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Article

Laboratory bioassay of six pesticides, an entomopathogenic fungus, and a botanical pesticide on two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae)

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ABSTRACT

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) is a serious pest that infests different agricultural crops in Bangladesh. The application of different types of pesticides (botanical and chemical) with various modes of action and bio-pesticides was used for the first time to develop sustainable pest control strategies against spider mites. In this study, the effects of six chemical insecticides viz. abamectin, bifenthrin, bifentazate, etoxazole, hexythiazox, and spinosad, and entomopathogenic fungus *Beauveria bassiana* and botanical neem oil were evaluated against adult and egg stages of *T. urticae* under laboratory conditions. Treated mites were kept in controlled conditions at 25 ± 1 °C, 60–70% RH and a photoperiod of 16:8 h (L: D). The LC₅₀ values of all chemicals [for adults, abamectin (0.51 ml/L), bifenthrin (3.26 ml/L), bifentazate (3.82 ml/L), hexythiazox (3.27 ml/L) and spinosad (3.83 ml/L); for eggs, abamectin (0.56 ml/L), bifenthrin (3.48 ml/L), bifentazate (26.81 ml/L), hexythiazox (4.18 ml/L) and spinosad (18.28 ml/L)] suggest that they were effective against eggs and adult females of the *T. urticae*. Etoxazole (LC₅₀ = 3.99 ml/L) is recommended against egg stage. The neem oil was found to be moderately effective (for adult, LC₅₀ = 1.26%; for egg, LC₅₀ = 1.77%) against eggs and adult females of *T. urticae*. The *B. bassiana* was also found to be effective against eggs and adult females of *T. urticae*. Finally, it could be concluded that all chemicals used in this study are effective for the management of two spotted spider mite but the entomopathogenic fungus *B. bassiana* and botanical neem oil were promising alternatives for successful management of *T. urticae* in IPM programs.

KEY WORDS: *B. bassiana*; chemicals; IPM; spider mite; toxicity.

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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 2020). It is a serious pest that infests different agricultural crops in Bangladesh (Islam *et al.* 2017). It primarily feeds near the midrib and plant veins, often causing 50 to 100 percent yield loss (Kumar *et al.* 2010). This species infests 86

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host plants including the families from Araceae, Cucurbitaceae, Fabaceae, Malvaceae, Solanaceae, Rosaceae, etc. (Migeon and Dorkland 2020). The cost of damages caused by this pest in crops such as beans, citrus, cotton, avocado, apples, pears, plums, and many other horticultural and ornamental crops are estimated at over 4500 USD per hectare (Rincón *et al.* 2019). Such costs correspond to 30% of the total cost of pesticides in crops of ornamental flowers. This constitutes a spending of almost 62% of the global market value on *T. urticae* control based on data of 2008 (Van Leeuwen *et al.* 2015). Along with its exceptionally broad host range, *T. urticae* has established pesticide resistance.

The frequent occurrence of pesticide resistance has led to a new interest in developing pesticides with alternative modes of action, lower environmental impact, better compatibility with integrated pest management programs and reduced health risk to humans and wildlife. Integrated pest management programs practice chemical control as a component giving priority to the use of selective pesticides that kill target pests but have relatively little or no effect on beneficial organisms and other non-target organisms. For the sustainable control of spider mite populations, it is becoming more and more important to design specific resistance management strategies i.e. keeping the number of individuals below a certain threshold value. The resistance management guidelines published by the Insecticide Resistance Action Committee (IRAC) especially for spider mite control provides a guide to the selection of insecticides for use in an effective and sustainable insecticide resistance management (IRM) strategy (IRAC 2020a). Therefore, proper care is essential in selecting acaricides for managing the spider mites as they have a notable intrinsic potential for swift evolution of resistance (Croft and Van de Baan 1988; Van Leeuwen *et al.* 2009). This mite has been reported to develop a very high degree of resistance to a newly introduced compound after only a few years of use, with cross-resistance to other compounds having the same mode of action. Nearly 800 cases of acaricide resistance in phytophagous mites are reported in the Arthropod Pesticide Resistance Database (APRD), 93% of which refer to spider mite resistance (Whalon *et al.* 2020). Among them, 549 cases of acaricide resistance belong to *T. urticae* (Mota-Sanchez and Wise 2020). Therefore, there is always a continuous effort to develop and apply new acaricides with novel biochemical modes of action, to optimize their use in order to prevent or delay the development of resistance (Dekeyser 2005).

Several entomopathogenic fungus and plant-derived products possess acaricidal properties against different soft-bodied arthropod pests. Approximately 500 fungi species are pathogenic in insects. The entomopathogenic fungi species of *Lagenidium*, *Entomophaga*, *Neozygites*, *Entomophytora*, *Erynia*, *Aschersonia*, *Lecanicillium*, *Nomuraea*, *Hirsutella*, *Metarhizium*, *Beauveria* and *Isaria* have gained importance in the field of plant protection (Erkiliç and Uygun 1993; Kılıç and Yıldırım 2008). Entomopathogens such as *M. anisopliae* (Metsch.) and *B. bassiana* (Balsamo) Vuillemin are well characterized in respect to pathogenicity to several insects and mites, and have been used as myco-biocontrol agents for biological control of agriculture pests worldwide (Sandhu *et al.* 2012; Ullah and Lim 2015; Islam *et al.* 2017). *Beauveria bassiana* is an abundant pathogen of many arthropod pests including the spider mites (Faria and Wraight 2001; Feng *et al.* 2004; Hatting *et al.* 2004; Pu *et al.* 2005; Ullah and Lim 2015) whose repeated application has been found to manage several arthropod pests (Grodén *et al.* 2002). In recent years, attention in applying mycoinsecticides including *B. bassiana* for the control of mites has increased (Alves *et al.* 2002, 2005; Shi and Feng 2006, 2009; Seiedy *et al.* 2010; Ullah and Lim 2015; Wu *et al.* 2016).

In addition, botanical insecticides have eco-toxicological advantages compared to traditional synthetic insecticides, because they can have favorable eco-toxicological properties (low human toxicity, rapid degradation and reduced environmental impact), which make them suitable insecticides for organic agriculture (Zanuncio *et al.* 2016). 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). The neem oil is a feeding inhibitor, delaying development and growth, reducing fecundity and fertility, changing behavior and causing

anomalies in eggs, larvae and adults of insects or mites (Ventura and Ito 2000; Mitcheli *et al.* 2004; Mourão *et al.* 2004; Masood *et al.* 2006).

Several studies reported on the efficacy of different chemical insecticides to control *T. urticae*. However, reports on the efficacy of entomopathogenic fungi, botanicals with synthetic chemicals against the *T. urticae* populations were scarce. The findings of this study may help to develop an alternative IPM strategy such as biological control (entomopathogenic fungi) and botanical to supplement the currently used pesticides.

MATERIALS AND METHODS

Rearing of two-spotted spider mites

The mite strain of *T. urticae* was collected from the Laboratory of Applied Entomology and Acarology, Bangladesh Agricultural University and maintained on leaf discs (ca. 25 cm²) of bean, *Dolichos lablab* L. The leaf discs were placed on water-saturated polyurethane mats in plastic Petri dishes (90-mm diameter, 20-mm depth) and were maintained at 25 ± 1 °C, 65 ± 5% relative humidity, and 16L: 8D h photoperiod. The edges of bean leaves were covered with moist tissue paper to provide free water and prevent mites from escaping. The leaves were replaced whenever they appeared to be damaged by feeding spider mites (Ullah *et al.* 2014).

Tested chemicals, plant extract and entomopathogenic fungus

The pesticides tested in this study were abamectin (Biomax-M 1.2 EC), bifenthrin (Serput 2.5 EC), bifenazate (Bifenazate 20 EC), etoxazole (Etoxazole 10 EC), hexythiazox (Mite Scavenger 10 EC) and spinosad (Tracer 45 SC). In addition, the entomopathogen *B. bassiana* (Botanigard^{ES}) and neem oil (Bioneem) were also used in this experiment. These pesticides (abamectin (0.12, 0.24, 0.48, 0.96, 1.92 and 3.84 ml/L), bifenthrin (1, 2, 4, 8, 16, 32 and 64 ml/L), bifenazate (2, 4, 8, 16, 32, 64 and 128 ml/L), etoxazole (0.5, 1, 2, 4, 8, 16, 32 and 64 ml/L), hexythiazox (1, 2, 4, 8, 16 and 32 ml/L), spinosad (0, 4.5, 9, 18, 36, 72, 144, and 288 ml/L)), *B. bassiana* (1 × 10⁴, 1 × 10⁵, 1 × 10⁶, 1 × 10⁷ and 1 × 10⁸ conidia/ml) and neem oil (0.5, 1, 2, 4, 6, 8 and 10%) used against eggs and adult of *T. urticae* were suspended in distilled water just before application. Five to seven concentrations per treatment with four replicates for each concentration were tested. Leaf discs sprayed with distilled water were served as control.

Generating uniform aged females of T. urticae

To get uniform aged female *T. urticae*, deutonymphs were randomly taken from separate mass rearing arenas and placed singly on 15-mm diameter bean leaf discs in plastic cups. The developmental stages of the spider mites were checked daily. Differences in the sex-specific body size were used to determine their sex after reaching adulthood. Three to five-day old female adults obtained within a day after emergence were used in the bioassay experiment (Ullah and Lim 2017; Tahmina *et al.* 2020).

Toxicity to adults

Fifteen 3–5-day-old mated females of *T. urticae* were placed on a new bean leaf disc (20-mm diameter) to determine the efficacy of six chemicals, *B. bassiana* and neem oil on adult females. Dead or injured individuals were removed from leaf discs after 24 h. Suspensions of the pesticides [abamectin (0.12, 0.24, 0.48, 0.96, 1.92, 3.84 and 7.68 ml/L), bifenthrin (1, 2, 4, 8, 16, 32 and 64 ml/L), bifenazate (2, 4, 8, 16, 32, 64 and 128 ml/L), etoxazole (0.5, 1, 2, 4, 8, 16, 32 and 64 ml/L), hexythiazox (1, 2, 4, 8, 16, 32 and 64 ml/L), spinosad (0, 4.5, 9, 18, 36, 72, 144, and 288 ml/L)], *B. bassiana* (1 × 10⁴, 1 × 10⁵, 1 × 10⁶, 1 × 10⁷ and 1 × 10⁸ conidia/ml) and neem oil (0.5, 1, 2, 4, 6, 8 and 10%) were sprayed onto the leaf disc with adult female spider mites (342–480 adult female) for 25 cm above, using a hand sprayer (RFL hand sprayer, Bangladesh) at a rate of 1 ml/cm². The

sprayed leaf discs with adult females were dried in the shade and then maintained at 25 ± 1 °C with a 16L: 8D photoperiod for 72 h. Mites scored as "dead" did not move their appendages when touched with a fine brush (Tahmina *et al.* 2020).

Toxicity to eggs

Ten 3–5-day-old mated females were placed on a new bean leaf disc (20-mm diameter) as described above and were allowed to lay eggs for 24 h to test ovicidal activity of the pesticides, entomopathogen and plant extract. After that the adult female spider mites were removed, and the eggs were counted on each leaf disc. Subsequently, chemicals [abamectin (0.12, 0.24, 0.48, 0.96, 1.92 and 3.84 ml/L), bifenthrin (1, 2, 4, 8, 16, 32 and 64 ml/L), bifenazate (2, 4, 8, 16, 32, 64 and 128 ml/L), etoxazole (0.5, 1, 2, 4, 8, 16, 32 and 64 ml/L), hexythiazox (1, 2, 4, 8, 16 and 32 ml/L), spinosad (0, 4.5, 9, 18, 36, 72, 144, and 288 ml/L)], *B. bassiana* (1×10^4 , 1×10^5 , 1×10^6 , 1×10^7 and 1×10^8 conidia/ml) and neem oil (0.5, 1, 2, 4, 6, 8 and 10%) suspensions were sprayed onto the leaf disc with eggs (671–2967 eggs) at a rate of 1 ml/cm². The eggs were kept on leaf discs at 25 °C with a 16L: 8D photoperiod at 60–70% relative humidity for 192 h. Eggs that had not hatched were scored as "dead".

Statistical analysis

Pooled data for either adult females or eggs were subjected to Probit analysis (transforming the percentage killed into a "probability unit") and values of LC₅₀ and LC₉₀ with 95% confidential limits (CL) were estimated (Finney 1971) using Abbott's correction for natural mortality (Abbott 1925):

$$\text{Corrected mortality (\%)} = [100 \times (\text{Treated} - \text{Control}) / (100 - \text{Control})]$$

RESULTS

Effectiveness of chemical pesticides, B. bassiana and neem oil against adult of T. urticae

The response of *T. urticae* adult female in all chemicals were dose dependent. The mortality of adult female against different pesticides between observed and expected values are closely fitted (Table 1). The one exception was that the etoxazole had an approximately higher LC₅₀ for *T. urticae* (50 ml/L). Abamectin was found the most toxic to adult females of *T. urticae* compared to bifenthrin, hexythiazox, spinosad and bifenazate (Table 1). The LC₉₀ values also showed similar response, but etoxazole was not effective against adults of *T. urticae*.

The plant extract, neem oil was found to be moderately effective against adult females of *T. urticae*. The LC₅₀ value of neem oil was 1.26 % and LC₉₀ value was 9.25 % against adult females of *T. urticae*.

The entomopathogen, *B. bassiana* was found to be effective against adult females of *T. urticae*. The LC₅₀ value of *B. bassiana* was 2.25×10^6 conidia/ml and LC₉₀ value was 1.35×10^7 conidia/ml against adult females of *T. urticae*.

Effectiveness of chemical pesticides, B. bassiana and neem oil against eggs of T. urticae

The response of egg mortality considering all pesticides were dose dependent. The observed and expected values of the response of eggs were closely fitted (Table 2). Spinosad and bifenazate were found to be moderately affecting the eggs of *T. urticae*. Abamectin was found the most toxic to eggs of *T. urticae* compared to bifenthrin, hexythiazox and etoxazole (Table 2). The LC₉₀ values were also showed similar response, but spinosad and bifenazate were not effective against eggs of *T. urticae*.

Table 1. Mortality caused by six pesticides, plant extract (neem oil) and entomopathogen (*Beauveria bassiana*) in adult females of *Tetranychus urticae*.

| *Chemicals, neem oil and entomopathogen | N | LC ₅₀ (95% CL) | LC ₉₀ (95% CL) | Slope ± SE | χ ² | R ² | df |
|---|-----|--|--|--------------|----------------|----------------|----|
| Abamectin (12) | 359 | 0.51 (0.40–0.65) | 1.70 (1.33–2.19) | 2.43 ± 0.411 | 11.78 | 0.9213 | 3 |
| Bifenthrin (25) | 342 | 3.26 (2.51–4.25) | 11.87 (9.11–15.47) | 2.29 ± 0.543 | 20.21 | 0.8550 | 3 |
| Bifenazate (200) | 359 | 3.82 (2.83–5.14) | 28.85 (21.42–38.85) | 1.46 ± 0.179 | 2.74 | 0.9568 | 3 |
| Etoxazole (50) | 469 | 27.85 (18.86–41.13) | 2368.06 (1603.62–3496.90) | 0.66 ± 0.076 | 2.44 | 0.9381 | 5 |
| Hexythiazox (50) | 480 | 3.27 (2.47–4.33) | 28.58 (21.60–37.84) | 1.36 ± 0.170 | 8.31 | 0.9274 | 5 |
| Spinosad (90) | 420 | 3.83 (2.83–5.19) | 38.82 (28.69–52.54) | 1.27 ± 0.154 | 3.77 | 0.9451 | 4 |
| Plant extract (%) | | | | | | | |
| Neem oil | 480 | 1.26 (0.96–1.65) | 9.25 (7.06–12.11) | 1.48 ± 0.097 | 1.52 | 0.9791 | 5 |
| Entomopathogen (conidia/mL) | | | | | | | |
| <i>Beauveria bassiana</i> | 360 | 2.25 × 10 ⁶ (1.33 × 10 ⁶ –3.79 × 10 ⁶) | 1.35 × 10 ⁷ (7.99 × 10 ⁷ –2.28 × 10 ⁸) | 2.69 ± 0.361 | 5.93 | 0.9487 | 3 |

LC₅₀, is the lethal concentration of with 50% of the population tested are dead; LC₉₀, is the lethal concentration of with 90% of the population tested are dead.

N = Total number of adult used.

CL = 95% confidential limit.

Estimation of LC₅₀ (ml of a.i./l distilled water) and slope.

* Chemicals (Recommended concentration, ml/l).

Table 2. Mortality caused by six pesticides, plant extract (neem oil) and entomopathogen (*Beauveria bassiana*) in eggs of *Tetranychus urticae*.

| *Chemicals, neem oil and entomopathogen | N | LC ₅₀ (95% CL) | LC ₉₀ (95% CL) | Slope ± SE | χ ² | R ² | df |
|---|------|--|--|---------------|----------------|----------------|----|
| Abamectin (12) | 960 | 0.56 (0.47–0.68) | 3.67 (3.07–4.40) | 1.58 ± 0.151 | 9.36 | 0.9644 | 4 |
| Bifenthrin (25) | 1259 | 3.48 (2.91–4.15) | 54.53 (45.68–65.09) | 1.07 ± 0.143 | 10.06 | 0.9336 | 4 |
| Bifenazate (200) | 2967 | 26.81 (23.93–30.02) | 233.29 (208.31–261.27) | 1.36 ± 0.078 | 15.03 | 0.9840 | 5 |
| Etoxazole (50) | 1160 | 3.99 (3.35–4.75) | 29.69 (24.94–35.36) | 1.47 ± 0.108 | 11.69 | 0.9738 | 5 |
| Hexythiazox (50) | 1164 | 4.18 (3.55–4.92) | 18.72 (15.89–22.05) | 1.97 ± 0.181 | 16.11 | 0.9673 | 4 |
| Spinosad (90) | 1063 | 18.28 (15.28–21.86) | 147.51 (123.33–176.43) | 1.41 ± 0.075 | 5.22 | 0.9860 | 5 |
| Plant extract (%) | | | | | | | |
| Neem oil | 1027 | 1.77 (1.51–2.06) | 6.90 (5.91–8.06) | 2.16 ± 0.133 | 5.79 | 0.9816 | 5 |
| Entomopathogen (conidia/mL) | | | | | | | |
| <i>Beauveria bassiana</i> | 761 | 4.89 × 10 ⁷ (3.32 × 10 ⁷ –7.21 × 10 ⁷) | 7.93 × 10 ⁸ (5.38 × 10 ⁸ –1.17 × 10 ⁹) | 0.400 ± 0.053 | 5.68 | 0.9506 | 3 |

LC₅₀, is the lethal concentration of with 50% of the population tested are dead; LC₉₀, is the lethal concentration of with 90% of the population tested are dead.

N = Total number of adult used.

CL = 95% confidential limit.

Estimation of LC₅₀ (ml of a.i./l distilled water) and slope.

* Chemicals (Recommended concentration, ml/l).

The plant extract, neem oil was found to be moderately effective against eggs of *T. urticae*. The LC₅₀ value of neem oil was 1.77 % and LC₉₀ value was 6.90 % against eggs of *T. urticae*.

The entomopathogen, *B. bassiana* was found to be effective against eggs of *T. urticae*. The LC₅₀ value of *B. bassiana* was 4.89 × 10⁷ conidia/ml and LC₉₀ value was 7.93 × 10⁸ conidia/ml

against eggs of *T. urticae*.

DISCUSSION

The preceding results from this study shows that *T. urticae* had no resistance to most of the commonly used acaricides. Although, eggs of the *T. urticae* showed high susceptibility to hexythiazox and spinosad compared to adult, whereas opposite tend was showed against abamectin, bifenthrin, bifenazate and etoxazole at LC₅₀ level.

The *T. urticae* strain used in this study was very susceptible to pesticides. LC₅₀ value of the pesticides was low when compared to values obtained after spraying commercial acaricides on the two-spotted mite, for example abamectin, bifenthrin, bifenazate, etoxazole, hexythiazox and spinosad. Although most of the acaricides were effective against eggs and adults of *T. urticae*, the LC₅₀ value was moderately higher in bifenazate against egg stage. The high degree of resistance may be attributed to the intensive use of chemicals with the same mode of action. Higher frequencies of pesticide applications increase the risk of resistance development in insects and spider mites. Bifenazate inhibits mitochondrial complex III (MoA no. unknown) (Van Nieuwenhuysse *et al.* 2009), causing the rapid knock-down of immature and adult spider mites (Ochiai *et al.* 2007). In choosing an acaricide, those for which resistance has developed should be avoided to reduce further increases of resistant strains in nature. Accordingly, we recommend all acaricides tested in this study to be used to manage *T. urticae* in Bangladesh as no resistance has already been documented. In addition, repeated use of the same or similar pesticides should be avoided as it may lead to increased resistance to the pesticides. Development of resistance can be delayed by rotating acaricides, i.e. successively using acaricides with different modes of action or by using mixtures of several compatible acaricides (Georghio 1980; Ives *et al.* 2011). In this study, the pesticides having different modes of action were effective against *T. urticae*, e.g. abamectin allosterically activate glutamate-gated chloride channels (GluCl_s) causing paralysis, bifenthrin keep sodium channels open, causing hyperexcitation and, in some cases, nerve block, bifenazate act as mitochondrial complex iii electron transport inhibitors; etoxazole and hexythiazox inhibit the enzyme that catalyzes the polymerization of chitin, and spinosad allosterically activate nAChRs, causing hyperexcitation of the nervous system (IRAC 2020b).

The neem oil, *Azadirachta indica* A. Juss (Sapindales: Meliaceae) has insecticide effects against pests of Coleoptera (Ventura and Ito 2000; Venzon *et al.* 2007), Diptera (Souza and Vendramim 2005; Yasmin *et al.* 2008), Hemiptera (Tedeschi *et al.* 2001; Kumar *et al.* 2005; Singha *et al.* 2007), and Lepidoptera (Viana and Prates 2003; Charbonneau *et al.* 2007). The main compound of the neem oil, Azadirachtin, has insecticidal activity and can be found in leaves and fruits. Azadirachtin has higher toxicity by ingestion than by contact, which permits it to be used to some extent selectively against phytophagous pests (Akol *et al.* 2002; Gaspari *et al.* 2007). Although natural enemies may ingest contaminated prey, adverse indirect effects may be insignificant because 90% of azadirachtin consumed is removed from the body of phytophagous insects seven hours after ingestion (Bond *et al.* 2012).

There are many studies regarding the use of plant extracts for the control of the *T. urticae*. Although many of these studies have delivered successful results, others have not demonstrated the level of expected control over this mite population. Therefore, a greater understanding of the mechanisms of action presented by molecules that demonstrate biological activity on these mites and the way in which these molecules interact is extremely important. Brito *et al.* (2006) reported the highest pesticidal potential as neem based on a comparative toxicity of different commercial products. It was applied not only on the *T. urticae* but also on its predators, *Euseius alatus* DeLeon and *Phytoseiulus macropilis* Banks (Acari: Phytoseiidae). They reported that the formulation of the product Neemseto (1%) showed the best result on the *T. urticae* by topical contact. They also used different concentrations (0.25%, 0.5%, and 1.0%) and found that the product had a repellent effect

on *T. urticae* and *E. alatus*; however, it did not affect the *P. macropilis*. In addition, the Neemseto revealed an important reduction of fecundity in *T. urticae*, but a significant decrease was only found when mites were exposed to the highest concentrations on the predatory mites. It was suggested that neem could be used as a promising option for the management of the two-spotted spider mites within integrated pest management systems given its relative compatibility with predatory mites.

Entomopathogenic fungi establish the largest single group of insect pathogens among microorganisms. Entomopathogenic fungi enter into their hosts by infection process: penetration of the host cuticle or put pressure on cuticle by making appressorium and then penetrate by penetration peg (Sandhu *et al.* 1995). When the pathogen reaches and adheres to the host surface, it proceeds with quick germination and growth which are intensely influenced by the accessibility of water, nutrients, oxygen as well as pH, and temperature, and by the effects of toxic host-surface compound. Entomopathogenic fungi with restricted host range appear to have more specific requirements for germination (Leger *et al.* 1989).

Gatarayihya *et al.* (2012) reported that mortality caused by 62 *B. bassiana* isolates on *T. urticae* adults ranged between 0.5 and 92.8%; and 23 isolates resulted in more than 50% mortality. The discrepancy in virulence among the different *B. bassiana* isolates to *T. urticae* may be associated with the enzymes produced by each isolate. De La Rosa *et al.* (1997) suggested that the differences in virulence of entomopathogenic fungal isolates are possibly associated with the presence of enzymes that affect the penetration process of the fungus. Secondary metabolites produced by entomopathogen fungi for example, toxins such as beauvericin, present in *B. bassiana*, which vary could contribute to the observed variation in virulence (Roberts and St. Leger 2004). The results observed in present study showed effectiveness against eggs and adults of *T. urticae*. Similarly, Oliveira *et al.* (2002) worked with *B. bassiana* isolates at 1×10^8 conidia ml⁻¹ and the red mite *Oligonychus yothersi* (Acari: Tetranychidae), recorded 77.0% to 98.0% mortality. All tested fungi proved to be pathogenic to *T. urticae* adults. A similar level of control by *B. bassiana* isolates against tetranychid mites in laboratory trials was observed by Barreto *et al.* (2004) and Wekesa *et al.* (2006). The use of *B. bassiana* is effective against spider mites because it propagates their spores very easily between insect bodies (Wang and Xu 2012).

In the present study, all the selected acaricides, entomopathogen, botanical were found effective in the laboratory condition against two spotted spider mites. Among all the chemical insecticides, abamectin was found very effective against eggs and female adult mites. The promising result found in case of entomopathogen, *B. bassiana*. The botanical as neem oil also found effective against two spotted spider mites.

From the experimental findings in this study, it can be concluded that all tested treatments can be used for the management of two spotted spider mite, however, the entomopathogenic fungus, *B. bassiana* and botanical will be promising alternatives for successful management of *T. urticae* in IPM programs. It is advisable that sucking insects can be controlled by insecticidal compounds with different MoA and repeated use of any insecticide can lead to resistance to that specific insecticide. In addition, if insects become resistant because of a change in the target site of the insecticide, there is a high risk that insects develop resistance to all insecticides within the same MoA group. Therefore, rotating the use of pesticides having different mode of action is suggested for effective management of spider mites. More studies regarding formulation and mode of action, safety and ecotoxicity are needed before field application.

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سنجش آزمایشگاهی شش آفتکش، یک قارچ پاتوژن حشرات و یک آفتکش گیاهی روی کنه تارتن دو لکه‌ای، *Tetranychus urticae* Koch (Acari: Tetranychidae)

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چکیده

کنه تارتن دو لکه‌ای، *Tetranychus urticae* Koch (Acari: Tetranychidae) آفتی مهمی است که به محصولات مختلف کشاورزی در بنگلادش حمله می‌کند. رویکرد استفاده از انواع آفتکش‌ها (گیاهی و شیمیایی) با شیوه‌های مختلف عملکرد و آفتکش‌های زیستی برای نخستین بار برای گسترش راهبردهای کنترل پایدار آفات در برابر کنه‌های تارتن مورد استفاده قرار گرفت. در این بررسی، اثر شش حشره‌کش شیمیایی یعنی آبامکتین، بایفنترین، بایفنازیت، اتوکسازول، هکسی‌تیاژوکس، و اسپینوساد، و قارچ پاتوژن حشرات *Beauveria bassiana* و روغن گیاهی نیم علیه مرحله‌های کنه کامل و تخم *T. urticae* ارزیابی شد. کنه‌های تیمار شده در شرایط کنترل شده در 1 ± 25 درجه سلسیوس، ۶۰-۷۰ درصد رطوبت نسبی و دوره نوری ۱۶:۸ ساعت (روشنایی: تاریکی) نگهداری شدند. مقادیر LC_{50} کلیه آفتکش‌های شیمیایی [برای کنه‌های کامل، آبامکتین (۰/۵۱ میلی‌لیتر در لیتر)، بایفنترین (۳/۲۶ میلی‌لیتر در لیتر)، بایفنزیت (۳/۸۲ میلی‌لیتر در لیتر)، هکسی‌تیاژوکس (۳/۲۷ میلی‌لیتر در لیتر) و اسپینوساد (۸۳/۳ میلی‌لیتر در لیتر)؛ برای تخم‌ها، آبامکتین (۰/۵۶ میلی‌لیتر در لیتر)، بایفنترین (۳/۴۸ میلی‌لیتر در لیتر)، بایفنزیت (۲۶/۸۱ میلی‌لیتر در لیتر)، هکسی‌تیاژوکس (۴/۱۸ میلی‌لیتر در لیتر) و اسپینوساد (۲۸/۱۸ میلی‌لیتر در لیتر)] حاکی از آن است که آنها در برابر تخم‌ها و کنه‌های کامل ماده *T. urticae* موثرند. اتوکسازول ($LC_{50} = ۳/۹۹$ میلی‌لیتر در لیتر) توصیه شده در مقابل تخم مناسب تشخیص داده شد. مشخص شد که روغن نیم به نسبت موثر است (برای کنه کامل، $LC_{50} = ۲۶/۱$ درصد؛ برای تخم، $LC_{50} = ۱/۷۷$ درصد) در برابر تخم‌ها و ماده‌های کامل *T. urticae*. همچنین قارچ *B. bassiana* در برابر تخم‌ها و کنه‌های ماده *T. urticae* موثر واقع شد. سرانجام، می‌توان نتیجه گرفت که همه آفتکش‌های شیمیایی مورد استفاده در این مطالعه برای مدیریت کنه تارتن دو لکه‌ای هر چند موثر بودند اما قارچ *B. bassiana* و روغن نیم جایگزین‌های امیدوارکننده‌ای برای مدیریت موفقیت آمیز *T. urticae* در برنامه مدیریت تلفیقی آفت هستند.

واژگان کلیدی: قارچ *B. bassiana*؛ آفتکش‌های شیمیایی؛ مدیریت تلفیقی آفات؛ کنه تارتن؛ سمیت.

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