Efficacy of *Stephania pierrei* Tuber Extract for Leaf Spot Disease Control in Greenhouse and Field Condition

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Abstract:

This study evaluated the efficacy of *Stephania pierrei* tuber extract for control of leaf spot disease in two cultivars of tomatoes and chili under greenhouse and field condition. The experiment was conducted Both experiments were conducted in Completely Randomized Design with 3 replications. Treatments included 1) control, 2) fungal, 3) *Stephania pierrei* tuber extract, 4) fungicide (mancozeb), 5) fungal with *Stephania pierrei* tuber extract and 6) fungal with fungicide (mancozeb). The results showed that tomatoes plants treated with the fungal with *Stephania pierrei* tuber extract decreased the disease severity to 8.0% and 3.8% in Puang-chom-poo and Sida cultivar, respectively after prevention but not differed from chemical fungicide under greenhouse and field condition. The efficacy of extracts depends on plant genotype, plant species and environmental factors. Further studies are needed to find out the way to use under conventional condition.

Keywords: Tomato, Leaf spot disease, *Stephania pierrei*

Introduction

The plants of the genus Stephania (Family: Menispermaceae) are slender climbers with peltate and membranous leaves. The inflorescences are axillary and arising from old leafless stem. The plants in this family are mostly herbs or shrubs but rarely trees. They are widely distributed and are traditionally used for the treatment of various ailments such as asthma, tuberculosis, fever, dysentery, hyperglycemia, cancer, and malaria. The biological activities of their extraction were found such as antimicrobial activity (fungi, bacteria), anti-malarial activity, anthelmintic activity, anti-viral activity and anti-inflammatory and analgesic activity. The extracts were used from tubers, stem or flowers [1].

Fungi are the main pathogen in plant causing developmental stage, fruit quality and yield. *Alternaria alternata* causes black spot in many fruits and vegetables around the world [2]. Fungi pathogen is usually controlled by fungicides which harmful effects on human health and environment. Therefore, the plant extracts/products are interested as the alternative control. Many plant extracts are used for against plant pathogen (fungi, bacteria or virus) or insects. The extracts of tuba root and clove can inhibit growth of *Alternaria* sp. causal agent of leaf spot disease of lettuce under hydroponics systems [3]. Many medicinal plant extracts were investigated the antifungal activity for control *Alternaria alternata* in laboratory. Minimal inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined ranging between 1.25-25 µg/mL and 1.25-10 µg/mL, respectively. The extracts of *Salvia sclarea*, *Salvia officinalis* and *Rosmarinus officinalis* had potential for treating diseases in plant [4].

Previously, we found that the minimum fungicidal concentration (MFC) was 4 mg/ml of *Stephania pierrei* tuber extract, while Mancozeb® (chemical fungicide) was 0.125 mg/ml in laboratory scale. The present study tested the antifungal activity of *Stephania pierrei* tuber extract under greenhouse and field condition

Materials and methods

Plant material culture and experiment design

The experiments were carried out in the greenhouse and field at Burapha University, Sakaeo campus, Sakaeo. Both experiments were conducted in Completely Randomized Design with 3 replications (10 plants/replication). Treatments included control (T1), fungal (T2), *Stephania pierrei* tuber extract (T3), fungicide (mancozeb) (T4), fungal with *Stephania pierrei* tuber extract (T5) and fungal with fungicide (0.125 mg/ml of mancozeb) (T6). Seeding of Tomato (Puang-chom-poo cultivar; PCP and Sida cultivar; SiD) and chili (Prik Num cultivar; PN) in tray for 3 weeks and then transplanted to plastic bag for greenhouse experiment and to soil for field experiment. At 1 month after transplanting, plants were treated according to the experimental design in both experiments.

***Alternaria* inoculation**

Alternaria alternata TISTR 3435 strain was obtained from the Thailand Institute of Scientific and Technological Research (TISTR). Fungal suspension was prepared according to Kamonwannasit *et al.* [5]. *Alternaria alternata* was grown in potato dextrose agar (PDA) plates for 7 days at room temperature. After that, the cultured plates were washed with sterile distilled water. The spore suspension was transferred into sterilized 250 ml flask for use for inoculum after its concentration of 10^5 spores/ml. The tomato leaves were inoculated with *Alternaria alternata* suspension according to the experimental design in both experiments.

***Stephania pierrei* tuber extraction**

Stephania pierrei tuber extract was prepared according to Kamonwannasit *et al.* [5] to 2 mg/ml in 1% of dimethyl sulfoxide (DMSO). The tubers of *Stephania pierrei* were extracted by soxhlet apparatus using chloroform as solvent. The tuber extract was evaporated using rotary evaporator and kept at 4°C. The extract was dissolved in 1% of dimethyl sulfoxide. After ten days of fungal inoculation the disease symptom appeared. Then the *Stephania pierrei* tuber extract and fungicide were used according to experimental design.

Disease incidence

Disease incidence was recorded on a scale of 0 – 5 in accordance to the degree of leaf spot. Scale zero refers to not suffer any leaf spot symptoms and scale 5 indicates the disease severity with the >50% of spot leaves.

$$\text{Disease severity} = [\text{Sum of all disease rating}/\text{Total number of rating} \times \text{Maximum disease grade}] \times 100$$

Disease severity of leaf spot was rating at two times; first time at 10 days after fungal inoculation and second time at 7 days after plant extract or fungicide prevention. Data between before and after prevention were compared by using Pair t-test and were analyzed by one-way ANOVA (analysis of variance) test. Pairwise comparison was carried out with Duncan's Multiple Range Test.

Results and discussion

Table 1 Mean of leaf spot disease severity (%) on plant leaves under greenhouse and field condition before and after prevention.

Type	Times	T1	T2	T3	T4	T5	T6	P-value
				Greenhouse condition				
PCP	After	4.89c	10.44ab	8.00bc	6.67bc	13.78a	7.33bc	*
	Before	5.56c	10.22abc	6.89bc	8.44abc	11.78a	11.33ab	*
	Pair t-test	ns	ns	ns	ns	ns	ns	
SiD	After	7.33	7.78	6.00	6.89	7.56	5.33	ns
	Before	5.56	14.89	10.67	13.11	12.00	13.33	ns
	Pair t-test	**	*	ns	ns	ns	*	
PN	After	1.11	2.44	2.89	2.22	3.56	3.56	ns
	Before	1.78	4.67	2.00	2.22	5.11	4.89	ns

	Pair t-test	ns	ns	ns	ns	ns	ns	
				Field condition				
PCP	After	7.56	11.78	6.00	9.78	8.00	7.78	ns
	Before	2.44b	9.78ab	4.67b	8.89ab	14.67a	6.22ab	***
	Pair t-test	*	ns	ns	ns	*	ns	
SiD	After	7.11	7.11	5.56	7.33	3.78	4.22	ns
	Before	6.44	9.33	11.33	8.22	11.33	11.78	ns
	Pair t-test	ns	ns	ns	ns	*	*	
PN	After	3.56	5.11	4.89	2.44	5.33	4.00	ns
	Before	2.89	4.44	2.67	2.67	5.78	4.89	ns
	Pair t-test	ns	ns	ns	ns	ns	ns	

The same letter in a row are not significantly different according to Duncan's Multiple Range Test

Table 1 showed the mean of leaf spot disease severity on plant leaves under greenhouse and field condition before and after prevention. Under greenhouse condition, the leaf spot disease severity of Puang-chom-poo cultivar showed a significant difference between treatments both at before and after prevention but there was no difference in another tomato cultivar and chili. And under field condition, there was only a significant difference of leaf spot disease severity between treatments in Puang-chom-poo cultivar at before prevention. This susceptibility to environment and fungal inoculation might depended on genotype of plants.

The treatment of fungal with *Stephania pierrei* tuber extract (T5) showed a significant difference of disease severity between before and after prevention in both genotypes under field condition. It determined that *Stephania pierrei* tuber extract could reduce disease symptom comparison to before prevention. The efficacy of plant extracts appeared only under field condition due to weather condition (moisture and temperature) of greenhouse might be suitable for fungi growth [6]. While the Sida cultivar showed a significant difference between before and after prevention on fungal with fungicide treatment (T6) in greenhouse and field condition. It determined that chemical fungicide effected in some species and some cultivars.

Considering Sida cultivar under field condition, the plant extract reduced disease severity 7.55% while chemical fungicide reduced 7.56% which efficacy of plant extract was similarly to chemical fungicide. However, the potential of plant extracts was not widely controlled the leaf spot disease in all species and all genotypes under greenhouse and field condition. Further studies are needed to find out the way to use under conventional condition.

Conclusions

The results of this work showed that efficiency of *Stephania pierrei* tubers extracts depends on plant species and environmental factors. Nevertheless, the natural plant-derived fungicide is still an alternative instead of chemical fungicide for organic agriculture.

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