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Comparative Efficacy of Reduced-risk pesticides, Entomopathogenic Fungus and Botanical for the Management of Red Spider Mite *Tetranychus macfarlanei* Baker and Pritchard (Acari: Tetranychidae)

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ABSTRACT

Tetranychus macfarlanei Baker & Pritchard, a serious phytophagous pest of many crops with economic importance. In this study, the effects of six reduced risk pesticides viz. abamectin, bifenthrin, bifenazate, etoxazole, hexythiazox, and spinosad with various mode of action, entomopathogenic fungus *Beauveria bassiana* and botanical neem oil were evaluated against adult and egg stages of *T. macfarlanei* under laboratory conditions. The mortality of adult female and eggs of *T. macfarlanei* in all chemicals are dose dependent. The response of adult female against different pesticides between observed and expected values were closely fitted. The LC_{50} values of all chemicals except spinosad were effective against eggs and all chemicals except etoxazole were effective against adult females of the *T. macfarlanei*. Etoxazole, known for inhibiting hatching and moulting might be recommended against egg stage as they found suitable but were not much effective against adult mites (LC_{50} = 340.83 and 0.55 ppm for adult and egg, respectively). Neem oil was found to be moderately effective against eggs and adult females of *T. macfarlanei* (LC_{50} = 2.75 and 2.73% for adult and egg, respectively). The entomopathogen, *B. bassiana* was also found to be effective against eggs and adult females of *T. macfarlanei* (LC_{50} = 3.63×10^7 and 3.98×10^7 spore mL^{-1} for adult and egg, respectively). Finally, it could be concluded that all pesticides except spinosad (LC_{50} > 2800 ppm for egg) can be used for the management of spider mite but the entomopathogenic fungus *B. bassiana* and the botanical neem oil will be promising alternatives for successful management of *T. macfarlanei* in IPM program.

Keywords: Spider mite, reduced risk pesticide, botanical, entomopathogen, IPM

INTRODUCTION

The spider mite, *Tetranychus macfarlanei* Baker & Pritchard has been reported from India, Madagascar, Mauritius and the Canary Islands, and can infest a wide range of crops in the plant families Malvaceae, Fabaceae, Cucurbitaceae, Convolvulaceae and Solanaceae (Jeppson et al., 1975; Bolland et al., 1998). Symptoms of infestation by *T. macfarlanei* first appear on leaves, as a pronounced yellowish hue, then the leaves wilt and finally drop, especially during dry periods (Ullah et al., 2012). *Tetranychus macfarlanei* has spread throughout Bangladesh and causes serious damage to a variety of crops such as jute, bean, eggplant and bottle gourd (Ullah et al., 2012; Ullah & Gotoh, 2013).

Acaricides play an important role in the management of spider mite populations but their high reproductive potential and very short life cycle tied with frequent applications of acaricides facilitate resistance development resulting in failure of control (Nauen et al., 2001). The development of resistance is proving to be a great obstacle in the effective integrated mite management programs in a field. The collective incidence of pesticide resistance has led to an interest in developing pesticides with alternative modes of action, lower environmental impact, greater compatibility with integrated pest management programmes and reduced health risk to humans and wildlife (Dekeyser, 2005; Ullah & Gotoh, 2013). In order to provide sustainable control of spider mite populations it is becoming more important to design specific resistance management strategies, e.g. the resistance management guidelines published by the Insecticide Resistance Action Committee (IRAC, 2020) especially for spider mite control by which the number of individuals retain below a certain threshold value. Proper attention is required in selecting acaricides for managing the spider mites as spider mites have a remarkable intrinsic potential for rapid evolution of resistance (Croft & Van de Baan, 1988; Van Leeuwen et al., 2009). The Arthropod Pesticide Resistance Database (APRD) reports nearly 920 cases of acaricide resistance in phytophagous mites specially from the family Tetranychidae (Mota-Sanchez & Wise, 2020). Therefore, it is essential to develop and apply new acaricides with novel biochemical modes of action and to optimize their use with the intention of prevent or delay the development of resistance and prolong their life span (Dekeyser, 2005).

There are many tetranychid pest species has been identified in Bangladesh and cause serious threat to the production of many agricultural crops including vegetables. Although many insecticides and acaricides have been recommended to farmers to control spider mites in Bangladesh, a major problem in controlling

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spider mites is their ability to rapidly develop resistance to various chemicals after only a few applications. Many entomopathogenic fungus and plant-derived essential oils possessed acaricidal properties against different soft-bodied arthropod pests including spider mites. The approach of different reduced risk pesticides (botanical and chemical) and bio-pesticides will be an effective approach to develop sustainable pest control strategies against spider mites. The challenge lies in developing technologies that make positive contributions in the right direction for the management of spider mites considering the environmental issues. Therefore, we aimed to determine the efficacy of six reduced risk pesticides viz. abamectin, bifenthrin, bifenazate, etoxazole, hexythiazox, spinosad, entomopathogenic fungus *Beauveria bassiana* and botanical neem oil against adult and egg stages of *T. macfarlanei* under laboratory conditions. The findings of this study may develop an alternative IPM strategies such as biological control (entomopathogenic fungus) and botanicals to supplement the use of reduced-risk pesticides that are presently being used.

MATERIALS AND METHODS

Rearing of spider mites

The mite strain of *T. macfarlanei* was independently maintained on upside down leaf discs (ca. 25 cm^2) of bean, *Dolichos lablab* L., placed on water-saturated polyurethane mats in plastic Petri dishes (90-mm diameter, 20-mm depth) and were kept at 25 ± 1 °C, 60-70% relative humidity, and 16L: 8D 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 dried out or overexploited by feeding spider mites (Ullah et al., 2014).

Tested chemicals, plant extract and entomopathogenic fungus

The pesticides tested 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), Spinosad (Tracer 45 SC); entomopathogen *B. bassiana* (Botanigard^{ES}) and neem oil (Bioneem) used in this experiment. These pesticides (abamectin (0.12, 0.24, 0.48, 0.96, 1.92 and 3.84 ppm), bifenthrin (1, 2, 4, 8, 16, 32 and 64 ppm), bifenazate (2, 4, 8, 16, 32, 64 and 128 ppm), etoxazole (0.5, 1, 2, 4, 8, 16, 32 and 64 ppm), hexythiazox (1, 2, 4, 8, 16 and 32 ppm), spinosad (0, 4.5, 9, 18, 36, 72, 144, and 288ppm)), *B. bassiana* (1×10^4, 1×10^5, 1×10^6,1×10^7 and 1×10^8) and neem oil (0.5, 1, 2, 4, 6 , 8 and 10%) were suspended in distilled water just before treatments.

Generating uniformly aged females of *T. macfarlanei*

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. Sex-specific body size differences were used to determine their sex after reaching adulthood. Three to five days old female adults obtained within a day after emergence were used in the bioassay experiment (Ullah et al., 2017).

Toxicity to adults

To test the activity of the six chemicals, *B. bassiana* and neem oil on adult females, 15 3-5 day-old mated females of *T. macfarlanei* were placed on a new bean leaf disc (20-mm diameter). After 24 h, dead or injured individuals were removed. Suspensions of the pesticides [abamectin (0.12, 0.24, 0.48, 0.96, 1.92, 3.84 and 7.68 ppm), bifenthrin (1, 2, 4, 8, 16, 32 and 64 ppm), bifenazate (2, 4, 8, 16, 32, 64 and 128 ppm), etoxazole (0.5, 1, 2, 4, 8, 16, 32 and 64 ppm), hexythiazox (1, 2, 4, 8, 16, 32 and 64 ppm), spinosad (0, 4.5, 9, 18, 36, 72, 144, and 288 ppm)], *B. bassiana* (1×10^4, 1×10^5, 1×10^6,1×10^7 and 1×10^8 spore/ml) and neem oil (0.5, 1, 2, 4, 6 , 8 and 10%) were sprayed onto the leaf disc with mites (359-480 adult female), using a hand sprayer at a rate of 1 ml/cm^2. The sprayed samples were dried in the shade and then maintained at 25 ± 1 °C with a 16L: 8D photoperiod for 72 h. Mites that did not move their appendages when touched with a fine brush were scored as "dead".

Toxicity to eggs

To test ovicidal activity of the pesticides, entomopathogen and plant extract, 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. Then the 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 ppm), bifenthrin (1, 2, 4, 8, 16, 32 and 64 ppm), bifenazate (2, 4, 8, 16, 32, 64 and 128 ppm), etoxazole (0.5, 1, 2, 4, 8, 16, 32 and 64 ppm), hexythiazox (1, 2, 4, 8, 16 and 32 ppm), spinosad (0, 4.5, 9, 18, 36, 72, 144, and 288ppm)], *B. bassiana* (1×10^4, 1×10^5, 1×10^6,1×10^7 and 1×10^8) and neem oil (0.5, 1, 2, 4, 6 , 8 and 10%) suspensions were sprayed onto the leaf disc with eggs (752-2083 eggs) at a rate of 1 ml/cm^2. The eggs were kept at 25 °C with a 16L: 8D photoperiod for 192 h. Eggs that had not hatched were scored as "dead". All eggs for *T. macfarlanei* tested had either hatched or died within 144 h at 25 °C. The mortality of hatched larvae was also measured 192 h after treatments.

Statistical analysis

Five to eight concentrations per treatment with four replicates each were tested. Leaf discs sprayed with distilled water were served as control. Pooled data for either adult females or eggs were subjected to Probit analysis and values of LC_{50} and LC_{90} with 95% confidential limits (CL) were estimated (Finney, 1971) using Abbott's correction for natural mortality (Abbott, 1925):

Corrected mortality (%) = [100 × (Treated - Control))/((100-Control)]

RESULTS

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

The response of adult female in all chemicals were dose dependent. The mortality of adult female against different pesticides between observed value and expected values are closely fitted. Of the six (6) chemicals tested for LC_{50}, five (5) were found to be much lower than the recommended concentration for each of the chemicals while etoxazole (340.83 mg/L) had an approximately higher LC_{50} (Table 1). Among the five pesticides, abamectin (0.33 mg/L) was found to be most toxic to *T. macfarlanei* compared to bifenazate (5.84 mg/L), bifenthrin (2.92 mg/L), hexythiazox (3.03 mg/L), and spinosad (12.36 mg/L). The LC_{90} values have also showed similar responses, but etoxazole was not effective against adult of *T. macfarlanei*.

The entomopathogen, *B. bassiana* was found to be effective against adult female of *T. macfarlanei*. The LC_{50} value of *B. bassiana* was at $3.63×10^7$ spore/ml and LC_{90} value was at $2.68×10^8$ spore/ml. The concentration of *B. bassiana* and response of adult female was concentration dependent and the observed and expected values of response were closely fitted.

Neem oil was also found to be less effective against adult female of *T. macfarlanei*. The LC_{50} value of neem oil was at 2.75% and LC_{90} value was at 13.98% against adult female of *T. macfarlanei* (Table 1). The concentration of neem oil and response of adult female was concentration dependent and the observed and expected values of response were closely fitted.

Table 1. Mortality in adult females of the *Tetranychus macfarlanei* to 6 pesticides, plant extract (neem oil) and entomopathogen (*Beauveria bassiana*)

*Chemicals, neem oil and entomopathogen	N	LC_{50} (95% CL)		LC_{90} (95% CL)		Slope ± SE	R^2	df
Abamectin (12)	359	0.33	(0.25-0.43)	1.42	(1.09 - 1.85)	2.02±0.282	0.95	3
Bifenthrin (25)	419	2.92	(2.24-3.82)	16.70	(12.78 – 21.83)	1.69±0.076	0.99	4
Bifenazate (200)	360	5.84	(4.36-7.82)	44.62	(33.33 – 59.73)	1.45±0.062	0.99	3
Etoxazole (50)	480	340.83	(219.12-530.17)	102043.12	(65601.52 - 158728.02)	0.52±0.018	0.99	5
Hexythiazox (50)	370	3.03	(2.35-3.92)	13.48	(10.43 – 17.44)	1.98±0.423	0.88	3
Spinosad (90)	420	12.36	(9.24-16.53)	143.42	(107.24 – 191.80)	1.20±0.168	0.93	4
Plant extract (%)								
Neem oil	480	2.75	(2.19-3.46)	13.98	(11.12 – 17.57)	1.82±0.272	0.90	5
Entomopathogen (Spore/mL)								
Beauveria bassiana	360	$3.63×10^7$	($2.14×10^7$-$6.14×10^7$)	$2.68×10^8$	($1.58×10^8$-$4.54×10^8$)	0.45±0.011	0.99	3

LC_{50}, is the lethal concentration of with 50% of the population tested are dead
LC_{90}, is the lethal concentration of with 90% of the population tested are dead
N = Total number of eggs used
CL = 95% confidential limit
Estimation of LC_{50} (mg of a.i./l distilled water) and slope
*Chemicals (Recommended concentration, mg/l)

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

The response of egg mortality considering all pesticides are dose dependent. The observed and expected values of the response of eggs were closely fitted. Of the six (6) pesticides tested for LC_{50}, five (5) were found to be much lower than the recommended concentration for each of the pesticides while spinosad had an approximately higher LC_{50} (2839.28 mg/L) (Table 2).The LC_{90} values have also showed similar responses to eggs. Abamectin (0.25 mg/L) was found the most toxic to *T. macarlanei* compared to bifenthrin (3.39 mg/L), etoxazole (0.55 mg/L), hexythiazox (1.04 mg/L) and bifenazate (35.16mg/L) (Table 2). The LC_{90} values were also showed similar response, but spinosad was not effective against eggs of *T. macarlanei*.

The *B. bassiana* was found to be effective against eggs of *T. macarlanei*. The LC_{50} value of *B. bassiana* was at $3.98×10^7$ spore/ml and LC_{90} value was at $8.82×10^8$ spore/ml against eggs of *T. macfarlanei*. The concentration of *B. bassiana* and response of egg mortality of *T. macarlanei* was concentration dependent.

The neem oil was found to be less effective against eggs of *T. macarlanei*. The LC_{50} and LC_{90} values of neem oil were at 2.73% and 9.33%, respectively against eggs of *T. macarlanei* (Table 2). The response of eggs mortality was concentration dependent and the observed and expected values of response are closely fitted.

Table 2. Mortality in eggs of the *Tetranychus macfarlanei* to 6 pesticides, plant extract (neem oil) and entomopathogen (*Beauveria bassiana*)

*Chemicals, neem oil and entomopathogen	N	LC_{50} (95% CL)		LC_{90} (95% CL)		Slope ± SE	R^2	df
Abamectin (12)	776	0.25	(0.21-0.30)	1.03	(0.86 – 1.24)	2.08±0.274	0.95	3
Bifenthrin (25)	884	3.39	(2.85-4.03)	12.69	(10.69 – 15.08)	2.24±0.503	0.83	4
Bifenazate (200)	2083	35.16	(30.28-40.82)	985.60	(848.82 – 1144.42)	0.89±0.151	0.87	5
Etoxazole (50)	850	0.55	(0.45-0.66)	2.26	(1.87 – 2.73)	2.08±0.209	0.97	3
Hexythiazox (50)	752	1.04	(0.83-1.29)	5.98	(4.79 – 7.46)	1.68±0.253	0.94	3
Spinosad (90)	1537	2839.28	(2087.20-3862.36)	75078.15	(55191.19 – 102.130)	0.90±0.320	0.61	5
Plant extract (%)								
Neem oil	1012	2.73	(2.36-3.16)	9.33	(8.06 – 10.80)	2.40±0.234	0.96	5
Entomopathogen (Spore/mL)								
Beauveria bassiana	798	3.98×10^{7}	(2.85×10^{7}-5.55×10^{7})	8.82×10^{8}	(6.33×10^{8}-1.22×10^{9})	0.55±0.046	0.98	3

LC_{50}, is the lethal concentration of with 50% of the population tested are dead
LC_{90}, is the lethal concentration of with 90% of the population tested are dead
N = Total number of eggs used
CL = 95% confidential limit
Estimation of LC_{50} (mg of a.i./l distilled water) and slope
*Chemicals (Recommended concentration, mg/l)

DISCUSSION

The results showed that *T. macfarlanei* are susceptible to most of the reduced-risk chemicals except for spinosad in egg stage and etoxazole in adult stage. The LC_{50} values were much lower than the recommended concentrations for each of the chemicals tested for both adults and eggs of the *T. macfarlanei* except spinosad in egg and etoxazole in adult stage. Since, etoxazole was not recommended against the adults of spider mite species as they inhibit hatching and moulting but do not kill adult mites (Yamada et al., 1987; Yamamoto et al., 1995; Gotoh et al., 2001, 2008).

The inefficacy of chemicals may be attributed to the intensive use of chemicals with the same mode of action. Higher frequencies of pesticide applications reduce the efficacy against insects and spider mites. Individual farmer incentives to control pests may lead to levels of pesticide use that reduce the efficacy of chemicals within a region. For example, some farmers spray pesticide in eggplant about 180 times during a cropping season in Bangladesh (SUSVEG-Asia, 2007). In April 2020, the Arthropod Pesticide Resistance Database (APRD) recorded 16734 cases of resistance developed in 611 arthropods species, of which 1796 cases refer to 81 Acari species. Out of this, 920 cases concern 24 species belonging to the family Tetranychidae (Mota-Sanchez & Wise, 2020). Cross-resistance appears to play a role in resistance development to acaricides against spider mites.

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 pesticides except spinosad and etoxazole has been tested in this study to manage *T. macfarlanei* 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 ineffectiveness to the pesticides. The effectiveness of chemicals may be enhanced by rotating the use of chemicals having different mode of action, i.e. successively using acaricides with different modes of action or by using mixtures of several compatible acaricides (Georghio, 1980; Ives et al., 2011).

At present, acaricide resistance problems are characterized by the development of complex- and/or multi-resistance to acaricides in distinct classes. Chemical control using acaricides is the most extensively used method in integrated management programs of spider mites under field conditions, because of its easy manipulation and low economic cost (Marčić et al., 2011; Ilias et al., 2012; Bernardi et al., 2013; Vassiliou & Kitsis 2013; Van Leeuwen et al., 2015). Multi-resistance to acaricides became an increasing problem in the management of spider mites owed to frequent applications of various chemicals (Osakabe et al., 2009). Development of resistance to one acaricide may lead to cross-resistance to another acaricide that has never been used against mite population (Van Leeuwen et al., 2005; Nicastro et al., 2010; Khalighi et al., 2014; Sugimoto & Osakabe, 2014). Cross-resistance can be explained by various mechanisms, including upregulating of detoxification enzymes (Khalighi et al., 2014; Sugimoto & Osakabe, 2014) and genetic linkage in resistance genes (Uesugi et al., 2002); however, most of the mechanisms are not well investigated (Osakabe et al., 2009). However, understanding the cross-resistance between chemicals provide a basis for the development of resistance management programs (Sparks et al., 2001, 2012). Uesugi et al. (2002) clarified the genetic basis of resistance of chlorfenapyr and etoxazole, and close linkage between the two resistant genes. They argued the strong selective pressure by both acaricides would lead to apparent cross resistance.

In the present study, all selected pesticides (except spinosad and etoxazole), *B. bassiana* and neem oil were found very effective in the laboratory condition against *T. macfarlanei*. Spinosad is highly effective against a wide range of Arthropods including spider mites (Salgado & Sparks, 2005; Dripps et al., 2011). Although monitoring and management of resistance plans were put in prior place to the introduction of spinosad into the market (Salgado & Sparks, 2005; Thompson et al., 2000; Zhao et al., 2006), overuse or misuse of any new insecticide product such as spinosad can be ineffective in such measures, and lead to the development of resistance. Resistance to spinosad was first documented in both laboratory and field-selected strains of *Heliothis virescens* several years after its introduction (Young et al., 2001). It might be possible that the high levels of resistance to spinosad could be developed through extensive selection for many generations. Likewise, a spinosad-selected laboratory strain of *Musca domestica* was also revealed to exhibit spinosad resistance (Shono & Scott, 2003). In the 14 years since the introduction of spinosad, a number of cases of spinosyn resistance have been documented in the Arthropod Pesticide Resistance Database. According to APRD, the apparent rate of resistance development for spinosad is slightly higher than that observed for the organophosphates chlorpyrifos or profenofos, but far less than that observed for the pyrethroids permethrin or delta-methrin, and most similar to the abamectin and imidacloprid (Sparks et al., 2012; Mota-Sanchez & Wise, 2020).

Despite the detrimental consequences involved in the use of synthetic chemicals to control pests, still they are extensively used in all countries over the world. The pressure increased to replace the chemical pesticides with other alternatives that are safe to environment, humans and nontarget organisms. These activities have led to increased development of compounds based on the naturally occurring active ingredients of biological origin, having various biological activities. The use of biopesticides for pest control today is an evolving field in pest management.

Several studies were conducted regarding the use of plant extracts for the control of spider mites (Brito et al., 2006; Islam et al., 2017). Although many of these trials have delivered successful results, others have not demonstrated the level of expected control over this mite species. It was found that the formulation of the product Neemseto (1%) was the one that obtained the best result on the *Teranychus urticae* Koch by topical contact. In the same way, they tested the product at different concentrations (0.25%, 0.5%, and 1.0%) and found that the product had a repellent effect on *T. urticae* (Brito et al., 2006).

Different strains of *B. bassiana* were found effective against spider mites. *B. bassiana* used against *T. urticae* adults at different concentrations were found effective (Gatarayiha et al., 2012). The concentration 1×10^{8} spores mL^{-1} of *B. bassiana* showed the maximum mortality percentage on *T. urticae* (Ullah & Lim, 2015). 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). Our results also showed that the efficacy of *B. bassiana* against *T. macfarlanei* is promising and it would be a suitable option for managing this pest.

CONCLUSION

It can be concluded that all reduced risk pesticides except spinosad and etoxazole can be used but the entomopathogenic fungus, *B. bassiana* and neem oil will be promising alternatives for successful management of *T. macfarlanei* in IPM programme. In addition, rotating the use of pesticides having different mode of action would be more effective for successful management of spider mites. Overall, the establishment of an integrated mite management strategy aimed at lowering spider mite population density may be the best way to manage pesticide resistance.

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