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Article

Laboratory evaluation of *Beauveria bassiana*, some plant oils and insect growth regulators against two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae)

Tarikul Islam, Md. Jamil Hossain Biswas, Mohammad Tofazzal Hossain Howlader and Mohammad Shaef Ullah

Department of Entomology, Bangladesh Agricultural University, Mymensingh, Bangladesh; E-mails: tarikul.entom@bau.edu.bd, 40765@bau.edu.bd, tofazzalh@bau.edu.bd, ullahipm@gmail.com

ABSTRACT

The acaricidal activity of entomopathogenic fungus *Beauveria bassiana* (strain GHA), three essential plant oils namely, neem (*Azadirachta indica* A. Juss), mahogany (*Swietenia mahogoni* L.) and karanja (*Millettia pinnata* L.), and two insect growth regulators (buprofezin and lufenuron) were evaluated against the two-spotted spider mite, *Tetranychus urticae* Koch under laboratory conditions by leaf disc bioassay method. Results revealed that *B. bassiana* was highly effective in controlling *T. urticae* adults causing mortality from 3 days after treatment application. Among the plant oils tested, karanja and mahogany oils were found most effective against *T. urticae* and both the oils showed significantly better performance compared to all other treatments ($P < 0.05$). Although neem oil showed comparatively lower toxicity than other plant oils, it was more effective than both the IGRs, buprofezin and lufenuron. However, all the treatments were found effective in controlling *T. urticae* populations and the study, therefore, suggested that the selected treatments could be considered for biorational management of the *T. urticae*.

KEY WORDS: *Beauveria bassiana*; bioassay; essential plant oils; insect growth regulators; *Tetranychus urticae*.

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INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is one of the most destructive polyphagous pest species attacking more than 1000 plant species belonging to more than 140 plant families (Grbić *et al.* 2011, Migeon and Dorkeld 2014). It primarily feeds near the midrib and plant veins, often causing 50 to 100 percent yield loss (Kumar *et al.* 2010). Different systemic chemical pesticides especially synthetic pesticides are used for controlling *T. urticae*, but quick development of pesticide resistance by the mite was reported (van Leeuwen *et al.* 2010). Many aspects of the biology such as rapid development, high fecundity and haplo-diploid sex determination have facilitated quick evolution of pesticide resistance by the *T. urticae* (van Leeuwen *et al.* 2010). Moreover, use of synthetic chemicals have resulted in serious environmental problems and have been a threat to human life (Kim *et al.* 2005; Heil *et al.* 2008). Therefore, alternative strategies such as use of different bio-control agents, essential plant oils and insect growth regulators needs to explore for their acaricidal activity against the *T. urticae* in order to reduce the chemical acaricides that are currently being used as well as to develop integrated management tactics.

The entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin is a well-known bio-control agent currently using for the management of many insects including aphids, leafhoppers, and whiteflies (Faria and Wraight 2001; Feng *et al.* 2004; Hatting *et al.* 2004; Pu *et al.* 2005). It was also reported having efficacy against the *T. urticae* (Irigaray *et al.* 2003; Chandler *et al.* 2005; Ullah and Lim 2015).

The plant derived pesticides are environmentally safe and non-toxic to human being, fish and wild-life (Isman 2000; Kumar *et al.* 2000). Many plant-derived essential oils possessed both insecticidal and acaricidal properties against different types of soft-bodied arthropod pests (Alexenizer and Dorn 2007). They usually exert multiple types of beneficial properties such as repellence, anti-feedant activity, growth regulatory activity and toxicity to many insect and mite pests (Prakash *et al.* 2008).

Insect growth regulators (IGRs) are the substances that adversely affect insect growth and development. Buprofezin and lufenuron, which are potential chitin synthesis inhibitors, were found effective against many arthropod pests (Das and Islam 2014; Islam *et al.* 2015) including mites (Naher *et al.* 2006; Kavya *et al.* 2015).

There are reports available on the evaluation of different chemical insecticides (Hossain *et al.* 2006; Uddin *et al.* 2015) and predators (Parvin and Haque 2008) to control the *T. urticae* in Bangladesh. However, reports on the efficacy of entomopathogens, plant oils and IGRs against the populations of *T. urticae* were rarely found. Hence, the present study was planned to evaluate the efficacy of *B. bassiana*, three plant oils and two IGRs against the Bangladeshi populations of *T. urticae*.

MATERIALS AND METHODS

Rearing of two-spotted spider mites

The mites were collected from the experimental research fields of Bangladesh Agricultural University, Mymensingh and were reared continuously on leaf discs (ca. 16 cm²) of country bean, *Phaseolus vulgaris* L. in the Laboratory of Applied Entomology and Acarology, Department of Entomology, Bangladesh Agricultural University, Mymensingh. Bean leaf discs were kept on water-saturated polyurethane mats in plastic Petri dishes (90 mm diameter, 20 mm depth). To obtain fixed-age females for the bioassay, quiescent deutonymphs were separated from the mite culture and transferred on fresh leaf discs (Ullah and Lim 2015). Rearing trays were kept under controlled conditions at 25 ± 1°C temperature, 75 ± 5% RH and 16L: 8Dh photoperiod. Withered leaves were replaced with new fresh ones, at 4 days interval.

Tested bio-control agent, plant-derived essential oils and IGRs

Three different types of compounds were selected to test their efficacy against the *T. urticae*. The first one, *B. bassiana* 1.15 WP (1 × 10⁸ conidia/ml), is an entomopathogenic fungal microbe that was supplied by Ispahani Biotech Ltd., Bangladesh. The second group is the essential plant derived oils. The plant oils from three different plants namely neem (*Azadirachta indica* A. Juss), mahogany (*Swietenia mahagoni* L.) and karanja (*Millettia pinnata* L.) were selected. The plant oils were collected from the local market. The third group is the insect growth regulators (IGRs). Two IGRs, buprofezin (as Award 40 SC marketed in Bangladesh by Square Pharmaceuticals Ltd.) and lufenuron (as Heron 5 EC marketed in Bangladesh by Haychem Bangladesh Ltd.) were selected and collected from the dealer shop of the local market of Mymensingh, Bangladesh.

Bioassay procedures

The acaricidal efficacy of the *B. bassiana* (strain GHA), plant oils and insect growth regulators (IGRs) were evaluated against the two-spotted spider mite, *T. urticae* under laboratory conditions as per leaf disc bioassay method reported previously (Ullah and Lim 2015) with slight modifications.

Briefly, leaf discs (ca. 16 cm²) using cotyledonous leaves of common bean (*Phaseolus vulgaris* L.) were prepared and placed on wet cotton pads in a Petri dish (90 mm diameter). Four to five day-old mated females of *T. urticae* were placed on a new bean leaf disc (ca. 4 × 4cm²) and incubated for 24 h. Before bioassay, the conidial viability of *B. bassiana* was confirmed by spread-plating 0.1 ml of 1% conidial suspension on Sabouraud Dextrose Agar (SDA) plates. More than 90% conidial germination was recorded after 24 hours from 100-spore counts by placing sterile coverslip on each plate under a microscope (Sundew, micros Austria).

Five conidial suspensions of *B. bassiana* having 0.25, 0.5, 0.75, 1 and 1.25% *B. bassiana* 1.15 WP (1×10^8 conidia/ml) were selected for the bioassay. For all other treatments, five solutions were prepared at concentrations of 0.1, 0.25, 0.5, 0.75, 1% based on preliminary concentration-mortality experiments to select the range of concentrations producing 5–95% mortalities (data not shown). One ml conidial suspensions of each five concentrations of *B. bassiana* and 1 ml solutions of each five concentrations of plant oils and IGRs were applied directly to mite-infested leaf discs by using a hand operated micro-applicator sprayer (Burkard Scientific, UK). The control group was treated with distilled water only. There were three replicates for each treatment, and 10 adult mites were used in each replicate. After air drying, the mite-infested discs were kept at $25 \pm 2^\circ\text{C}$ temperature, $80 \pm 5\%$ RH and 16L: 8D photoperiod in an incubator. Mortality was recorded daily until death of all the tested organisms.

Statistical analyses

The percentage of mortalities was corrected using Abbott's (1925) formula. The median lethal values (LC₅₀) were determined by probit analysis using 'Ldp line' software (<http://www.ehabsoft.com/ldpline/>). Statistical differences between LC₅₀ estimates were determined by using a 95% CI for the ratio of two estimates (Robertson *et al.* 2007).

RESULTS

Acaricidal activity of *B. bassiana* against *T. urticae*

The responses of two-spotted spider mite, *T. urticae* to *B. bassiana* is presented in the Table 1. The LC₅₀ values of *B. bassiana* against *T. urticae* were found to be 0.562, 0.467, 0.358 and 0.272% at 72, 96, 120 and 144 hours after treatment (HAT), respectively. The death of *T. urticae* caused by the infection of *B. bassiana* initiated after 72 hours of fungal application (Table 1). It was found that the acaricidal activity of *B. bassiana* against *T. urticae* was time dependent, i.e. the toxicity increased with the advancement of time. We observed that the infected dead females of *T. urticae* were covered with fungal conidia causing mycosis, whereas dead mites in control did not produce any mycotic symptoms (Fig. 1).



Figure 1. Infection caused by *Beauveria bassiana* on *Tetranychus urticae*, (a, b) dead female mites due to mycosis, (c) dead mite in control.

Acaricidal activity of plant oils against *T. urticae*

All three tested plant oils showed acaricidal effect against *T. urticae* although and their efficacy

varied significantly. Karanja oil showed highest toxicity (LC_{50} values were 0.008, 0.006, 0.004 and 0.004% at 24, 48, 72 and 96 HAT, respectively) against *T. urticae* which was followed by mahogany oil (LC_{50} values were 0.009, 0.007, 0.005 and 0.004% at 24, 48, 72 and 96 HAT, respectively; see Table 2). Neem oil was the worst treatment having highest LC_{50} estimates (0.230, 0.174, 0.109 and 0.075% at 24, 48, 72 and 96 HAT, respectively) and both mahogany and karanja oil were found significantly better than neem oil (Table 2).

Table 1. Susceptibility (% LC_{50}) of two spotted spider mite, *Tetranychus urticae* to *Beauveria bassiana*.

Test Material	72 Hours				96 Hours				120 Hours				196 Hours			
	LC_{50} 95%CI	Slope \pm SE	χ^2	df	LC_{50} 95%CI	Slope \pm \pm SE	χ^2	df	LC_{50} 95%CI	Slope \pm SE	χ^2	df	LC_{50} 95%CI	Slope \pm SE	χ^2	df
<i>B. bassiana</i>	0.562	2.511 \pm	0.593	3	0.467	2.48 \pm	1.145	3	0.358	2.687 \pm	0.83	3	0.272	2.285 \pm	3.048	3
	0.456–	0.485			0.367–	0.471			0.271–	0.473	2		0.171–	0.468		
	0.697				0.571				0.436				0.361			

Acaricidal activity of insect growth regulators (IGRs) against *T. urticae*

Among two insect growth regulators, lufenuron was comparatively more toxic (LC_{50} values were 0.049, 0.028, 0.020, 0.013% at 24, 48, 72 and 96 HAT, respectively) than buprofezin (LC_{50} values were 0.092, 0.060, 0.049, 0.019% at 24, 48, 72 and 96 HAT, respectively) against *T. urticae* (Table 2). For all the treatments LC_{50} values decreased gradually with increased time length.

Table 2. Susceptibility (% LC_{50}) of two spotted spider mite, *Tetranychus urticae* to three essential plant oils and two insect growth regulators.

Test Material	24 Hours				48 Hours				72 Hours				96 Hours			
	LC_{50} 95%CI	Slope \pm SE	χ^2	df	LC_{50} 95%CI	Slope \pm SE	χ^2	df	LC_{50} 95%CI	Slope \pm \pm SE	χ^2	df	LC_{50} 95%CI	Slope \pm SE	χ^2	df
Neem Oil	0.230 ^c	2.328	0.396	3	0.174 ^c	1.957 \pm	0.458	3	0.109 ^c	2.019	1.626	3	0.075 ^b	1.574 \pm	0.111	3
	0.171–	\pm 0.599			0.132–	0.529			0.084–	\pm			0.039–	0.501		
	0.487				0.326				0.146	0.509			0.102			
Mahogany Oil	0.009 ^a	2.016	1.131	3	0.007 ^a	2.276 \pm	3.180	3	0.005 ^a	2.228	0.511	3	0.004 ^a	2.322 \pm	0.076	3
	0.006–	\pm 0.448			0.004–	0.467			0.003–	\pm			0.002–	0.545		
	0.012				0.009				0.007	0.471			0.006			
Karanja Oil	0.008 ^a	2.036	1.923	3	0.006 ^a	2.127 \pm	2.004	3	0.004 ^a	2.098	0.450	3	0.004 ^a	2.593 \pm	0.957	3
	0.005–	\pm 0.450			0.003–	0.470			0.002–	\pm			0.002–	0.584		
	0.011				0.008				0.006	0.526			0.006			
Lufenuron	0.049 ^b	1.386	0.308	3	0.028 ^a	1.420 \pm	3.012	3	0.020 ^a	1.436	1.229	3	0.013 ^a	1.601 \pm	0.522	3
	0.025–	\pm 0.423			^b	0.439			^b	\pm			0.001–	0.490		
	0.069				0.008–				0.004–	0.434			0.024			
Buprofezin	0.092 ^b	1.217	1.458	3	0.060 ^b	1.211 \pm	0.574	3	0.049 ^b	1.141	1.549	3	0.019 ^a	1.054 \pm	0.351	3
	0.061–	\pm 0.375			0.031–	0.368			0.030–	\pm			0.003–	0.382		
	0.17				0.090				0.066	0.370			0.035			

Different letters within columns indicate significant difference (based on overlapping confidence intervals; $P < 0.05$)

DISCUSSION

The *T. urticae* is a global pest in greenhouse production and field crops, infesting many annual and perennial crops such as tomatoes, peppers, cucumbers, strawberries, apples, grapes and citrus (Aslan *et al.* 2004; Wekesa *et al.* 2011). It is very difficult to control *T. urticae* with chemical acaricides due to their ability to develop resistance to chemical groups after a few years of use. On the other hand, there are predictions that their injurious effect in agriculture will increase due to accelerated development at high temperatures (Kiritani 2006). Therefore, it is an urgent matter to test novel and alternative agents for their efficacy to this pest. In this report, we have evaluated the efficacy of three

different groups of novel agents, i.e. *B. bassiana* as representative of entomopathogenic fungus, three essential plant oils and two IGRs against *T. urticae* in leaf discs under laboratory conditions. The bioassay provided LC₅₀ estimates for each of the treatments which might be effective for selecting the proper doses for the management of *T. urticae*.

In case of the *B. bassiana*, the LC₅₀ value was highest among the treatments and mycosis developed after three days of application which was similar with the findings of Ullah and Lim (2015). Though *B. bassiana* had been reported as a promising fungal pathogen against two-spotted spider mites, in the present study, it required higher conidial concentration and more time duration for causing death of *T. urticae* as compared to plant oils and insect growth regulators. This might be because, the small size and cryptic habitat of spider mites make them less exposed to the fungal conidia at lower concentrations. Moreover, *B. bassiana* might require minimum time duration for its development on *T. urticae* and ultimately causing death of adult female mites. The exact mechanisms of mycosis against *T. urticae* need to be further investigated.

Numerous laboratory and field studies have been conducted over the last 30 years using many plant extracts or products having potentiality as botanical pesticides to control agricultural mites (Rasikari *et al.* 2005; Shi *et al.* 2006; Sertkaya *et al.* 2010; Jia *et al.* 2011; Pavela 2016). Among the plant oils tested, both mahogany and karanja oils were performed significantly better than neem oil. The exact reason of better performance of mahogany and karanja oils is unknown. One possibility could be the presence of some novel natural components in the oils which have more toxic effects to *T. urticae*. Methanol extracts from the seeds of karanja (*M. pinnata* L.), formerly *Pongamia pinnata* L. possessed good larvicidal activity toward *Culex pipiens pallens* and *Aedes aegypti* larvae (Perumalsamy *et al.* 2015). The phytochemistry, pharmacological activities and uses of the different chemical components present in leaves, stem, and seeds of *M. pinnata* plant have been well documented by Arote and Yeole (2010) and Meera *et al.* (2003). The proper identification and characterization, efficacy against the *T. urticae* of these components needs to be further investigated.

Although many researchers have reported the efficacy of neem oil and neem kernel extracts against spider mites (Roobakkumar *et al.* 2010; Tehri and Gulati 2014), our results showed that the neem oil performed poorly compared to mahogany and karanja oils. This might be attributed to slow action of azadirachtin, the main active ingredient of neem products, which includes complete or partial anti-feedant response, delayed and/or disrupted moulting and inhibited reproduction (Copping and Duke 2007). The two insect growth regulators (IGRs), buprofezin and lufenuron were found moderately effective against the *T. urticae*. However, as IGRs are considered to be safe for beneficial organisms (Ishaaya *et al.* 2001), both the selected IGRs could be considered for the development of IPM program for *T. urticae*.

In conclusion, mahogany and karanja oils had the highest acaricidal activities against *T. urticae*, whereas neem oil and IGRs showed moderate toxicity. The *B. bassiana* was also found effective, although a relatively higher dose is the pre-requisite for the development of mycosis and death of *T. urticae*. Therefore, the selected treatments could be used as most promising eco-friendly alternatives of synthetic chemical pesticides for the management of *T. urticae*.

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ارزیابی آزمایشگاهی *Beauveria bassiana* چند روغن گیاهی و تنظیم‌کننده‌های رشد حشرات روی کنه تارتن دولکه‌ای، *Tetranychus urticae* Koch (Acari: Tetranychidae)

تریکل اسلام، ام‌دی. جمیل حسین بیسواس، محمد تفضل حسین هولدر و محمد شاعف الله

گروه حشره‌شناسی، دانشگاه کشاورزی بنگلادش، میمنه سینگ، بنگلادش؛ رایانامه‌ها: 40765@bau.edu.bd tarikul.entom@bau.edu.bd ullahipm@gmail.com tofazzalh@bau.edu.bd

چکیده

فعالیت کنه‌کشی قارچ بیماری‌گر حشرات *Beauveria bassiana* (جدایه GHA)، سه اسانس روغنی گیاهی یعنی نیم (*Azadirachta indica* A. Juss)، ماهوگانی (*Swietenia mahogoni* L.) و کارانجا (*Millettia pinnata* L.)، و دو تنظیم‌کننده رشد حشرات (بوپروفزین و لوفنورون) روی کنه تارتن دولکه‌ای، *Tetranychus urticae* Koch در شرایط آزمایشگاهی به روش زیست‌سنجی دیسک برگی بررسی شد. نتایج نشان داد که قارچ *B. bassiana* در کنترل کنه‌های کامل *T. urticae* با ایجاد مرگ سه روز پس از کاربرد بسیار مؤثر بود. در بین اسانس‌های روغنی گیاهی مورد آزمون، اسانس‌های روغنی کارانجا و ماهوگانی بر علیه *T. urticae* بسیار مؤثر بودند و هر دو اسانس روغنی ترجیح معنی‌داری را در مقایسه با همه تیمارهای دیگر نشان دادند ($P > 0.05$). اگرچه اسانس روغنی نیم سمیت کمتری نسبت به دیگر اسانس‌های روغنی گیاهی داشت اما از هر دو تنظیم‌کننده رشد، بوپروفزین و لوفنورون، مؤثرتر بود. با این حال، همه تیمارها در کنترل جمعیت‌های *T. urticae* مؤثر بودند و بنابراین، نتایج به دست آمده نشان می‌دهد که تیمارهای انتخاب شده می‌توانند در مدیریت کنترل *T. urticae* با کمترین اثرات جانبی زیست‌محیطی در نظر گرفته شوند.

واژگان کلیدی: *Beauveria bassiana*؛ زیست‌سنجی؛ اسانس‌های روغنی گیاهی؛ تنظیم‌کننده‌های رشد حشرات؛ *Tetranychus urticae*.

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