chitosans on the incubation period was comparable with the effect of Phosphonate (33 days) and Benlate (34.20 days). Both GABA and BTH, Boost were effective at 3 weeks spray interval regardless of concentration. The delay on the onset of the disease is a form of SAR in plants to pathogen invasion while short incubation period means failure to induce resistance to plants. Thus, the application of elicitors to plants can cause development of resistance to disease through the activation of multiple signalling pathways of intracellular defenses.

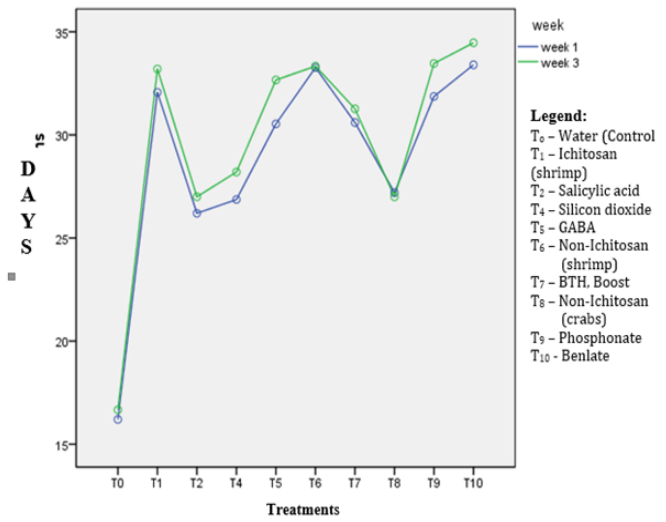

Figure 1. Mean interaction effects between elicitors and spray intervals on incubation period *Fusarium* wilt.

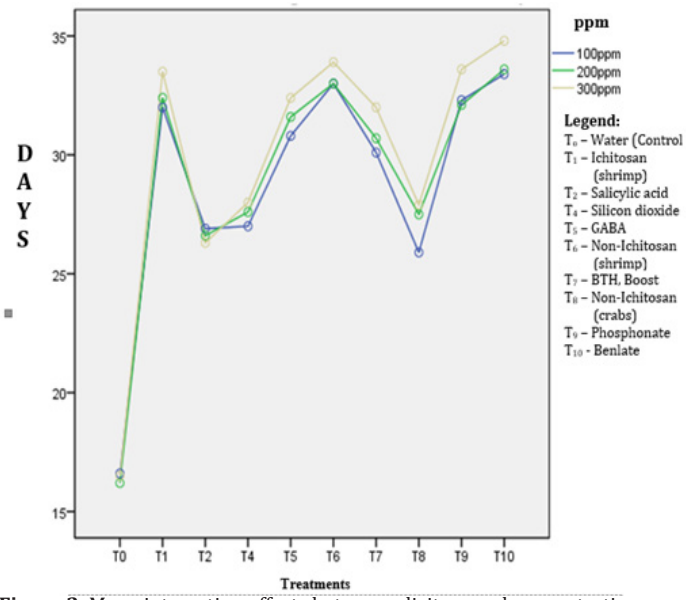

Figure 2. Mean interaction effects between elicitors and concentrations on the incubation period of *Fusarium* wilt

3.3 Percent Infection

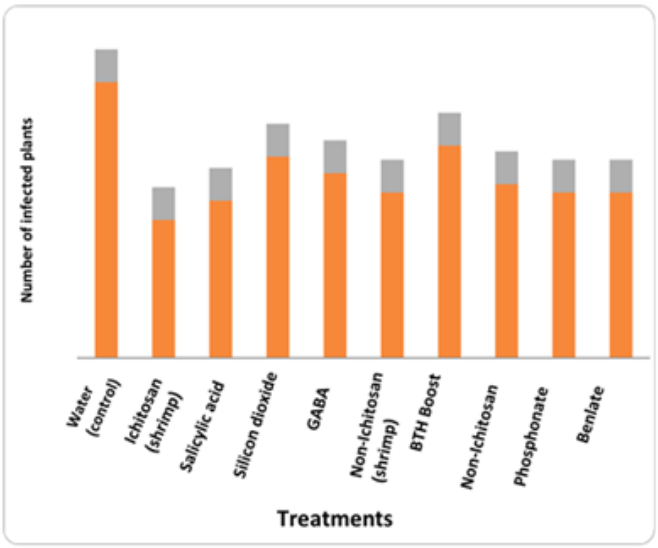

Figure 3. Number of infected plants as affected by the application of elicitors regardless of concentration and spray interval

Elicitors at the rate of 300 ppm (irradiated chitosan (shrimp), Silicon dioxide, GABA, BTH, Boost) delayed the infection up to five weeks, which is similar to the effects of Phosphonate and Benlate (Fig.3). The non-irradiated chitosan (shrimp and crabs) at all concentrations had 0% infection 5 weeks after inoculation indicating their efficacy. All the elicitors tested provided protection against the *Fusarium* wilt disease, however, *Foc* infection progressed over time starting at the 5th week after inoculation of plants. Treated and untreated plants were similarly infected on the sixth and seventh weeks. This implies that the elicitors could only provide protection to the plants for 4-5 weeks and thus, a second application is necessary. This result conformed with the findings of Stitcher et al., (1997) that induced resistance retains its efficacy for weeks or even for the whole cropping season after activation of SAR.

3.4 Effects of Concentration of Elicitors on Disease Severity Index of Foc

At 1 week interval, the mean LSI was lower in plants applied with irradiated chitosan (shrimp), Salicylic acid, Phosphonate (Figs. 4& 5). The same trend was observed at 3 week interval regardless of concentrations using irradiated chitosan (shrimp), SA, non-irradiated chitosan (shrimp) and BTH, Boost. The disease severity based on the rhizome discoloration index (RDI) was lower in all the elicitors (Table 5 & Fig. 6). Irradiated chitosan (shrimp) and SiO_2 were effective at 1and 3 weeks spray interval regardless of concentration while non-irradiated chitosan (shrimp) and GABA at 300 ppm were effective when applied at 3 weeks interval (Figs.). These results were as good as the Phosphonate and Benlate treatments. SA and BTH, Boost also in all levels of concentration RDI was observed at higher concentration (300 ppm).RDI in all levels of concentration, the infection visible. Further examination of the rhizomes revealed a slight to medium discoloration of the roots and the stellar region (Fig.).

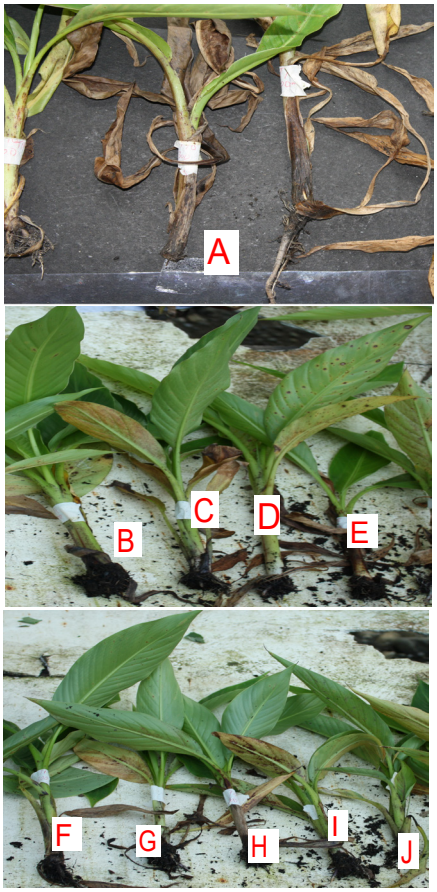

Figure 4. Disease Severity based on Leaf Symptom Index (LSI) as affected by Different Concentrations of Elicitor treatments. A- water (control), B- Irradiated chitosan (shrimp), C- GABA, D- Non-irrradiated chitosan (shrimp), E- SiO2, F- SA, G- Boost, H- Non-irradiated chitosan (crabs), I-Phosphonat and J- Benlate.

The host generally developed resistance, because application of elicitors on plant surface activated multiple signaling pathways of intracellular defense (Holopainen et al., (2009). Elicitors are very stable molecules that induce an immune defense response in plants. They have low molecular weight and synthesized as such or released from polymeric precursors during infection (Boller and Felix, 2009).

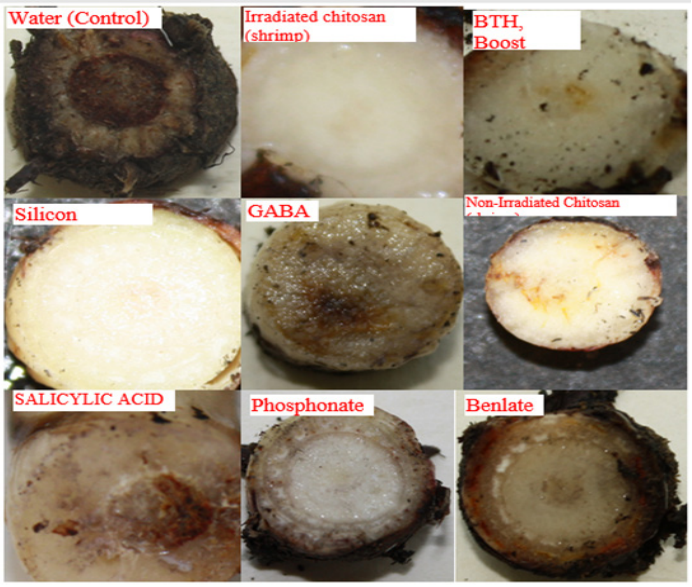

Figure 5. Disease severity based on Rhizome Discoloration Index (RDI) as affected by different concentrations of elicitor treatments.

In this study, chitosan (both irradiated and non-irradiated) was found to induce resistance in abaca and was capable of protecting the plant against the *Foc* disease comparable to Phosphonate and Benlate, both known as effective fungicides against some diseases.

4.0 Conclusion

In this study the use of 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*. These elicitors induce SAR in abaca plants against *Fusarium* wilt by delaying the onset of the disease, lowering percent infection, reducing leaf symptom index (LSI) and rhizome discoloration index (RDI) compared to untreated plants. Moreover, the application of irradiated chitosan (shrimp), non-irradiated chitosan (shrimp), GABA and BTH, Boost at 1 week or 3 weeks resulted in the delay of the onset of the disease, lower percent infection, reduce leaf symptom index (LSI) and rhizome discoloration index (RDI) as compared to control plants. The degree of protection of these elicitors was comparable to Phosphonate and Benlate-treated plants. Hence, 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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