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Acute and Chronic Effect of Biopesticide on Infestation of Whitefly *Bemisia tabaci* (Gennadius) on the Culantro Cultivation

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***Abstract*—**Acute and chronic effects of biopesticide from entomopathogenic nematode (*Steinernema thailandensis* n. sp.), bacteria ISR (*Pseudomonas fluorescens*), wood vinegar and fermented organic substances from plants: (neem *Azadirachta indica* + citronella grass *Cymbopogon nardus* Rendle + bitter bush *Chromolaena odorata* L.) were tested on culantro (*Eryngium foetidum* L.). The biopesticide was investigated for infestation reduction of the major insect pest whitefly (*Bemisia tabaci* (Gennadius)). The experimental plots were located at a farm in Nakhon Sawan Province, Thailand. This study was undertaken during the drought season (late November to May). Effectiveness of the treatment was evaluated in terms of acute and chronic effect. The populations of whitefly were observed and recorded every hour up to 3 hours with insect nets and yellow sticky traps after the treatments were applied for the acute effect. The results showed that bacteria ISR had the highest effectiveness for controlling whitefly infestation on culantro; the whitefly numbers on insect nets were 12.5, 10.0 and 7.5 after 1 hr, 2 hr, and 3 hr, respectively while the whitefly on yellow sticky traps showed 15.0, 10.0 and 10.0 after 1 hr, 2 hr, and 3 hr, respectively. For chronic effect, the whitefly was continuously collected and recorded at weekly intervals; the result showed that treatment of bacteria ISR found the average whitefly numbers only 8.06 and 11.0 on insect nets and sticky traps respectively, followed by treatment of nematode where the average whitefly was 9.87 and 11.43 on the insect nets and sticky traps, respectively. In addition, the minor insect pests were also observed and collected. The biopesticide influenced the reduction number of minor insect pests (red spider mites, beet armyworm, short-horned grasshopper, pygmy locusts, etc.) with only a few found on the culantro cultivation.

***Keywords*—**Whitefly (*Bemisia tabaci* Gennadius), Culantro (*Eryngium foetidum* L.), Entomopathogenic nematode (*Steinernema thailandensis* n. sp.), Bacteria ISR (*Pseudomonas fluorescens*), wood vinegar, fermented organic substances.

I. Introduction

CULANTRO (*Eryngium foetidum* L.) is one of the tropical herbs, belonging to the family Apiaceae [1]. In Thailand, it is known as phak chi farang. It is a popular green herb prized for the serrate, spatulate-shaped leaves that are used in many culinary dishes [2]. It is mainly cultivated for its leaves as a condiment and for its essential oils. The leaves and roots are used to stimulate appetite, improve digestion, combat colic, soothe stomach pains, eliminate gases and also as an aphrodisiac [1]. However, whitefly is a serious pest of *Eryngium foetidum* L. cultivation and causes crop damage and lost yields. The outbreak of whiteflies on the crop has resulted in overuse of conventional insecticidal control and the development and use of new insecticide chemistries over the past 20 years. Hence, whitefly has developed resistance to numerous insecticides throughout the world, and they are now unable to control this pest [3].

For over 100 years, whitefly, *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), has been one of the most important pests of agricultural crops worldwide as well as in greenhouse production [4]. Whitefly is widely distributed throughout the tropics and subtropics [5]. It causes crop damage and lost yields in fields, vegetables, and ornamental crop production. It is a vector of numerous plant viruses and also reduces crop production by direct feeding [6]. *B. tabaci* was readily controlled with insecticides in field and glasshouse situations. Therefore, problems with its effective control on many crops are now being experienced worldwide due to insecticide resistance. Population outbreaks of *B. tabaci* and *B. tabaci*-transmitted viruses have become a limiting factor in the production of many crops in many parts of the world [3], [6].

Bacteria ISR (induced systemic resistance) to *Pseudomonas fluorescens* SP007s use as a biocontrol agent in protecting various plants from several diseases caused by bacteria and fungi has been reported for multiple studies [7]. The beneficial bacteria can be a significant component of management practices to achieve the attainable yield. The phenomenon called ISR which regulates through the activation of multiple compounds at sites distant from the point of pathogen attack has been reported [8]. Biocontrol mechanisms by this PGPR strain SP007s revealed antibiosis, inducing systemic resistance of plants [7]. Recently, [9] the efficacy of *P. fluorescens* SP007s in two formulations against dirty panicle disease of rice was reported.

Entomopathogenic nematodes were reported to be effective against sweet potato weevil *Cylas puncticollis* Boheman [10] and many other crop pests, particularly those found in soil inter-phase and cryptic habitats [11], [12]. Entomopathogenic nematodes (*Steinernema thailandensis* n. sp.) seem to be the organisms with the greatest potential for practical biological suppression of sweet potato weevil on organic cultivation of sweet potato [13].

Wood vinegar is suitable for organic farming; it showed

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high efficiency as an insect repellent and had the highest efficacy in reducing pest infestation on soybean [14]. The fermented organic substance from plants is a dynamic practice employed by farmers who need to promote pest control using local plants; it is an effective method for increasing growth and yields of soybean as well [14].

The aim of this study was to investigate the effectiveness in terms of acute and chronic effect of biopesicides from wood vinegar, fermented organic substances from plants, entomopathogenic nematodes (*Steinernema thailandensis* n. sp.), and bacteria ISR (*Pseudomonas fluorescens*) for reducing the infestation of the major pest whitefly (*Bemisia tabaci* (Gennadius)) on culantro (*Eryngium foetidum* L.).

II. Materials and Methods

A. Crop and Experiment Establishment

The experiment was conducted at a farm in Nakorn Sawan Province, Thailand. This study was undertaken in the drought season between November and May 2014. Culantro *Eryngium foetidum* L. was planted in 1x2 meter experimental plots. Four weeks after sprouting leaves, five treatments of wood vinegar, fermented organic substances from herbal plants: (neem *A. indica* + citronella grass *C. nardus* + bitter bush *C. odorata*), entomopathogenic nematode (*Steinernema thailandensis* n. sp. and bacteria ISR (*Pseudomonas fluorescens*) were applied as foliage application at 7 day intervals for a total of 8 times until the *E. foetidum* L. was harvested. The three herbal plants were fermented in molasses at 50 ml/25 liters of water with 12 gm of a microbial activator. The Thai strain of entomopathogenic nematode *S. thailandensis* (Rhabditida: Steinernematidae) was isolated at the Department of Agriculture, Thailand [15]. All of the treatments were diluted with water in a 1:200 ratio prior to spraying. Plant growth promoting rhizobacteria (PGPR) strains were cultured from bacteria ISR (*P. fluorescens*). They were used as biocontrol agents against *B. tabaci*. Formulations of bacteria ISR *P. fluorescens* SP007s were isolated from cauliflower rhizoshere at the Department of Plant Pathology, Kasetsart University, Thailand [7].

B. Experimental Design

The experiment was split plot using a Randomized Complete Block Design (RCBD) with five treatments and four replications as follows:

1) Water (control)
2) Wood vinegar
3) Fermented organic substances (neem *Azadirachta indica* + citronella grass *Cymbopogon nardus* Rendle + bitter bush *Chromolaena odorata* L.)
4) Entomopathogenic nematode (*Steinernema thailandensis* n. sp.)
5) Bacteria ISR (*Pseudomonas fluorescens*)

C. Data Collection

The data were collected from whitefly (*Bemisia tabaci* (Gennadius)), the major pest of *E. foetidum* L., found in the treated area. Insect nets and yellow sticky traps were used to monitor adult populations of *B. tabaci*. Efficiency of the biopesticide was determined in terms of acute effect and chronic effect. The *B. tabaci* was collected and recorded every hour up to 3 hours after application of the treatments for acute effect and continuously collected and recorded at weekly intervals for chronic effect. The treatments were applied and observed until the *E. foetidum* L. was harvested.

D. Data Analysis

The data were analyzed using a split plot in a Randomized Complete Block Design (RCD) and compared for significant differences using Duncan's New Multiple Range Test (DMRT).

III. Result

A. Acute Effect of Biopesticides on Whitefly (Bemisia tabaci (Gennadius))

TABLE I
Acute Effect of Treatment on Mean Numbers of Whitefly (*B. TABACI*) by Insect Nets

Treatment	Whitefly no. after application			Mean
	Time after treatment (Hour)			
	1 hr	2 hr	3 hr	
Control	42.5^{a}	40.0^{a}	40.0^{a}	40.83^{a}
Wood vinegar	27.5^{ab}	22.5^{ab}	20.0^{ab}	23.33^{ab}
FOS	30.0^{ab}	20.0^{ab}	20.0^{ab}	23.33^{ab}
Nematode	20.0^{ab}	15.0^{ab}	12.5^{b}	15.83^{b}
Bacteria ISR	12.5^{b}	10.0^{b}	7.50^{b}	10.00^{b}
F-test	*	*	*	

Note * = significant difference, means followed by the same letter are not significantly different at 5% level by DMRT
Mark: FOS = Fermented organic substances

TABLE II
Acute Effect of Treatment on Mean Numbers of Whitefly (*B. TABACI*) by Yellow Sticky Traps

Treatment	Whitefly no. after application			Mean
	Time after treatment (Hour)			
	1 hr	2 hr	3 hr	
Control	40.0^{a}	35.0^{a}	32.5^{a}	35.83^{a}
Wood vinegar	27.5^{ab}	22.5^{ab}	20.0^{ab}	23.33^{ab}
FOS	30.0^{ab}	25.0^{ab}	17.5^{ab}	24.17^{ab}
Nematode	17.5^{b}	15.0^{b}	10.0^{b}	14.17^{b}
Bacteria ISR	15.0^{b}	10.0^{b}	10.0^{b}	11.67^{b}
F-test	*	*	*	

Note * = significant difference, means followed by the same letter are not significantly different at 5% level by DMRT
Mark: FOS = Fermented organic substances

The biopesticide showed an acute effect on the numbers of *Bemisia tabaci* Gennadius with reduction within 1, 2 and 3 hours after application. The highest efficiency on reduction numbers of *B. tabaci* found on the insect nets was the acute effect of the treatment of bacteria ISR, followed by nematode, wood vinegar and fermented organic substances; they showed

significant differences when compared with the control (water). Bacteria ISR indicated the highest effect with a continuously reduced population of *B. tabaci*: only 12.5, 10.0 and 7.5 by insect nets within 1, 2 and 3 hours after treatment, respectively (Table I) while treatment of nematode resulted in continuous reduction of *B. tabaci:* 20.0, 15.0 and 12.5 by insect nets within 1, 2 and 3 hours after treatment, respectively. Similarly, the lowest infestation of *B. tabaci* occurred with the bacteria ISR application by yellow sticky traps as shown on Table II. The infestation of *B. tabaci* found on yellow sticky traps treated with bacteria ISR was 15.0, 10.0 and 10.0 after 1, 2 and 3 hours, respectively. This was followed by treatment of nematode which showed continuous reduction of *B. tabaci:* 17.5, 15.0 and 10.0 within 1, 2 and 3 hours after treatment by yellow sticky traps, respectively (Table II).

B. Chronic Effect of Biopesticides on Whitefly (Bemisia tabaci (Gennadius))

Throughout the experiment, the treatment showed a chronic effect on whitefly infestation on the *E. foetidum* L. Population abundances of *B. tabaci* were in response to the chronic effect of the 5 treatments from the 2nd week until the 8th week although whitefly infestation clearly increased with outbreak time in all treatments with both by insect nets and yellow sticky traps. Particularly, the average number of *B. tabaci* left untreated (control) was 7-8 times higher in the last fortnight when compared to the previous six weeks of the experiment. However, bacteria ISR showed a chronic effect with the highest effectiveness to control the population abundances of *B. tabaci*, followed by nematode, wood vinegar and fermented organic substances.

Table III shows the mean population of *B. tabaci* found on *E. foetidum* L. with the insect nets in the range of only 5.00 to 7.75 from the 2nd week until the 6th week in all treatments, but the highest abundance of *B. tabaci* sharply increased up to 43.75 in the 8th week, particularly for the control treatment. In addition, during this period, treatment of bacteria ISR indicated a high chronic effect reducing the number of *B. tabaci* by insect nets at 13.75 followed by nematode, fermented organic substances and wood vinegar with 21.50, 27.50 and 28.75, respectively. Similarly, the population of *B. tabaci* on the yellow sticky traps fluctuated in the range of 1.0 to 8.0 from the 2nd week until the 6th week under all treatments. However, the outbreak time happened in 8th week when the abundance of *B. tabaci* suddenly increased up to 75.0 under the control treatment (Table IV). Moreover, the lowest infestation of *B. tabaci* occurred with the bacteria ISR treatment by the yellow sticky traps. The treatment of bacteria ISR showed a chronic effect with a high reduction of the abundance of *B. tabaci* at 30.0, followed by treatments of nematode, fermented organic substances and wood vinegar at 31.25, 43.75 and 51.25, respectively. Therefore, the average infestation of *B. tabaci* on *E. foetidum* L. by insect nets and yellow sticky traps after application with various biopesticides shows both an acute effect and chronic effect as shown on Figs. 1 (a) and (b), respectively. The acute effect on the average population of *B. tabaci* after application with treatment of bacteria ISR, nematode, fermented organic substances and wood vinegar was 10.0, 15.83, 23.33 and 23.33, respectively, with insect nets. Meanwhile, the control treatment found *B. tabaci* increased to 40.83. While the averages of the population of *B. tabaci* found with yellow sticky traps under application of bacteria ISR, nematode, fermented organic substances and wood vinegar were 11.67, 14.17, 24.17, and 23.33, respectively; there was a high number of *B. tabaci* found on control treatment, up to 35.83. Similarly, chronic effect on the average of *B. tabaci* after application with the treatment of bacteria ISR, nematode, fermented organic substances and wood vinegar was 13.67, 21.5, 27.5 and 28.75 by the insect nets, respectively.

TABLE III
CHRONIC EFFECT OF TREATMENT ON MEAN NUMBERS OF WHITEFLY (*B. TABACI)* BY INSECT NETS

Treatment	Whitefly no. found on insect nets				Mean
	Time after treatment (weeks)				
	2 weeks	4 weeks	6 weeks	8 weeks	
Control	6.25^{ab}	4.00^{b}	5.50^{a}	43.75^{a}	15.12^{a}
Wood vinegar	5.75^{b}	5.75^{a}	5.00^{ab}	28.75^{ab}	11.31^{ab}
FOS	7.50^{a}	5.50^{a}	6.25^{a}	27.50^{ab}	11.68^{ab}
Nematode	7.25^{a}	5.21^{a}	5.75^{a}	21.50^{ab}	9.87^{b}
Bacteria ISR	7.75^{a}	5.75^{a}	5.00^{ab}	13.75^{b}	8.06^{b}
F-test	ns	ns	ns	*	

Note * = significant difference, means followed by the same letter are not significantly different at 5% level by DMRT
Mark: FOS = Fermented organic substances

TABLE IV
CHRONIC EFFECT OF TREATMENT ON MEAN NUMBERS OF WHITEFLY (*B. TABACI*) BY YELLOW STICKY TRAPS

Treatment	Whitefly no. found on yellow sticky traps				Mean
	Time after treatment (weeks)				
	2 weeks	4 weeks	6 weeks	8 weeks	
Control	1.22^{c}	3.75^{ab}	2.25^{a}	75.00^{b}	21.25^{a}
Wood vinegar	3.50^{b}	4.75^{ab}	1.75^{a}	51.25^{ab}	15.31^{b}
FOS	6.50^{a}	4.25^{ab}	1.50^{ab}	43.75^{ab}	14.0^{b}
Nematode	6.25^{a}	6.25^{a}	2.00^{a}	31.25^{a}	11.43^{b}
Bacteria ISR	8.00^{a}	5.00^{a}	1.00^{ab}	30.00^{a}	11.0^{b}
F-test	*	ns	ns	*	*

Note * = significant difference, means followed by the same letter are not significantly different at 5% level by DMRT
Mark: FOS = Fermented organic substances

The control treatment found *B. tabaci* up to 43.75 while the average number of *B. tabaci* found with yellow sticky traps under application of bacteria ISR, nematode, fermented organic substances and wood vinegar was 30.0, 31.25, 43.75, and 51.25, respectively, with a high number of infested *B. tabaci* on the treatment of control up to 75.0. In addition, biopesticide has influence on the reduction number of minor insect pests: Red spider mites (family Tetranychidae); beet army worm (family Noctuidae), short-horned grasshopper (family Acrididae) and pygmy locusts (family Tetrigidae) etc. with only a few found on the culantro throughout this

experiment as shown in Tables V and VI.

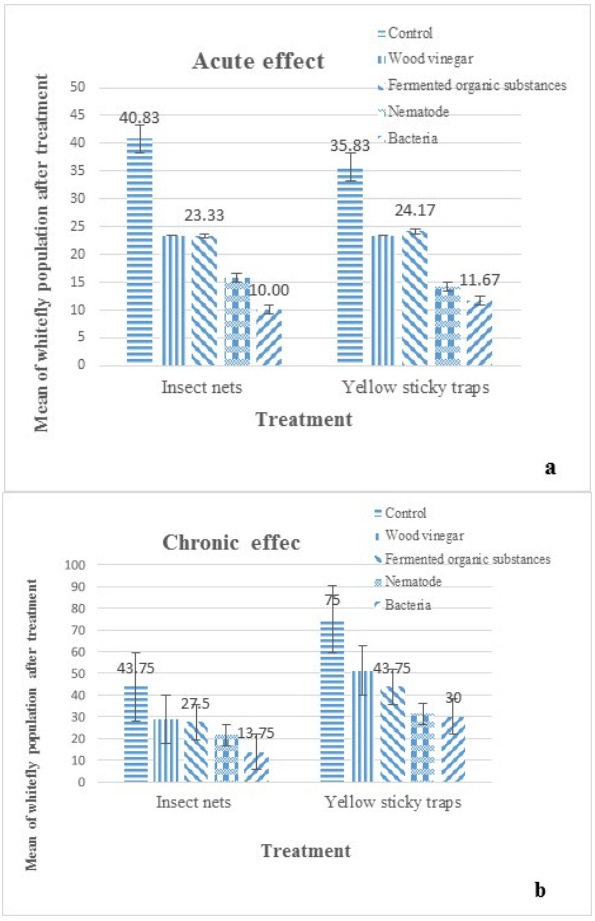

Fig. 1 Average of whitefly *Bemisia tabaci* (Gennadius) population response to acute effect (a) and chronic effect (b) of the treatments

IV. Discussion

In this study, the abundance of *B. tabaci* on *Eryngium foetidum* L. both on the treated and untreated (control) sharply increased at the 7th -8th weeks. Only a small population of *B. tabaci* occurred in the experimental crop within 6 weeks after treatment. In contrast, reports in India, Sri Lanka and Bangladesh show that the plants are infected by *B. tabaci* within 4-5 weeks after germination with losses to exceed 80% [16]. It may be presumed that the time was during higher humidity and temperatures between late summer to the rainy season. Also, low levels of indigenous natural enemy activity leads to plants being seriously affected by *B. tabaci* [17]. This is in line with reports [18] that entomopathogenic fungi can be used to suppress *B. tabaci* populations. For example, *Paecilomyces fumosoroseus* can lead to substantial reductions in *B. tabaci* populations during or immediately following rainy seasons or even prolonged periods of cool, humid conditions in the field or greenhouse.

TABLE V
Chronic Effect of Treatment on Mean Numbers of the Minor Insect Pest by Insect Nets

Treatment	Mean numbers of the minor insects pest with insect nets			
	Red spider mites	Beet army worm	Short-horned grasshopper	Pygmy locusts
Control	1.25	0.37	0.87	1.75
Wood vinegar	0.93	0.12	1.87	2.06
FOS	1.31	0.25	0.93	1.68
Nematode	1.00	0.31	0.50	1.50
Bacteria	0.87	0.25	0.62	1.43
F-test	ns	ns	ns	ns

Note ns = non-significant difference at 5% level by DMRT
Mark: FOS = Fermented organic substances

TABLE VI
Chronic Effect of Treatment on Mean Numbers of the Minor Insect Pests by Yellow Sticky Traps

Treatment	mean numbers of the minor insects pest with yellow sticky traps			
	Red spider mites	Beet army worm	Short-horned grasshopper	Pygmy locusts
Control	0.43	0.68	0.93^{ab}	1.56^{a}
Wood vinegar	0.43	0.56	1.37^{a}	1.43^{ab}
FOS	0.25	0.56	0.62^{b}	0.87^{b}
Nematode	0.37	0.56	0.62^{b}	1.25^{a}
Bacteria	0.56	0.56	0.87^{ab}	1.62^{a}
F-test	ns	ns	*	*

Note * = significant difference, means followed by the same letter are not significantly different at 5% level by DMRT; ns = non-significant difference
Mark: FOS = Fermented organic substances

The serious nature of the *B. tabaci* problem worldwide has resulted in accelerated research to provide acceptable management methods [19]. The use of PGPR strains (bacteria ISR) as biocontrol agents against dirty panicle of rice has not widely been reported although biological control of rice diseases has recently been investigated [20]. There are only a few studies of bacteria ISR for controlling insect pests. In this study, the response of *E. foetidum* L to the treatment of biopesticides was studied against infestation of *B. tabaci*. The treatment of bacteria ISR was the most effective, both acute and chronic effect control of *B. tabaci*. It caused a successful reduction in *B. tabaci* populations on treated *E. foetidum* L. The bacteria ISR *P. fluorescens* had a potential effectiveness to reduce the abundance of *B. tabaci* in both acute and chronic effect when compared to the control treatment.

The formulation of *P. fluorescens* as a biocontrol agent for particular crop systems with greenhouse or field crops and seed treatment or seed coating using PGPR appears to be a feasible method for dirty panicle disease [7]-[9]. Additionally, the *P. fluorescens* strain SP007s significantly reduced the percentage incidence of dirty panicle and increased rice yield compared to fungicide spray. Most importantly, these *Pseudomonas* bioformulations produced multiple effects, are easy to use, and are chemical-free [21]. The mechanism of biocontrol using *P. fluorescens* SP007s revealed that it protects plants from pathogen attack by inducing an increased accumulation of different defense enzymes in treated plants [8].

There are many reports of natural fungal Hyphomycetes, especially *Paecilomyces*, *Verticillium* and *Aschersonia* spp. infecting *B. tabaci* [22]. However, Entomophthorales attacking *B. tabaci* are rare [23], and these fungi are difficult to develop for microbial control applications. However, fungi in the genera *Acremonium, Cladosporium, Aspergillus* and *Fusarium* may also be found associated with whiteflies [19]. In Peru, [24] it was reported that the effect of bioinsecticide and entomopathogenic fungi, rotenone, and mineral oil contributed to the control of *B. tabaci* by killing the nymph population, but these treatments were only fairly effective on *B. tabaci* adults. Hence, the treatments can be integrated with other IPM components to control *B. tabaci*. Also these treatments are expected to allow the recovery of natural enemies of the whitefly.

V. Conclusion

The use of insecticides alone or in mixtures has been the means to control *B. tabaci*. Consequently, *B. tabaci* have developed resistance to numerous conventional insecticides worldwide. Exploiting biopesticides from biocontrol, particularly from bacteria ISR is the most effective means to control whitefly infestation on culantro and also entomopathogenic agent based IPM offers appropriate and better ways against infestation of whitefly. Therefore, IPM research for *B.tabaci* to find a sustainable and ecologically based environmentally acceptable means is the goal for the future.

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