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Article

Toxicity and ovicidal activity of different entomopathogenic fungi, *Hirsutella* extracts on *Tetranychus urticae* (Acari: Tetranychidae)

Suradet Buttachon^{1*}  and War War May Zin² 

1. Department of Entomology, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom, 73140, Thailand; E-mail: fagrsdbu@ku.ac.th
2. Department of Chemistry, Patheingyi University, Pathien 10014, Myanmar; E-mail: wmmzin.chem.yu@gmail.com

* Corresponding author

ABSTRACT

Crude ethyl acetate extracts of six entomopathogenic fungi, *Hirsutella* species were screened against *Tetranychus urticae* Koch (Acari: Tetranychidae). In this study, all extracts presented potential to control eggs and adult female activities. Laboratory evaluation of the crude extracts of *Hirsutella citrififormis*, *H. guignardii*, *H. petchabunensis*, *H. satumaensis*, *H. saussurei* and *H. thompsonii* showed slightly toxicity and median lethal concentration (LC₅₀) values of 7,579.94, 8,910.38, 8,579.45, 5,055.96, 1,555.46 and 44,197.85 mg/L at 72 h after treatment against adult females of *T. urticae*, respectively. Related LC₅₀ values that exhibited on eggs were 12,701.86, 30,922.72, 10,870.96, 12,635.56, 9,244.59 and 22,676.66 mg/L at 5 days after exposure. The most effective on *T. urticae* eggs and adult females is *H. saussurei*. Moreover, all extracts deceased the fecundity of *T. urticae* after being fed with mulberry leaf discs treated the crude extract of six species of *Hirsutella* under residual effect bioassay. The results suggest that the ethyl acetate extracts of *Hirsutella citrififormis*, *H. guignardii*, *H. petchabunensis*, *H. thompsonii*, *H. satumaensi* and *H. saussurei* have the potential to be used for the control of *T. urticae* in the future.

KEY WORDS: Biological control; crude extracts; fungal metabolites; *Hirsutella*; two spotted spider mite.

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INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is an important mite pest worldwide that can harm more than 1000 plant species around the world in both outdoor crops and greenhouses (Migeon and Dorkeld 2007; Attia *et al.* 2013). Management of *T. urticae* is still mainly based on the use of synthetic pesticides (Van Leeuwen *et al.* 2006; Akyazi *et al.* 2018). Indeed, this pest is very difficult to control and many of these chemicals are not effective because the pest can easily develop resistance to 96 active ingredients on account of its small, very short life cycle, high fecundity, fast breeding, arrhenotokous reproduction and high mutation rate (Van Leeuwen *et al.* 2009, 2010). Furthermore, the synthetic pesticides used contribute to atmospheric pollution and residual toxicity in environment, human safety, and non-target organisms (Silva *et al.* 2006; Tirello *et al.* 2012; Horikoshi *et al.* 2017).

Therefore, the development of new acaricides with eco-friendly management and reduce the effects on environmental health. One promising candidate, the entomopathogenic fungus genus *Hirsutella* is pathogenic to insect groups such as Acarida, Lepidoptera, and Hemiptera (Kaushal *et*

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al. 2011). This fungus is belonging to the family Ophiocordycipitaceae that 70 species of asexually present an important function as a controller for insects, mites, and nematodes and many of the *Hirsutella* species produce several important bioactive substances that could be utilized in acaricide (Omoto and McCoy 1998), antimalaria (Thongtan *et al.* 2006), antitumor (He *et al.* 2008) and antituberculosis (Isaka *et al.* 2006). Microbial metabolites show high toxicity to insects and mite pests (Gulati 2014; Ragavendran and Natarajan 2015). For example, hirsutellin A produced by *Hirsutella thompsonii* demonstrated highly toxic effects on *Phyllocoptruta oleivora* (Omoto and McCoy 1998). Moreover, an abamectin was isolated from *Streptomyces avermitilis* and revealed against *T. urticae*, *Ph. oleivora* Ashmead, *Panonychus citri* (McGregor), and *T. turkestanii* Ugarov and Nikolski (Putter *et al.* 1981; Wu and Liu 1997). Also, thuringiensin produced by *Bacillus thuringiensis* exhibits potent acaricidal efficiency on *T. urticae* (Neal *et al.* 1987), and *Panonychus ulmi* Koch (Vargas Mesina 1993). Microbial metabolites maybe not only promising resources in a novel pesticide development but also promising tools in bio-rational control of *T. urticae* (Attia *et al.* 2013; Horikoshi *et al.* 2017). Besides, very little is known about the toxicology of crude extract of entomopathogenic fungus *Hirsutella* against mite pests. In this study, aimed to evaluate the control potential of ethyl acetate extract produced by different fungal strains against *T. urticae* under laboratory conditions.

MATERIALS AND METHODS

Culture of spider mites

Tetranychus urticae was obtained from Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand and it was reared on healthy and clean mulberry leaves (*Morus alba* L.) for several generations to provide a laboratory colony. The colonies were maintained at laboratory conditions at 25 ± 1 °C, 60–70% relative humidity and a photoperiod of 16:8 h (light:dark). The adult females were transferred with a fine paint brush to lower surface of mulberry leaf placed on moistened cotton pads resting on sponges in the plastic box (17.5 cm width × 25 cm length × 4 cm height) and removed after 24 hours. The one-day-old adult females were used in all experiments.

Fungal material

Six *Hirsutella* species namely *Hirsutella citrififormis* (BCC 2539), *H. guignardii* (BCC 1883), *H. petchabunensis* (BCC 14502), *H. satumaensis* (BCC 2507), *H. saussurei* (BCC 12681) and *H. thompsonii* (BCC 6380) were obtained from National Center for Genetic Engineering and Biotechnology (BIOTEC) in Thailand.

Preparation of crude extracts

The crude ethyl acetate was extracted following the procedures of Buttachon *et al.* (2013). Briefly, each fungus was cultured on potato dextrose agar (PDA) plates and incubated at 27 to 28 °C for seven days. Ten fungal mats (1 cm in diameter) were taken from each plate and placed in an Erlenmeyer flask containing 100 ml of malt extract broth (MEB) before being incubated at 27 to 28 °C in the rotary shaker set at 180 rpm for four days. These fungal biomasses were used as inoculums throughout the study. A plastic bag containing 200 g of cooking rice that was autoclaved at 121 °C for 15 min. was inoculated with 3 µl broth culture and incubated at 28 °C for 30 days. Ten moldy rice bags were then emptied into 5 L Erlenmeyer flasks and 2500 ml of ethyl acetate were added to each flask. All flasks were kept for three days before the fungal materials and solvents were filtered through a Buchner funnel using Whatman® No. 2 filter paper. The two aqueous layers were then separated using a separating funnel and the ethyl acetate solution was concentrated at reduced pressure yielding crude extract.

Toxicity test

The toxicity by residual effect only was determined according to the method of Buttachon *et al.* (2013). Five concentrations (100, 500, 1000, 5000, 10,000 mg/L) were prepared from the crude extract of each fungal isolate. An aliquot of 50 µl of each concentration was painted on the upper surface of each mulberry leaf disc (2 cm in diameter). After solvent was dried for about 15 minutes and resting on moistened cotton pads in 9 cm Petri dishes (2 leaf discs/Petri dish, 5 Petri dishes/treatment). Twenty *T. urticae* females (two to three day-old) from stock culture were placed on each mulberry leaf disc. The treated mites were kept at 25 °C with a 16L: 8D photoperiod. After 24, 48, 72 hours of extracts application, the morality rate and number of eggs laid were recorded under a stereomicroscope [OLYMPUS, SZ51, 8× through 40× (using 10× eyepieces), with a zoom ratio of 5:1].

Ovicidal bioassay

To test ovicidal activity of the crude ethyl acetate extract of six *Hirsutella* species, 10 gravid females of *T. urticae* (3-to 5-day-old) were placed on a mulberry leaf disc (20 mm diameter) and were allowed to lay eggs for 24 h as above. Then the females were removed and 50 eggs were left on each leaf disc (2 leaf discs/Petri dish, 5 Petri dishes/treatment). Subsequently, 500 µl of suspensions of five concentrations (5000, 10,000, 20,000, 30,000, 40,000, 50,000 mg/L) were prepared from the crude extract of each fungal isolate which were sprayed onto the leaf disc with a plastic atomizer spray. The control discs were treated with ethanol 95%. A bioassay with five concentrations and a control was replicated five times. Hatchability was determined for a period of 5 days after treatment at 25 °C with a 16L: 8D photoperiod. Those eggs that did not hatch after this period were regarded as non-viable.

Statistical analyses

For toxicity and ovicidal activity experiments, the number of dead mite individuals and unhatched eggs were counted and tested for normality (Shapiro-Wilk test). The data-sets that were not normally distributed were subjected to arcsine transformation and when the data were normally distributed, they were analyzed with a one-way analysis of variance (ANOVA) (P = 0.05). Means were compared by Tukey HDS test. Control is corrected according to Abbott's formula (Abbott 1925).

$$\text{Corrected mortality} = ((T-C)/(100-C)) \times 100$$

where T = dead mites in treatment and C = dead mites in control. Data obtained from each dose-response bioassay were subjected to probit analysis (Finney 1971) to estimate LC₅₀ values. All statistical analyses were done on SPSS version 26.

RESULTS

Toxicity test

The residual toxicity activity of ethyl acetate crude extract from *Hirsutella citrifomis*, *H. guignardii*, *H. petchabunensis*, *H. satumaensis*, *H. saussurei* and *H. thompsonii* against *T. urticae* was investigated in this study. The results showed that low mortality rates were observed at low concentrations of all extracts. The acaricidal activity enhanced with increasing concentrations (Fig. 1). The highest concentration of *H. saussurei* showed the highest mortality to adult females of *T. urticae*. The accumulated mortality rates were 54.16, 69.92 and 81.76% at 24, 48 and 72 hours after treatment, respectively. While high percentages of mortality rates were not significantly different from mortality rates of *T. urticae* treated with 5000 mg/L concentration of this extract for 24, 48 and 72 hours (p > 0.05).

The LC₅₀ values at 72 h exposure to the ethyl acetate crude extracts of *Hirsutella citriformis*, *H. guignardii*, *H. petchabunensis*, *H. satumaensis*, *H. saussurei* and *H. thompsonii* against *T. urticae* are summarized in Table 1. It was also determined that the ethyl acetate crude extract of each of the isolates showed strong acaricidal efficacy. LC₅₀ values of the *H. saussurei* extract were lower than *H. satumaensis*, *H. citriformis*, *H. petchabunensis*, *H. guignardii* and *H. thompsonii*, respectively. Although the *H. thompsonii* extract had the lowest toxic effect, it showed increased mite mortality along with the rise in the concentrations. Overall, it can be said that all extracts have potential to be used in the control of *T. urticae* adults.

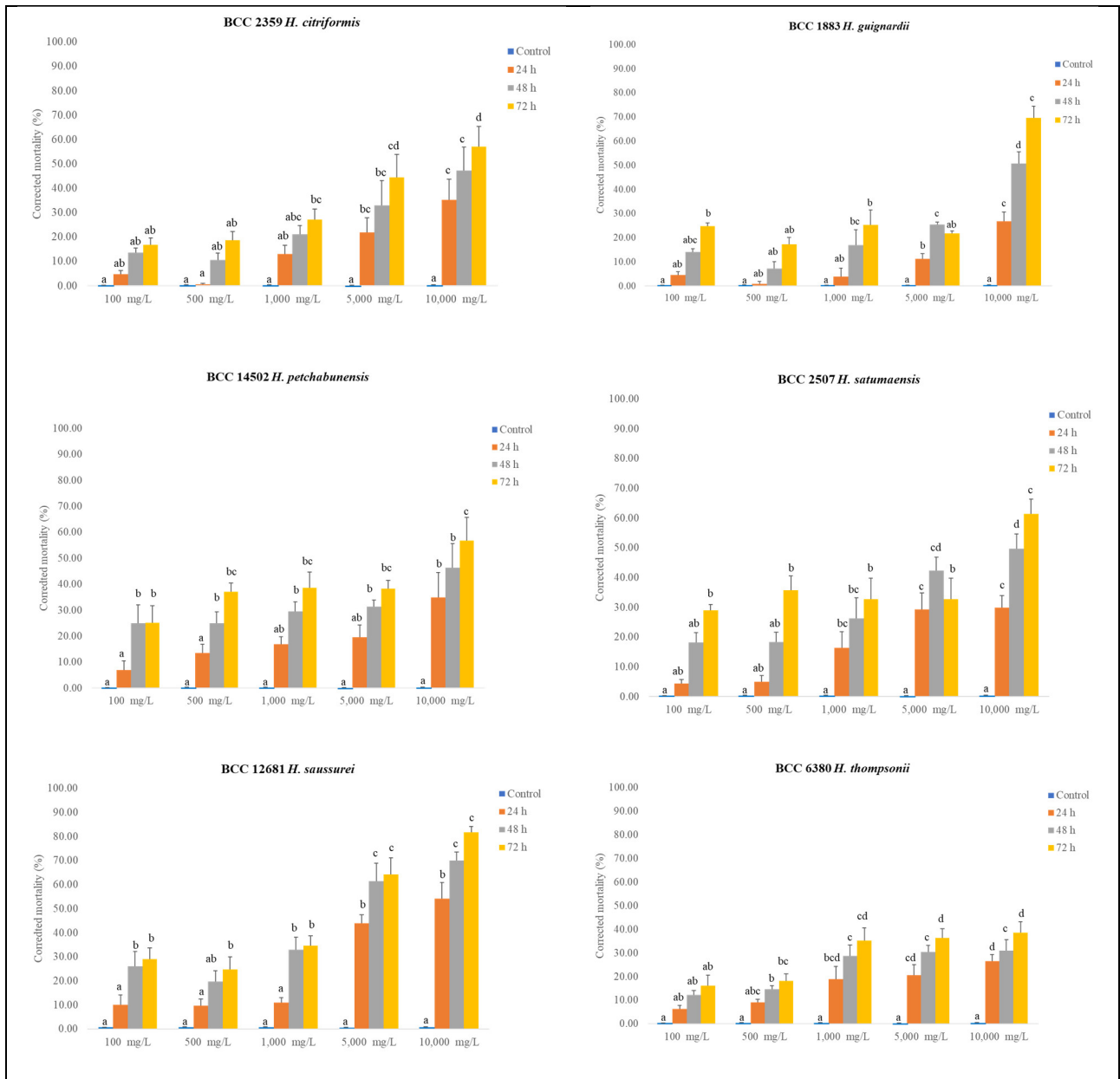


Figure 1. Accumulated mortality of *T. urticae* females at 24, 48 and 72 h after being fed with mulberry leaf discs treated with different concentrations of crude extract, six species of *Hirsutella* under residual effect bioassay. For each extract and mite, means followed by the same letter are not significantly different ($p = 0.05$; Tukey HSD test).

Table 1. Toxicity regression equation of the crude ethyl acetate extract from *Hirsutella* spp. against *T. urticae* 72 h after exposure.

| Treatment | N | LC ₅₀ (95% CL) | Toxicity regression equation | X ² | df | R ² linear |
|-------------------------------|-----|----------------------------------|------------------------------|----------------|----|-----------------------|
| <i>Hirsutella citriformis</i> | 200 | 7,579.94 (4,431.53–17,071.05) | $y = -2.30 + 0.59x$ | 4.011 | 3 | 0.918 |
| <i>H. guignardii</i> | 200 | 8,910.38 (7,599.41–10,332.61) | $y = -2.12 + 0.53x$ | 1.405 | 2 | 0.587 |
| <i>H. petchabunensis</i> | 200 | 8,579.45 (3,451.10–66,567.62) | $y = -1.31 + 0.33x$ | 4.844 | 3 | 0.782 |
| <i>H. satumaensis</i> | 200 | 5,055.90 (2,454.82–18,285.87) | $y = -1.14 + 0.38x$ | 5.094 | 3 | 0.814 |
| <i>H. saussurei</i> | 200 | 1,555.46 (83.40–670,850.50) | $y = -2.42 + 0.76x$ | 1.045 | 3 | 0.802 |
| <i>H. thompsonii</i> | 200 | 44,197.85 (12,775.24–952,256.17) | $y = -1.74 + 0.38x$ | 5.109 | 3 | 0.798 |

LC₅₀, is the lethal concentration of with 50% of the population tested are dead. N = Total number of adults used; CL = 95% confidential limit.

We found that the mean daily number of eggs laid by *T. urticae* females treated with ethyl acetate extract of *Hirsutella citriformis*, *H. guignardii*, *H. petchabunensis*, *H. satumaensis*, *H. saussurei* and *H. thompsonii* ranged between 2.27–7.15, 1.54–6.40, 3.56–6.79, 2.65–7.54, 2.22–6.66 and 3.50–6.99 eggs/female/day, respectively over three days at the tested different concentrations. These daily eggs numbers were generally significantly lower than control values which ranged between 6.94–7.60 eggs/female/day. After a 72 h exposure, the results showed that the number of eggs laid by *T. urticae* females decreased along with the rise in the concentrations. Female spider mites exposed to ethyl acetate extract of *H. guignardii* also produced significantly fewer eggs during the first 72 h of adult life than those exposed to the ethyl acetate extract of *H. citriformis*, *H. petchabunensis*, *H. satumaensis*, *H. saussurei* and *H. thompsonii*. This study indicates that all crude extracts of *Hirsutella* species at the tested dose rates significantly decrease the fecundity of *T. urticae* (Table 2).

Table 2. Mean daily egg production by *T. urticae* females at 24, 48 and 72 h after being fed mulberry leaf discs treated with different concentrations of crude extract, six species of *Hirsutella*.

| Treatment (mg/L) | Egg/female/day (Mean ± SEM) * | | | df | F | P |
|-------------------------------|-------------------------------|----------------------------|---------------------------|----------|-------|-------|
| | 24 h | 48 h | 72 h | | | |
| <i>Hirsutella citriformis</i> | | | | | | |
| 100 | 6.42 ± 0.14 ^{ab} | 6.43 ± 0.18 ^a | 6.36 ± 0.26 ^a | | | |
| 500 | 6.87 ± 0.15 ^{ab} | 6.36 ± 0.19 ^a | 6.38 ± 0.47 ^a | 24 h = 5 | 3.05 | 0.029 |
| 1,000 | 7.15 ± 0.23 ^{ab} | 6.81 ± 0.43 ^a | 5.96 ± 0.47 ^a | 48 h = 5 | 4.53 | 0.005 |
| 5,000 | 6.14 ± 0.31 ^{ab} | 4.72 ± 0.53 ^{ab} | 5.88 ± 0.52 ^a | 72 h = 5 | 10.16 | 0.000 |
| 10,000 | 4.72 ± 1.33 ^b | 3.45 ± 1.44 ^b | 2.27 ± 0.95 ^b | | | |
| <i>H. guignardii</i> | | | | | | |
| 100 | 6.29 ± 0.16 ^b | 6.40 ± 0.15 ^{ab} | 5.43 ± 0.60 ^b | | | |
| 500 | 5.39 ± 0.40 ^{cd} | 5.10 ± 0.25 ^{bc} | 5.37 ± 0.18 ^b | 24 h = 5 | 52.24 | 0.000 |
| 1,000 | 6.03 ± 0.31 ^b | 3.92 ± 0.51 ^c | 2.64 ± 0.34 ^c | 48 h = 5 | 25.92 | 0.000 |
| 5,000 | 4.64 ± 0.34 ^c | 5.03 ± 0.46 ^{bc} | 2.36 ± 0.39 ^c | 72 h = 5 | 33.98 | 0.000 |
| 10,000 | 1.54 ± 0.25 ^d | 2.42 ± 0.25 ^d | 2.20 ± 0.20 ^c | | | |
| <i>H. petchabunensis</i> | | | | | | |
| 100 | 6.38 ± 0.36 ^{ab} | 6.45 ± 0.48 ^{ab} | 5.94 ± 0.52 ^{ab} | | | |
| 500 | 6.35 ± 0.34 ^{ab} | 6.79 ± 0.13 ^a | 6.62 ± 0.26 ^a | 24 h = 5 | 8.04 | 0.000 |
| 1,000 | 6.54 ± 0.33 ^{ab} | 5.50 ± 0.71 ^{abc} | 5.04 ± 0.71 ^{ab} | 48 h = 5 | 7.75 | 0.000 |
| 5,000 | 6.13 ± 0.30 ^{bc} | 4.24 ± 0.59 ^{bc} | 3.91 ± 0.61 ^b | 72 h = 5 | 5.87 | 0.001 |
| 10,000 | 4.75 ± 0.39 ^c | 3.56 ± 0.65 ^c | 3.81 ± 0.79 ^b | | | |

* Means ± SEM in column followed by the same letters are not significantly different as determined by Tukey HSD test ($\alpha = 0.05$).

Table 2. Continued.

| Treatment (mg/L) | Egg/female/day (Mean \pm SEM)* | | | df | F | P |
|-----------------------|----------------------------------|-------------------------------|-------------------------------|----------|-------|-------|
| | 24 h | 48 h | 72 h | | | |
| <i>H. satumaensis</i> | | | | | | |
| 100 | 7.54 \pm 0.51 ^a | 7.11 \pm 0.44 ^a | 6.37 \pm 0.46 ^a | | | |
| 500 | 7.25 \pm 0.25 ^a | 5.20 \pm 0.45 ^a | 5.71 \pm 0.46 ^a | 24 h = 5 | 6.75 | 0.000 |
| 1,000 | 6.89 \pm 0.13 ^a | 6.17 \pm 0.49 ^a | 6.62 \pm 0.35 ^a | 48 h = 5 | 3.39 | 0.019 |
| 5,000 | 6.82 \pm 0.17 ^a | 4.61 \pm 0.35 ^a | 3.73 \pm 0.13 ^b | 72 h = 5 | 16.42 | 0.000 |
| 10,000 | 5.59 \pm 0.30 ^b | 4.69 \pm 1.21 ^a | 2.65 \pm 0.74 ^b | | | |
| <i>H. saussurei</i> | | | | | | |
| 100 | 6.66 \pm 0.31 ^{ab} | 6.63 \pm 0.36 ^{ab} | 5.42 \pm 0.89 ^{ab} | | | |
| 500 | 6.24 \pm 0.16 ^{ab} | 4.81 \pm 0.81 ^b | 3.86 \pm 0.43 ^{bc} | 24 h = 5 | 2.30 | 0.076 |
| 1,000 | 5.98 \pm 0.51 ^{ab} | 6.70 \pm 0.35 ^{ab} | 5.97 \pm 0.64 ^{ab} | 48 h = 5 | 15.72 | 0.000 |
| 5,000 | 6.32 \pm 0.73 ^{ab} | 4.80 \pm 0.49 ^b | 4.22 \pm 0.38 ^{bc} | 72 h = 5 | 9.60 | 0.000 |
| 10,000 | 5.46 \pm 0.63 ^b | 2.29 \pm 0.26 ^c | 2.22 \pm 0.48 ^c | | | |
| <i>H. thompsonii</i> | | | | | | |
| 100 | 6.17 \pm 0.53 ^{ab} | 4.71 \pm 0.33 ^b | 6.12 \pm 0.29 ^{ab} | | | |
| 500 | 6.99 \pm 0.31 ^{ab} | 5.64 \pm 0.27 ^{ab} | 5.71 \pm 0.31 ^{ab} | 24 h = 5 | 11.65 | 0.000 |
| 1,000 | 6.60 \pm 0.30 ^{ab} | 5.80 \pm 0.28 ^{ab} | 3.50 \pm 0.32 ^c | 48 h = 5 | 4.55 | 0.005 |
| 5,000 | 6.04 \pm 0.39 ^b | 5.83 \pm 0.19 ^{ab} | 5.79 \pm 0.24 ^{ab} | 72 h = 5 | 12.09 | 0.000 |
| 10,000 | 4.09 \pm 0.30 ^c | 5.52 \pm 0.63 ^{ab} | 4.46 \pm 0.65 ^{bc} | | | |
| Control | 7.60 \pm 0.17 ^a | 7.00 \pm 0.14 ^a | 6.94 \pm 0.09 ^a | | | |

* Means \pm SEM in column followed by the same letters are not significantly different as determined by Tukey HDS test ($\alpha = 0.05$)

Table 3. Toxicity determined by the lethal concentrations (LC₅₀ and LC₉₅) of the different ethyl acetate crude extracts of *Hirsutella* spp. on the eggs of *Tetranychus urticae*, five days after exposure.

| Scientific name | N | LC ₅₀ (95% CL) | LC ₉₅ (95% CL) | X ² | df | R ² | Regression equation |
|--------------------------|-----|------------------------------------|---------------------------------------|----------------|----|----------------|---------------------|
| <i>H. citriformis</i> | 500 | 12,701.86 (9,370.26–16,077.01) | 43,033.04 (31,526.27–73,485.96) | 12.11 | 4 | 0.94 | y = -13.92 + 3.39x |
| <i>H. guignardii</i> | 500 | 30,922.72 (23,500.59–42,404.96) | 91,541.35 (59,208.20–301,270.87) | 19.23 | 4 | 0.93 | y = -13.42 + 2.99x |
| <i>H. petchabunensis</i> | 500 | 10,870.96 (6,536.76–15,117.17) | 45,975.66 (29,836.60–118,397.23) | 18.36 | 4 | 0.87 | y = -13.2 + 3.28x |
| <i>H. satumaensis</i> | 500 | 12,635.56 (9,368.01–15,949.63) | 43,178.13 (31,700.56–73,122.54) | 11.70 | 4 | 0.93 | y = -13.9 + 3.39x |
| <i>H. saussurei</i> | 500 | 9,244.59 (7,017.75–11,484.45) | 25,777.42 (19,690.19–40,242.20) | 10.46 | 4 | 0.96 | y = -13.57 + 3.41x |
| <i>H. thompsonii</i> | 500 | 22,676.66 (20,056.70–25,664.17) | 140,234.37 (105,034.39–208,675.39) | 2.24 | 4 | 0.98 | y = -9.02 + 2.07x |

LC₅₀, is the lethal concentration of with 50% of the population tested are dead; LC₉₅, is the lethal concentration of with 95% of the population tested are dead. N = Total number of egg used. CL = 95% confidential limit.

Ovicidal toxicity

In all cases, considerable differences in mortality of eggs to *Hirsutella* species extract were observed with different concentrations. Probit analysis showed that *H. saussurei* (LC₅₀ = 9,244.59

mg/L) was more susceptible to *H. petchabunensis* ($LC_{50} = 10,870.96$ mg/L) than *H. satumaensis* ($LC_{50} = 12,635.56$ mg/L), *H. citriformis* ($LC_{50} = 12,701.86$ mg/L), *H. thompsonii* ($LC_{50} = 22,676.66$ mg/L) and *H. guignardii* ($LC_{50} = 30,922.72$ mg/L). The corresponding LC_{95} values were 25,777.42, 45,975.66, 43,178.13, 43,033.04, 140,234.37 and 91,541.35 mg/L, respectively. The 95% fiducial limits of the LC_{50} and LC_{95} values for five species are shown in Table 3.

DISCUSSION

Based on the results demonstrated above the six entomopathogenic fungi extracts in this study showed different activities against eggs and adults females of two-spotted spider mite, *T. urticae*. We confirmed the presence of potent toxic effect of crude ethyl acetate extracts of *Hirsutella* spp. on adults and eggs of *T. urticae*. Reddy *et al.* (2020) reported that *Hirsutella* sp. has been found efficient against nymph and adults of red spider mite, adults of varroa mite (*Varroa destructor* Anderson & Trueman) of honey bees and coconut eriophyid mite (*Aceria gueneronis* Keifer). Moreover, entomopathogenic fungi such as *H. thompsonii* are well-documented fungal agents against insect pests (Feng *et al.* 1994; Reddy *et al.* 2020) but are poorly understood on their potential to infect insect eggs and unknown about their ability to infect spider mite. There are very limited reports on their acaricidal activity (Lacey *et al.* 1999). The acaricidal toxicity of the crude ethyl acetate extract of *Hirsutella* six species is clear against *T. urticae*. This is the first record on such effects of *Hirsutella* on *T. urticae*. Interestingly, all evaluated isolates demonstrated more toxicity towards adults than eggs of *T. urticae*. Crude extracts of *H. saussurei* showed slight toxicity and the others extracts were practically non-toxic when topically sprayed on eggs (Table 3). This result was quite different from those of Mehdi (2006) who reported not only fungal filtrates had more toxic action on eggs of *T. urticae* than adults but also found that the filtrates of *Alternaria alternata*, *A. pluriseptata*, *Aspergillus terreus*, *Trichoderma harzianum*, *T. koningii*, *T. viride*, *Eurotium eurotiorum*, *Ulocladium chartarum*, and *Stachybotrys atra* inhibited hatchability by an average of 65.5, 58.3, 65.5, 60.6, 60.6, 54.1, 40, 52, 49.1, and 49.1%, respectively. Similarly, the ethyl acetate crude extracts of *Aspergillus melleus*, *A. terreus*, *Emericella nidulans*, and *Chaetomium globosum*, exhibited LC_{50} values of 10.27, 33.05, 14.68, and 22.40 mg/ml against females of *T. urticae*, respectively. Correspondent LC_{50} values exhibited on eggs were 8.81, 23.17, 11.66, and 11.05 mg/ml (Osman *et al.* 2019). The lowest survival rate of *T. urticae* adults was observed when the mites were put in contact with 10,000 mg/L crude ethyl acetate extract of *H. saussurei* (Fig. 1). Similarly, the 3% (w/v) of crude extract, *Hypocrella raciborskii* Zimm. showed residual toxicity on adults in 72 hours after treatment (Buttachon *et al.* 2013). Santamarina *et al.* (1987) found that the crude extract (10 mg/ml) of *Penicillium funiculosum* caused 100% mortality in the population of *Panonychus ulmi* on the second day after application. Similar results were obtained by Jimenez *et al.* (1993) who tested *P. funiculosum* on adult females of *T. urticae* recorded a mortality rate of 100% after three days of exposure. In another similar study, the ethyl acetate extract of *Paecilomyces lilacinum* exhibited LC_{50} values of 10.49 mg/ml for eggs and 30.75 mg/ml for adults of *T. urticae* (Nawar *et al.* 2018). The extracts showed different biological activities probably due to their bioactive compounds. So, this is one reason why some entomopathogenic fungi showed high activity whereas others appeared to exhibit low or no activity on the pest. For example, list of bioactive compounds having insecticidal and acaricidal activity produced by entomopathogenic fungi such as dustanin (15 α , 22 dihydroxyhopane) and 3 β -acetoxy-15 α ,22- dihydroxyhopane produced by *H. raciborskii* Zimm. showed reduced egg production of *T. urticae* by 71% and 70%, respectively (Buttachon *et al.* 2013). Osman *et al.* (2019) found that the mellamide, ochratoxin C, nodulisporic acid, 7-Oxocurvularin, and 6-(4'-hydroxy-2'-methyl phenoxy)-(-)-(3R)-mellein were separated from the active fraction of *Aspergillus melleus* crude extract are sources for biopesticides to control *T. urticae*. The chemical study of the ethyl acetate extract of *Hirsutella citriformis* (Ramachandran *et al.* 2013) and *Hirsutella thompsonii* (Mazet and

Vey 1995) are reported but the *H. guignardii*, *H. petchabunensis*, *H. satumaensis* and *H. saussurei* have not yet been studied. From the experimental results in this study, it can be concluded that all tested treatments can be used for the management of two spotted spider mite, however, the six entomopathogenic fungi extracts will be promising alternatives for successful management of *T. urticae* in IPM programs. Thus, this study showed promising acaricidal activities against the two spotted spider mite, *T. urticae*, females and eggs. Further investigations are still needed to identify their bioactive secondary metabolites and to evaluate their efficacy. These results both encourage further studies for isolation and characterization of the acaricidal compounds, and provide a new approach to the research and development of pesticides from natural products.

CONCLUSION

Our results suggested that ethyl acetate crude extract of entomopathogenic fungi caused high mortality percentage of egg and adult stages of *T. urticae* under laboratory conditions by increasing concentration and time of exposure. The ethyl acetate crude extract of *H. satumaensis* had higher toxicities for *T. urticae* adults and eggs compared to other extracts. Crude extract of entomopathogenic fungi, as a potent biopesticide, may have the potential to be more widely applied in treatments for other spider mites and insect pests.

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سمیت و فعالیت تخم‌کشی قارچ‌های مختلف انتوموپاتوزن، عصاره‌های *Hirsutella* روی کنه تارتن *Tetranychus urticae* (Acari: Tetranychidae)

سورادت بوتاجون^{۱*} و وار وار می زین^۲

۱. گروه حشره‌شناسی، دانشکده کشاورزی در کمفنگ سائن، دانشگاه کاستسارت، پردیس کمفنگ سائن، ناخون پاتوم، ۷۳۱۴۰، تایلند؛ رایانامه: fagrsdbu@ku.ac.th

۲. گروه شیمی، دانشگاه پاتین، پاتین ۱۰۰۱۴، میانمار؛ رایانامه: wwmzin.chem.yu@gmail.com

* نویسنده مسئول

چکیده

عصاره خام اتیل استات شش قارچ انتوموپاتوزن، گونه‌های *Hirsutella* در برابر *Tetranychus urticae* Koch (Acari: Tetranychidae) غربالگری شدند. در این مطالعه، تمام عصاره‌ها پتانسیل کنترل تخم‌ها و فعالیت‌های ماده بالغ را نشان دادند. ارزیابی آزمایشگاهی عصاره‌های خام *Hirsutella citriformis*، *H. guignardii*، *H. petchabunensis*، *H. satumaensis*، *H. saussurei* و *H. thompsonii* مقادیر کمی سمی را نشان دادند و میانگین غلظت کشنده (LC₅₀) به ترتیب ۷۵۷۹/۹۴، ۸۹۱۰/۳۸، ۸۵۷۹/۴۵، ۵۰۵۵/۹۶، ۱۵۵۵/۴۶ و ۴۴۱۹۷/۸۵ میلی‌گرم بر لیتر در ۷۲ ساعت پس از تیمار علیه کنه‌های ماده بالغ را نشان دادند. مقادیر مربوط به LC₅₀ روی تخم *H. saussurei*، *H. thompsonii*، *H. petchabunensis*، *H. guignardii* و *H. citriformis* به ترتیب ۲۲۶۷۶/۶۶ و ۹۲۴۴/۵۹، ۱۲۶۳۵/۵۹ و ۳۰۹۲۲/۷۲، ۱۲۷۰۱/۸۶، ۱۰۸۸۷۰/۹۶، ۱۰۸۸۷۰/۹۶ و ۳۰۹۲۲/۷۲ میلی‌گرم بر لیتر در ۷۲ ساعت پس از تیمار علیه کنه‌های ماده بالغ را نشان دادند. مقادیر مربوط به LC₅₀ روی تخم *T. urticae* و ماده بالغ آن بود. افزون بر این، تمام عصاره‌ها باروری *T. urticae* را پس از تغذیه از دیسک‌های برگ‌گی توت تیمار شده با عصاره خام شش گونه *Hirsutella* در روش زیست‌سنجی باقیمانده کاهش دادند. نتایج نشان می‌دهد که عصاره اتیل استات *H. citriformis*، *H. guignardii*، *H. petchabunensis*، *H. thompsonii*، *H. satumaensis* و *H. saussurei* پتانسیل استفاده برای کنترل *T. urticae* را در آینده دارند.

واژگان کلیدی: مهار زیستی؛ عصاره‌های خام؛ متابولیت‌های قارچی؛ *Hirsutella*؛ کنه تارتن دولکه‌ای.

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