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Influence of Biotic and Chemical Plant Inducers on Resistance of Chilli to Anthracnose

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ABSTRACT

The potential of biotic and chemical plant inducers to trigger the resistant in plant against disease was investigated. A biotic inducer as *Trichoderma harzianum* and chemical inducers as salicylic acid and Bion® were tested for their induced resistance ability to protect anthracnose disease on two tested chilli cvs. Mae Ping and Man Dum in both seedling and mature stages. The necrotic symptom development on seedling of both tested chilli varieties after treated with Bion® 0.05 mg a.i./ml was significantly lower than the others. The potential of anthracnose management on chilli cv. Man Dum was also treated by *T. harzianum* at 10^8 spore/ml and salicylic acid at 1 mM. Treatments with any plant inducer did not lead to an increase activity of polyphenol oxidase in chilli cotyledon 1, 3 and 5 day after challenge inoculation. In field experiment, the result showed that the foliar sprays with any of plant inducer reduced the infection percentage of anthracnose on chilli. Bion® also showed the best percentage of disease reduction compared to the untreated treatment at 76.3% and 75.9% on both chilli varieties, whereas in treatment of 10^8 spore/ml *T. harzianum* and 2 mM salicylic acid were reduced 24.2, 57.4% and 19.8, 36.6% respectively. However, fruit yield of Bion® treatment was very low and significantly different to control. The phytotoxicity occurred at high concentration. *T. harzianum* and salicylic acid treatment showed the fruit yield to be significantly increased in comparison with the control on chilli cv. Man Dum, but not on cv. Mae Ping. All plant inducers did not affect to fruit fly infestation on both varieties under field conditions.

Key words: plant inducers, disease resistance, anthracnose, chilli

INTRODUCTION

Anthracnose disease, caused by *Colletotrichum* spp., is an important disease of chilli (*Capsicum annuum* L.) and many other kinds of plants in tropical areas worldwide. It occurs as a pre-harvest or post-harvest fruit rot, causing extensive losses in chilli grown during the warm, wet season in tropical and subtropical climates (AVRDC, 1998). Management strategies for this disease include the use of disease-free seeds and transplants, resistant varieties, and chemical sprays. The pathogenic fungi, *C. capsici* (Syd.-1913) E. J Butler & Bisby and *C. gloeosporioides* (Penz) Penz. & Sacc. in Penz., were reported as a causal agent of chilli anthracnose in Thailand (Sangchote, 1999). They are also the main causal agent in the tropical Asia (Kim *et al*., 1989).

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The natural resistance of plants to pathogens is based on constitutive and inducible barriers. Induced resistance may be expressed locally at the infection site as well as systemically that so-called Systemic Acquired Resistance (SAR) (Sticher *et al*., 1997). SAR is a broad-spectrum resistance to pathogen includes viruses, bacteria and fungi that occurs when the plant is challenged by either a pathogen or chemical compounds such as salicylic acid (SA), jasmonic acid (JA), 2,6 dichloroisonicotinic acid (INA) and DL-3-amino-n-butanoic acid (BABA) (Kessmann *et al*., 1994; Oostendorp *et al*., 2001). Many studies showed that SA acts as an endogenous signal responsible for inducing SAR in plant (Malamy *et al*., 1990; Gaffney *et al*., 1993) and exogenous applied SA is also induced the defence in some crops (Manandhar *et al*., 1998; Murphy *et al*., 2000). A new product, Benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH), has trade name as Bion® in Europe or Actigard™ in the United States. Bion® is the first synthetic plant activator that triggers plant to elicit the identical response as biological induction of SAR (Tally *et al*., 1999). At low application rates it activates resistance in many crops against a broad spectrum of diseases, including fungi, bacteria and viruses. Exogenous application of BTH induce resistance to disease on a range of commercial crops (Cole, 1999; Godard *et al*., 1999; Romero *et al*., 2001). However, there is no report of the activity of BTH against anthracnose disease of the Thai chilli.

Apart from the above mentioned chemicals, some biotic agents also can induce disease resistance in plants. Few micro-organisms have been fully commercialized for the control of foliar plant pathogens. One of the most studied commercial biocontrol agents is *Trichoderma* spp. which can be regarded as a model to demonstrate a control of pathogenic pathogens under commercial conditions (Elad, 2000; Harman *et al*., 2004). In this study, the main objectives were to determine the efficiency of plant inducer i.e. including Bion®, salicylic acid and *T. harzianum* against the anthracnose disease of chilli under greenhouse and field conditions and their effects to yield under field conditions.

MATERIALS AND METHODS

1. Resistant efficiency determination and experimental design

1.1 Plant material

The plant material was conducted in both greenhouse and under field conditions. Two chilli varieties were used in the experiment, namely Mae Ping (Mae Ping-80, from Known You Seed Co., Ltd - Thailand) and Man Dum (Hot pepper, from Chia Tai Co., Ltd - Thailand).

1.1.1 Greenhouse experiment: Chili seeds were sown in polystyrene with mixed media containing soil, coconut husk and manure (1:1:1). Twenty-day-old seedlings with fully expanded cotyledon were tested in the greenhouse experiment.

1.1.2 Field experiment The experiment was conducted at the field of Plant Pathology Department, Kasetsart University, Kamphaeng Saen campus from April to September 2004. Plants were grown from seeds in polystyrene trays in the greenhouse for 7 to 8 weeks, and then they were transplanted to the field at the end of May 2004. Sprinkler irrigation was applied to the crop as needed to maintain the optimum growth. Each treatment consisted of two rows, with 7 plants per row. Beds were mulched by black plastic after soil preparation. The spacing between beds was 50 cm, and spacing on the beds was 40 × 35 cm. Fertilizer N-P-K (15-15-15) was applied two times 2nd week after transplanting, followed by 10 days after first application of each treatment.

1.2 Plant inducer preparation

The procedures of plant inducer preparation are shown in Figure 1.

Three plant inducers, *T. harzianum*,

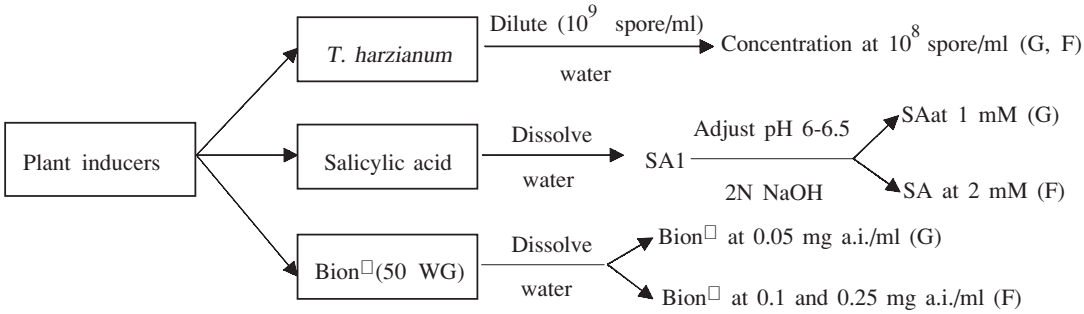

Figure 1 Diagram of plant inducer preparation (G: Greenhouse experiment; F: Field experiment).

salicylic acid and Bion® were tested in greenhouse and field conditions. They were prepared as in Figure 1. In greenhouse, inducer concentrations were prepared as follows: 1) *T. harzianum* of 10^8 spore/ml, 2) 1 mM salicylic acid and 3) Bion® (0.05 mg a.i./ml). Field experiment included 1) *T. harzianum* of 10^8 spore/ml, 2) 2 mM salicylic acid and 3) Bion® (0.25 mg a.i./ml). However, when phytotoxic symptom appeared in Bion® treatment, the concentration was then reduced to 0.1 mg a.i./ml after the fourth sprays.

1.3 Experimental design

The experiment was arranged in Randomized Complete Block Design (RCBD) with 4 treatments. Ten seedlings per treatment were considered as each replication in the greenhouse experiment and field experiments.

2. Plant inducer treatment and pathogen inoculation

2.1 Greenhouse experiment

The foliar spray on each treatment was applied as a fine mist one day before inoculation with a macerated mycelium and spore suspension of *Colletotrichum capsici.* Mycelia and spores of *C. capsici* were harvested from 10-day old culture on Potato Dextrose Agar (PDA). One droplet (15 μl) of mycelium and spore suspension of *C. capsici* was placed on the wound which was punctured by needle at the middle of leaves. After inoculation, the seedlings were covered with plastic bags to keep moisture.

2.2 Field experiment

The foliar spray of each treatment was applied as a fine mist for nine times in total from the beginning of flowering stage. About 10-day interval of each spraying times were applied before harvesting and 7-day interval within harvesting period. The plant inducers were applied in the late afternoon. The disease incidence of anthracnose under natural infection was observed.

3. Polyphenol oxidase activity on cotyledon

Twenty-day-old seedlings were treated with plant inducers one day before inoculation by pathogen. Polyphenol oxidase (PPO) enzyme activity was checked 1, 3 and 5 days after inoculation. Cotyledons were homogenized in mortar using 5 ml of 0.1 M phosphate buffer (PBS), pH 6.5. Thereafter, the homogenates were centrifuged at 7,500 rpm, for 10 min. at 4°C. The supernatant (crude enzyme extract) was collected and kept in refrigerator at – 20°C. The reaction mixture consisted of 1 ml of catechol 0.006 M, 1 ml of 0.1 M phosphate buffer (pH 6.5) and 3 ml sterilized water. Enzyme extract (200μl) was then added. PPO activity was determined by measuring with UV spectrophotometer at wavelength 310 nm after 30 min of incubation.

4. Data collection and statistical analyses

Disease incidence was observed under greenhouse and field conditions throughout the period of plantation. In greenhouse experiment, the disease severity of anthracnose on cotyledon was assessed based on the areas of necrotic symptom during 3 to 7 days after inoculation.

The disease incidence of anthracnose in the field was expressed as the percentage of infected fruit per total fruits in each harvesting time. Total of disease incidence, fruit yield, weight of fruit and fruit fly infestation were inspected until the last harvesting time. Some other diseases were also observed in the field. The first harvest was started after the fourth spray of plant inducers. Ripened fruits were harvested weekly for 8 times on cv. Mae Ping and 7 times on cv. Man Dum. Fruit yield was calculated of 10 plants from each treatment. Data were analysed by analysis of variance (Proc ANOVA in SAS program).

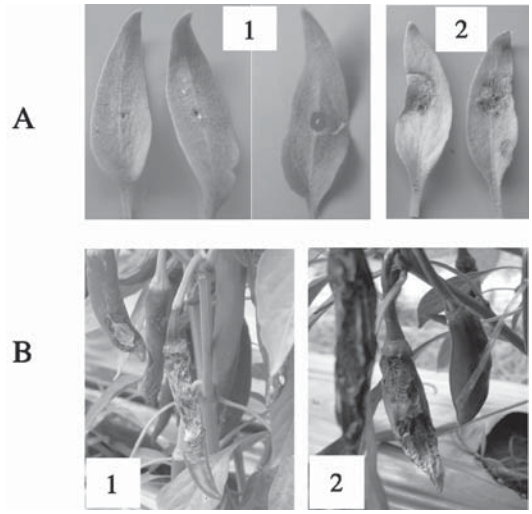

Figure 2 Various symptoms of anthracnose disease appeared on cotyledon from 3 to 7 days after inoculation of Bion® treatment (A1) and untreated control (A2). Symptom of anthracnose disease on chilli fruit (B) in the field of cvs. Mae Ping (B1) and Man Dum (B2)**.**

RESULTS

1. Symptom of anthracnose disease observation and evaluation

In the greenhouse, initial symptoms appeared on cotyledon as small halo around inoculated point from 3 days after inoculation. The disease symptom varied from minimum level to maximum level. It was clear that cotyledons treated with Bion® showed less area of necrotic symptom than the others (Figure 2A). Under field conditions, it was noted that the symptom on cv. Mae Ping occurred mostly on ripened chilli fruits, whereas symptoms were found on both ripened and unripened chilli fruits on Man Dum variety (Figure 2B).

2. Plant inducers and disease control on cotyledon

The plant inducers as *T. harzianum*, salicylic acid and Bion® were tested on induced resistant ability to anthracnose disease in chilli seedling. In Figure 3, the disease severity was expressed through areas of necrotic symptom on cotyledon 7 days after inoculation. The treatment of Bion® expressed the smallest area of necrotic symptom in both varieties compared to the untreated control. The percentage of inhibition in each treatment was calculated based on data of the untreated control. It was found that Bion® showed the highest activity of inhibition in the variety of Mae Ping and Man Dum. Figure 3B shows that percentages of inhibition in the treatment of Bion® in Mae Ping and Man Dum were 71.3 and 84.3 %, respectively.

3. PPO enzyme activity on cotyledon

Chilli seedlings were treated with plant inducers one day before inoculated with a macerated mycelium of *C. capsici*. PPO enzyme activity was determined 1, 3 and 5 days after inoculation (Figure 4). The PPO enzyme activity

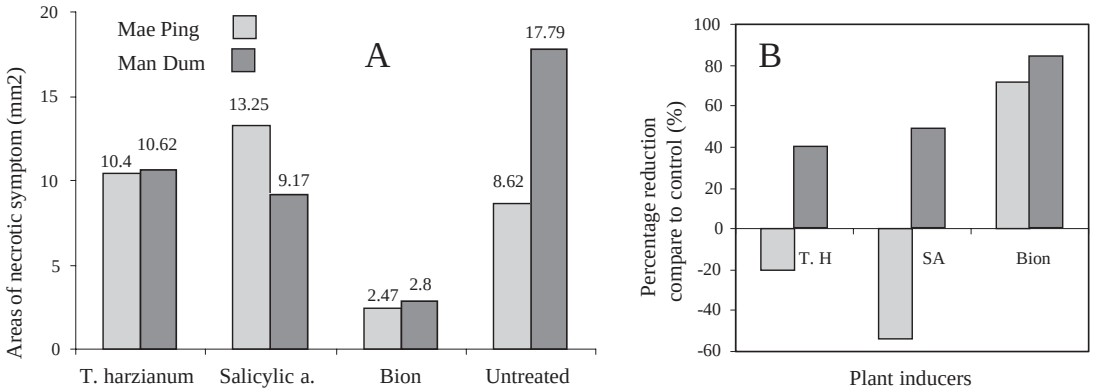

Figure 3 Areas of necrotic symptom (mm^2) on chilli cotyledon after 7 day after inoculation (A). Seedlings were treated with plant inducer as *T. harzianum*, salicylic acid, Bion® and untreated control before inoculation with spore and macerated fungal mycelium suspension of *C. capsici*. Percent reduction of inducer treatment compared to the control (B).

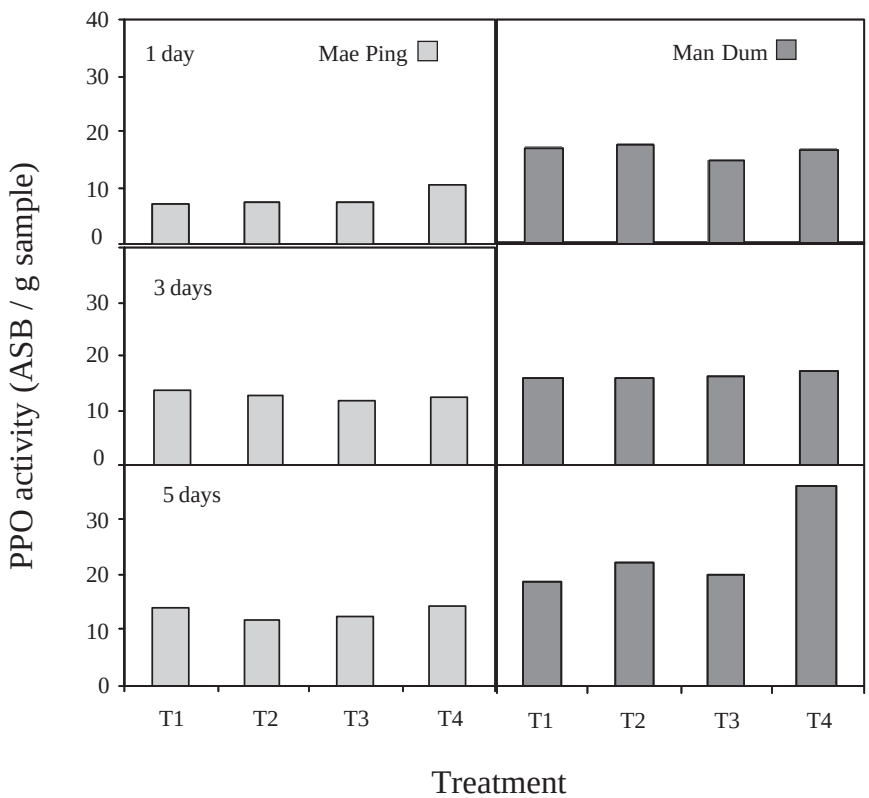

Figure 4 Effects of plant inducer as *T. harzianum* (T1), salicylic acid (T2), Bion® (T3), and the untreated control (T4) on PPO enzyme activity in cotyledon of chilli cvs. Mae Ping and Man Dum 1, 3, 5 days after inoculation with *C. capsici.*

was not significantly different within the treatments of plant inducer compared to the untreated control in both chilli varieties. However, the level of PPO enzyme activity was different between two chilli varieties in the same treatment of each time interval. PPO activity on cv. Man Dum was higher than that on cv. Mae Ping. In chilli cv. Man Dum, enzyme activity also increased over time from 1 to 5 days after inoculation, whereas on cv. Mae Ping enzyme activity did not change.

4. The effect of plant inducers on anthracnose disease under field conditions

The biotic and chemical plant inducers including *T. harzianum*, salicylic acid and Bion® were tested on their inducible ability of anthracnose resistance on chilli under field conditions.

Percentages of disease incidence among plant inducers in both varieties are shown in Figure 5. The results showed that foliar sprays with any of plant inducers reduced anthracnose disease in each harvesting time, especially in treatment with Bion®. The highest disease incidence was about 80% in the untreated control at the first harvesting time from both tested chilli varieties. The trend of disease incidence along the harvesting time was similar in both varieties. However, disease incidence on cv. Mae Ping was higher than that on cv. Man Dum. It was noted that after spraying stopped, the disease incidence increase in Mae Ping variety whereas the incidence seemed to sharply decreased in Man Dum variety.

The effect of plant inducers on fruit anthracnose disease, fruit yield, weight of fruit and insect infestation are shown in Table 1. Among three plant inducers, Bion® provided the best percentage of reduction of disease on both varieties (76.3 and 75.9%) compared to the control, followed by *T. harzianum* (24.2 and 57.4%) and salicylic acid (19.8 and 36.6%). However, certain phytotoxic effects resulted from Bion® application was district at high concentration (0.25 mg a.i./ml). Treated plant leaves became smaller, curved, and brittle. After phytotoxic symptom on leaves was observed, the concentration of Bion® was reduced to 0.1 mg a.i./ml, thereafter no phytotoxicity occurred, plants produced more flowers and fruits resulting in fruit yield especially on chilli cv. Mae Ping variety (Figure 5).

Apart from anthracnose, some other diseases and fruit fly infestation were also recorded. The result showed that all plant inducers were not relevant to control fruit fly infestation (Table 1). Some other diseases were observed in the field such as *Choanephora* blight caused by *Choanephora cucurbitarum*, collar rot caused by *Sclerotium rolfsii* and virus.

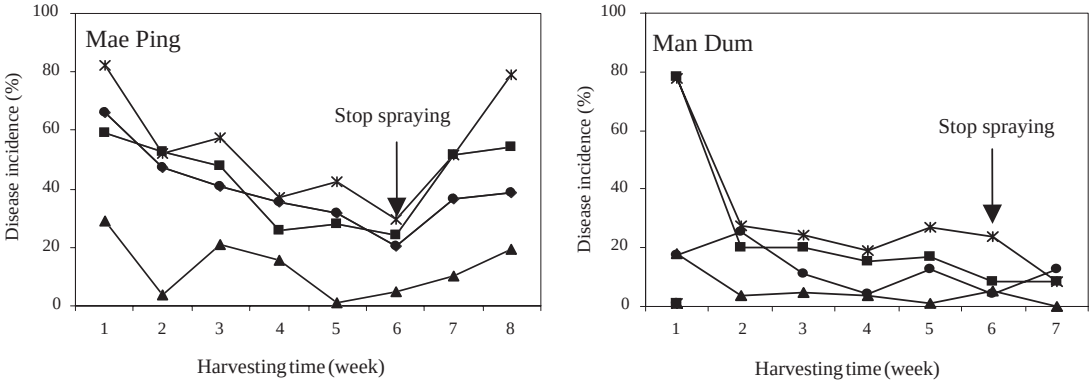

Figure 5 Disease incidence of chilli fruit anthracnose in each harvesting time on chilli cvs. Mae Ping and Man Dum treated by *Trichoderma harzianum* (—●—), salicylic acid (—■—), Bion® (—▲—) and control (—*—).

5. Effects of plant inducer on fruit yield of chilli under field conditions

The result of fruit yields of all treatments in both varieties is presented in Figure 6. Fruit yields of inducer treatment were not significantly higher than the untreated control in each harvesting time in both chilli varieties, except treatment with Bion®. It was noted that after five weeks of harvesting time, the peak of Bion® treatment in cv. Mae Ping increased sharply. However, the total fruit yield of Bion® treatments was significant lower than the others. Treatment with *T. harzianum*

Table 1 Effects of plant inducer on fruit anthracnose disease and the relation between disease incidence and fruit yield of chilli cvs. Mae Ping and Man Dum under field conditions.

Treatment	Disease% incidence[1/] (%)	Reduction of disease compared to control	Fruit yield (g / plot)[1/]	Weight of healthy fruit[2/] (g)	Fruit weight (g/fruit)[1/] (%)	Insect incidence[1/]
Mae Ping variety						
T. harzianum	38.1 a	24.2	4883.6 a	3022.9	9.8 a	10.4
Salicylic acid	40.3 a	19.8	4532.1 a	2705.6	10.1 a	10.0
Bion®	11.9 b	76.3	3092.9 b	2724.8	6.9 b	11.2
Untreated control	50.3 a	-	4492.1 a	2232.5	9.6 a	9.8
CV (%)	12.6		9.6	19.8	6.6	16.8
F-test	**		*	ns	*	ns
Man Dum variety						
T. harzianum	11.5 bc	57.4	3298.7 a	2919.4	5.4	8.2
Salicylic acid	17.1 b	36.6	2684.5 b	2225.5	5.8	7.4
Bion®	6.5 c	75.9	780.0 c	729.3	4.8	8.3
Untreated control	27.0 a	-	2533.7 b	1849.6	5.9	6.3
CV (%)	17.6		6.0	10.9	4.5	16.6
F-test	**		**	**	ns	ns

1/ Data (X) were collected until the last harvesting time and transformed to square root $\sqrt{x + 0.5}$ before analysis. Means of nontransformed data were presented for clarity. Values in each column followed by the same letter were not significantly difference ($P \leq 0.05$).

2/ Weight of healthy fruit was calculated based on percentages of uninfected fruit per total fruit yield.

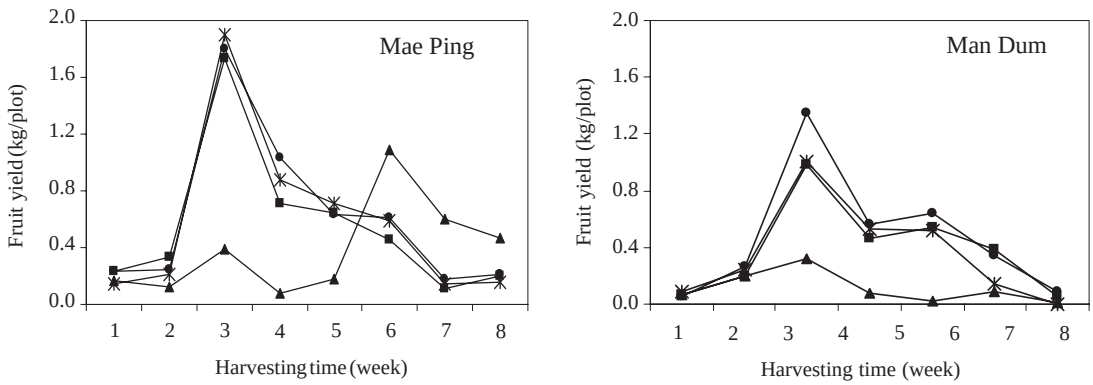

Figure 6 Fruit yield of chilli at each harvesting time on cvs. Mae Ping and Man Dum applied by *T. harzianum* (—●—), salicylic acid (—■—), Bion® (—▲—) and control (—*—).

showed the highest yield compared to the untreated control on cv. Man Dum (Table 1). In Mae Ping variety, the fruit yields and weights of healthy fruit of inducer treatment were not significantly higher than that of the untreated control. However, *T. harzianum* treatment also showed the potential for higher yield than the others. Fruit weight was not significantly increased among treatments and the control in each variety, but there was difference between two varieties. The fruit weight of chilli cv. Mae Ping was higher than that of cv. Man Dum. Therefore the total fruit yield of chilli cv. Mae Ping was also higher than that of cv. Man Dum.

DISCUSSION

In this research, from field trial all tested plant inducers induced the disease resistance of chilli against anthracnose when applied as foliar sprays. Among three inducers, Bion® showed the best activity in controlling anthracnose compared to the untreated control, followed by *T. harzianum* and salicylic acid. Many reports demonstrated the efficiency of Bion® to control plant disease caused by plant pathogenic fungi, bacteria and viruses in various of plant species (Görlach *et al*., 1996; Tally *et al*., 1999; Cole, 1999). Bion® also activates a very wide spectrum of resistance under practical field condition e.g. on some vegetable and fruit crops (Tally *et al*., 1999). However, in this study the fruit yield of Bion® treatment was reduced compared to the others. Phytotoxicity was observed in Bion® treatment at high concentration (0.25 mg a.i./ml) that caused direct effect on the growth of chilli. After concentration was reduced to 0.1 mg a.i./ml, there was no phytotoxic symptom and plants produced more flower and fruit setting, especially on cv. Mae Ping. Hence, further studies will be needed to determine the optimal growth stage for application with the best rate and a method of suitable application. The optimization of these factors will lead to the development of a reliable and effective control against anthracnose infection under field conditions.

Apart from Bion®, *T. harzianum* and salicylic acid also have potential in management of anthracnose in chilli under field conditions. An activation of the natural plant defense system has been shown to occur upon exogenous application of salicylic acid (Manandhar *et al.*, 1998; Malamy *et al*., 1990) and *T. harzianum* (De Mayer *et al.*, 1998; Elad, 2000). The first clear demonstration of induced resistance by *Trichoderma* sp. showed that treating soil with *T. harzianum* made leaves of bean plants resistance to disease that caused by *Botrytis cinerea* and *Colletotrichum lindemuthianum*, even *Trichoderma* sp. was present only on the root and not on the foliar (Harman *et al.*, 2004).

Disease incidence on chilli cv. Man Dum was lower than those on Mae Ping. It might suggest that Man Dum variety contained constitutive genes relevant to resistant response. Therefore, the less effect of inducers on disease resistance was found in this variety. The inducible ability of inducers on chilli cv. Mae Ping could be more consistent and reliable than that on cv. Man Dum under field conditions.

The results in greenhouse showed that induced resistance abilities by plant inducers were different among plant inducers in each variety and between two chilli varieties. All plant inducers could induce disease resistance to anthracnose in cv. Man Dum, whereas only treatment with Bion® could induce resistance to anthracnose in cv. Mae Ping. Among plant inducers, Bion® showed the best controlling to anthracnose in both tested varieties. This was similar to the result in the field experiment. Tally *et al*. (1999) pointed out that Bion® turned on various genes in plants which in turn produced specific proteins and enzymes. Many plant enzymes are involved in defense reactions against plant pathogens including oxidative enzyme such as polyphenol oxidase (PPO), peroxidase (PO) and other enzymes such as chitinase, β-1,3-glucanase and phenylalanine

ammonia lyase (PAL) (Avdiushko *et al*., 1993). These enzymes were reported to increase in plant which were treated with abiotic and biotic inducers in cucumber and tobacco plant. However, no clear relationships were found between increases in specific enzyme activities and the induction of resistance (Schneider and Ullrich, 1994). This suggested that resistance was not due to a particular enzyme but that the whole group could be responsible for defence. In current study, the results indicated that treatment with plant inducers did not significantly enhance the PPO activity on chilli cotyledon compared to the untreated control. This might suggest that PPO activity in tested chilli was not related to induce resistance treated by plant inducers. Further study for the relevant enzymes should be continued for experimentation.

CONCLUSION

Among tested plant inducers, Bion® expressed the best activity for controlling anthracnose in both greenhouse and field conditions on two chilli cvs. Mae Ping and Man Dum. However, the effect of plant inducer on controlling anthracnose showed difference between greenhouse and field condition within the two tested varieties. Polyphenol oxidase enzyme activity in chilli cotyledon was not clearly affected by the tested plant inducers.

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