Susceptibility of geographically isolated populations of the Tomato red spider mite (*Tetranychus evansi* Baker & Pritchard) to commonly used acaricides on tomato crops in Kenya

F.J. Toroitich,[1,2] M. Knapp,[3] J.H. Nderitu,[1] F.M. Olubayo,[1] M. Obonyo[4]

[1]Department of Plant Sciences and Crop Protection, Faculty of Agriculture, University of Nairobi, Nairobi, Kenya; [2]African Insect Science for Food and Health (ICIPE), Nairobi, Kenya; [3]Koppert Biological Systems, Postbus, Berkel en Rodenrijs, The Netherlands; [4]Department of Biochemistry, Egerton University, Njoro, Kenya

Abstract

Farmers in Kenya continue to raise concerns of difficulty in managing *Tetranychus evansi*, the most widespread pest species of tomato applying the most commonly used acaricides. This invasive pest species is not only found in Kenya, but in Eastern and Southern Africa, as well as parts of Europe and Asia. In the current study, populations of *T. evansi* were collected from farms in the four major tomato-growing areas of Kenya (Loitoktok, Kibwezi, Athi-River and Subukia) and their susceptibility compared to a laboratory culture (ICIPE) that had been maintained for three years without exposure to acaricides. Susceptibility of *T. evansi* eggs and adults (contact and residual) to Brigade (bifenthrin), Dimethoate (dimethoate), Karate (lambda-cyhalothrin), Kelthane (dicofol), Omite (propargite) and Polytrin (profenofos+cypermethrin) was tested in the laboratory using respective manufacturer's recommended concentrations. Dimethoate resulted in variable ovicidal mortality while Kelthane, Brigade, Karate, Omite and Polytrin had high mortality across all populations. Similarly, adult contact and residual mortality was lower than that of the other chemicals when exposed to Dimethoate regardless of the location. Furthermore, it also had no residual effect on the mites from ICIPE and Kibwezi. On the other hand, Kelthane was most lethal against the mites from all locations followed by Brigade and Polytrin in that order. Omite caused significantly lower mortality on mites from Subukia while Karate produced variable effects on mites from Kibwezi, Loitoktok and Subukia. The implications of these findings are further discussed.

Correspondence: Faith Jebet Toroitich, Department of Plant Science and Crop Protection, Faculty of Agriculture, University of Nairobi, P.O Box 29053, Kangemi, 00625, Kenya.
Tel.: +254.722.274454.
E-mail: fjtoroitich@gmail.com; fjtoroitich@uonbi.ac.ke

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Introduction

The tomato red spider mite, *Tetranychus evansi* Baker & Pritchard, is an important invasive pest species of solanaceous plants not only in Kenya and other parts of Africa (Varela *et al*., 2003; Knapp *et al*., 2003), but in parts of Europe (Ferreira & Carmona, 1995; Ferragut & Escudero, 1999; Migeon, 2005; Castagnoli *et al*., 2006; Tsagkarakou *et al*., 2007) as well as Asia (Ho *et al*., 2004; Gotoh *et al*., 2009). It is believed to have originated from South America (Moutia, 1958) and was first reported in continental Africa in 1979 on tobacco in Zimbabwe (Blair, 1983) from where it spread to other parts of the continent. In Kenya, *T. evansi* was initially reported in 2001 on tomato (Knapp *et al.*, 2003) and has since been reported in several other solanaceous plants in many parts of the country (Toroitich *et al*., 2009). Besides its invasive nature, recent reports indicate that *T. evansi* may be displacing native spider mite species hence posing new pest management challenges (Ferragut *et al*., 2013).

While there are efforts to control *T. evansi* using cultural practices (Saunyama & Knapp, 2003), application of synthetic acaricides remains the method of choice in Kenya. This, however, is faced by the risk of resistance development among pest populations due to prolonged exposure (Cranham & Helle, 1985; Blair, 1989; Tsagkarakou *et al.*, 2002; Nyoni *et al.*, 2011) or even poor application techniques

(Sibanda *et al*., 2000). It has been observed that farmers in Africa frequently apply lower than recommended tank concentrations of chemicals and even use sprayers with inappropriate or worn-out nozzles and do not spray carefully enough leading to poor crop coverage (Sibanda *et al*., 2000; Saunyama & Knapp, 2003). As a result, farmers frequently complain of ineffective acaricides, as the pest populations appear to resurge immediately after application.

It was hypothesised that geographically isolated *T. evansi* populations were likely to differ in their susceptibility to acaricides depending on their history of chemical exposure. A prerequisite was to conduct farmer interviews to establish the predominant chemical(s) used in each region and thereafter test the susceptibility of *T. evansi* populations to the reported chemicals at the respective manufacturer's recommended concentrations.

Materials and methods

The chemical compounds (and respective trade names) commonly used for Red Spider mite control in tomato production in Kenya are listed below (Table 1).

Spider mite collection

Spider mites were collected from tomato fields in Athi-River Division (Machakos County), Kibwezi Division in Makueni County, Loitoktok Division in Kajiado County and Subukia Division in Nakuru County, Kenya.

Twenty to thirty infested tomato leaves were collected from each farm where mites were observed. Mites from six farms in Athi-River, four in Loitoktok, two in Kibwezi and six in Subukia were collected between April and July 2004. Leaf samples were put in paper bags, then placed in a cool box and transported to ICIPE laboratory for examination. In the laboratory, at least 20 males from each of the sampled sites were mounted for identification. At the same time, 60-100 live females were picked from the infested leaves collected per site (no more than one spider mite was taken from a single leaf) for rearing.

The *T. evansi* populations from Athi River, Kibwezi, Loitoktok and Subukia were reared in water isolation cages designed after those described by Dennehy and Granett (1982). Potted tomato plants (variety Cal-J) were placed on top (bottom part) of inverted pots inside a Basin (90 cm diameter) half-filled with water. A clear-sided Perspex cage (30×30×90 cm) whose topside was covered with fine polyester lining (for ventilation) was placed over the potted plants to prevent escape of mites (Figure 1). The spider mites were reared at same conditions of temperature (25±2°C), relative humidity (50-80%) (RH) and photoperiod (12L: 12D) h in the laboratory. An ICIPE laboratory culture previously collected from Mwea (Kirinyaga County) and maintained on tomatoes without exposure to acaricides for three years was used for comparison.

Commercial acaricides were used in the experiments as follows: Polytrin 440 EC (profenofos Q 400 g/L+cypermethrin 40) (Syngenta Crop Protection, Switzerland), Brigade 025EC (bifenthrin 25 g/L) (FMC Corporation, USA), Karate 1.75 EC (lambda-cyhalothrin 17.5 g/L (Syngenta Crop Protection), Dimethoate 40% EC (dimethoate 400 g/L) (Eastchem, China), Omite 57 EC (propargite 57%, Crompton Corporation, USA) and Kelthane EC (dicofol 18.5%) (Rohm and Haas Company, USA).

Ovicidal tests

For each pesticide, twenty *T. evansi* females from respective colonies were transferred onto tomato leaf discs (25 mm diameter) placed lower side up on moist cotton wool in Petri dishes (9 cm diameter). The Petri dishes were placed in rectangular plastic boxes (30×50×10 cm) and maintained at 25±2°C in an incubator. The females were allowed 24 h oviposition time after which, they were removed and the number of eggs adjusted to 20. Pesticide solutions were prepared following respective manufacturer's recommended rates as follows: Polytrin (1.5 L/ha), Brigade (2.5 L/ha), Karate (2.5 L/ha), Dimethoate (1 L/ha), Omite (1 L/ha) and Kelthane (2.5 L/ha). In addition, Aquawet (nonylphenol ethoxylate, Osho Chemicals, Kenya) was added as a sticker to all the chemical solutions and water at 1 L/ha.

The leaf discs containing *T. evansi* eggs from respective locations were dipped in pesticide solutions for five seconds and placed lower side up in plastic Petri dishes containing moist cotton wool. The num-

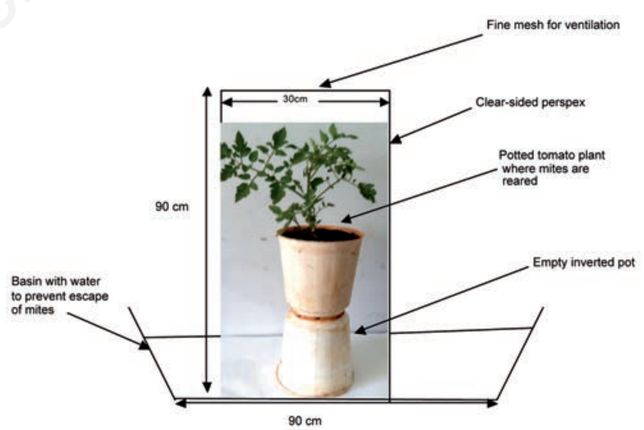

Figure 1. Sketch of the mites rearing cage (not drawn to scale).

Table 1. Chemicals commonly used for Red Spider mite control in tomato production in Kenya (ICIPE, 2003, unpublished survey data).

Compound	Trade name	Class	% of farmers
Dicofol	Kelthane	A	25.8
Lambda-cyhalothrin	Karate	I/A	24.7
Dimethoate	Dimethoate	I/A	22.7
Cypermethrin+profenofos	Polytrin	I/A	19.6
Bifenthrin	Brigade	I/A	13.4
Propargite	Omite	A	10.3

A, acaricide; I, insecticide.

ber of eggs was checked to ensure none were lost in the dipping process. The Petri dishes were placed on a wire mesh inside plastic boxes (30×50×10 cm) and maintained under the same conditions earlier described. Water and Aquawet sticker was used as the negative control. The experiments were arranged in a completely randomized design and replicated six times for all treatments.

The leaves were examined the first time after four days and subsequently daily for another five days for larval emergence. Egg mortality was determined by comparing the number of unhatched eggs with the initial number of post treatment eggs (Agnello *et al.*, 1994).

Adulticidal tests

The bioassays employed were similar to those described by Kabir *et al*. (1993) with some modifications as outlined below.

Modified leaf disc direct method

Twenty, day-old adult *T. evansi* females from the colonies that had been reared for six weeks from the time of collection were transferred onto the lower side of tomato leaf discs (25 mm diameter). The leaf discs containing mites were carefully dipped in the acaricide solutions for 5 seconds before being placed lower side up in plastic Petri dishes containing moist cotton wool. The Petri dishes were left on the bench for one hour to allow the leaves to dry then introduced into plastic boxes (mentioned above) and maintained in an incubator as already described. Similarly, the experiments were arranged in a completely randomized design and replicated six times.

After 24 h the mites were observed and scored as dead, alive or escaped for those which were trapped in the cotton barrier (Agnello *et al*., 1994). Mites were considered dead when they did not react to gentle prodding with a camel hairbrush. Escaped mites were excluded from the analyses.

Leaf disc residue – dipping method

Leaf disc residue – dipping method differs from leaf disc direct method (LDD) slightly in that the leaf discs were first dipped in acaricide solutions for five seconds, then placed in Petri dishes and allowed to dry at room temperature for about 30 min. Similar to the LDD, spider mites were transferred onto the discs and observed after 24 h and scored as dead, alive or escaped. Escaped mites were excluded from the analyses.

Data analysis

Data on egg and adult mortalities were arcsine square root transformed then subjected to one-way analysis of variance (Proc GLM) and means separated by Student-Newman-Keuls test (SAS 9.1, 1990). However, data presented in the tables are actual percent mortalities. In addition, only for ovicidal tests, control mortality was corrected using Abbott's formula (Abbott, 1925) while for adult mortalities actual uncorrected figures were used.

Results

Acaricides used for spider mites control

In Loitoktok, three of four sampled farmers used Dimethoate while the remaining one used Polytrin. On the other hand, in Subukia three farmers used Polytrin, two-used Tata Alpha (alpha-cypermethrin) and the remaining one used Karate. In Kibwezi, both farmers used Karate but one alternated its use with Brigade. In Athi-River, four of six farmers used Karate while each of the remaining ones used Dimethoate and Kelthane.

The farmers in Subukia complained of spider mites being a persistent problem in the area and explained their choice of Polytrin as the most effective chemical against insects and spider mites. In Kibwezi and Athi River, spider mites were not cited as a major problem, although the farmers conventionally applied acaricides albeit infrequently. Similarly, most farmers in Loitoktok had scanty knowledge of spider mites hence they were not ranked as important pest of tomato. However, the farmers routinely applied Dimethoate against all pest infestations.

Ovicidal tests

Compared to the other chemicals, Dimethoate resulted in variable ovicidal mortality among the tested *T. evansi* populations. Significantly lower mortality was observed in Subukia and Kibwezi populations while Loitoktok, ICIPE and Athi River ones had minimal susceptibility comparable to the negative control. On the other hand, treatments by Kelthane, Brigade, Karate, Omite and Polytrin resulted in significantly (<0.0001) high (about 100%) egg mortality in all populations (Table 2).

Adulticidal tests: contact mortality (leaf disc direct method)

In a trend similar to the ovicidal test, mortality of adult mites was significantly lower (P=0.0099) than the other chemicals when exposed to Dimethoate regardless of the location (Table 3). However, with the exception of Athi River where mortality did not differ significantly from the negative control, in the other locations some differences were reported. Significant differences (P=0.0202) were observed among populations when Karate was applied as follows: highest mortality attained was with ICIPE and Kibwezi mites at 70% and as low as 43% for those from Loitoktok. This is much lower than the response to

Table 2. Mortality of spider mite eggs following treatment by various acaricides (N=6 in all cases).

Treatment/Location	Athi River	ICIPE	Kibwezi	Loitoktok	Subukia	P-values
Brigade	100.0±0^{*a}	100.0±0^{*a}	100.0±0^{*a}	100.0±0^{*a}	100.0±0^{*a}	-
Control	0.0±0^{**a}	0.0±0^{**a}	0.0±0^{***a}	0.0±0^{**a}	0.0±0^{***a}	-
Dimethoate	0.0±0^{**c}	8.7±3.4^{**c}	33.3±7.6^{**b}	13.0±4.3^{**c}	54.05±10.9^{**a}	<0.0001
Karate	98.8±1.17^{*a}	100.0±0^{*a}	96.7±2.1^{*a}	99.0±1.0^{*a}	93.5±6.5^{*a}	0.6084
Kelthane	100.0±0^{*a}	100.0±0^{*a}	100.0±0^{*a}	100.0±0^{*a}	100.0±0^{*a}	-
Omite	100.0±0^{*a}	100.0±0^{*a}	100.0±0^{*a}	100.0±0^{*a}	100.0±0^{*a}	-
Polytrin	100.0±0^{*a}	100.0±0^{*a}	100.0±0^{*a}	100.0±0^{*a}	100.0±0^{*a}	-
P-values	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-

*,**,***Means±SE down the columns are significantly different (P=0.05) [Analysis of variance (ANOVA) and Student-Newman-Keuls (SNK)]; ^{a,b,c}means±SE followed by different letters across the rows are significantly different (P=0.05) (ANOVA and SNK).

Omite, Brigade, Polyrin and Kelthane which caused nearly 100% mortality among all spider mite populations.

Residual effect of the acaricides on *T. evansi* (leaf disc residue – dipping method)

The residual effect of various acaricides followed the pattern observed with contact mortality (Table 4). Compared to the other chemicals Dimethoate had the least significant residual effect on *T. evansi* adults from Subukia, Loitoktok and Athi River while it had no effect on those from ICIPE and Kibwezi. On the other hand, Kelthane was most lethal against the mites from all locations followed by Brigade and Polytrin in that order. Omite caused significantly lower (P=0.0363) mortality on mites from Subukia while Karate produced variable effects on mites from Kibwezi, Loitoktok and Subukia.

Discussion and conclusions

Farmers in the major tomato producing areas of Kenya appear to prefer Omite, Polytrin, Dimethoate and Karate as acaricides. It is evident that most of these acaricides are still effective against *T. evansi* both as ovicides and adulticides. The current study shows that Kelthane (dicofol) was the most effective acaricide. This corroborates earlier findings that reported high efficacy of dicofol against *Tetranychus urticae* Koch (Wilson *et al*., 1995). Dicofol, cyhexathin, fenbutatin oxide and propargite are among the few selective acaricides that have been successfully used in integrated pest management (IPM) of *T. urticae* (Rizzieri *et al*., 1988; Jacobson *et al*., 1999). They are considered useful in IPM due to the fact that they are only slightly-to-moderately toxic to phytoseiid mites that are used to control phytophagous mite species (Van Leeuwen *et al*., 2005).

From the current findings, Omite (propargite) was observed to be highly effective against *T. evansi* in the laboratory, although this contradicted complaints by farmers of poor control outcomes using this chemical in the field. It is highly likely that the varied results observed in the field could be caused by other factors commonly known to be behind pesticide abuse in Africa among them: poor application techniques, low levels of education, inadequate public awareness and lack of understanding of pest behavior (Schwab *et al*., 1995).

On the other hand, Polytrin (profenofos+cypermethrin) and Brigade (bifenthrin) are broad spectrum chemicals also used as insecticides and have shown high efficacy against *T. evansi*. However, resistance to bifenthrin was reported in *T. urticae* after four seasons of continuous use in Australian cotton fields (Herron *et al*., 2001). As such, its effectiveness in the current study could be attributed to the fact that it was the least applied acaricide in the farms sampled in Kenya. Another interesting observation is that *T. evansi* from Loitoktok appears tolerant to Karate (lambda-cyhalothrin) yet the farmers from that region had not used it for spider mite control. This is very likely the outcome of two possibilities: i) the farmers could have been using Karate to control other pests in which case *T. evansi* was not a target or ii) it could be a case of cross-resistance since most farmers used Dimethoate. The possibility of cross-resistance between an organophosphate (dimethoate) and a pyrethroid (bifenthrin) has been reported before in *T. urticae* (Yang *et al*., 2002).

From the findings of the current study, tolerance to Dimethoate appears to have been widespread across the different populations of

Table 3. Mortality of adult mites due to contact with various acaricides (N=6 in all cases).

Treatment/Location	Athi River	ICIPE	Kibwezi	Loitoktok	Subukia	P-values
Brigade	100±0^{*a}	100±0^{*a}	100±0^{*a}	96.82±0.05^{*a}	100±0^{*a}	0.4261
Control	4.46±0.05^{***a}	0.0±0^{****a}	7.01±0.05^{****a}	2.55±0.04^{****a}	3.38±0.05^{****a}	0.4404
Dimethoate	11.46±0.09^{***b}	7.01±0.05^{***b}	48.4±0.19^{***a}	27.4±0.14^{***ab}	27.39±0.09^{***ab}	0.0099
Karate	56.69±0.08^{**ab}	71.34±0.05^{**a}	70.1±0.11^{**a}	43.95± 0.07^{**b}	63.06±0.12^{**ab}	0.0202
Kelthane	100±0^{*a}	100±0^{*a}	100±0^{*a}	100±0^{*a}	100±0^{*a}	-
Omite	100±0^{*a}	97.45±0.04^{*a}	100±0^{*a}	100±0^{*a}	100±0^{*a}	0.4261
Polytrin	97.45±0.04^{*a}	100±0^{*a}	100±0^{*a}	100±0^{*a}	100±0^{*a}	0.4261
P-values	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	

*,**,***,****Means±SE down the columns are significantly different (P=0.05) [Analysis of variance (ANOVA) and Student-Newman-Keuls (SNK)]; ^{a,b}means±SE followed by different letters across the rows are significantly different (P=0.05) (ANOVA and SNK).

Table 4. Mortality of adult mites due to residual effect of various acaricides (N=6 in all cases).

Treatment/Location	Athi River	ICIPE	Kibwezi	Loitoktok	Subukia	P-values
Brigade	96.82±0.05^{*a}	100.0±0^{*a}	70.01±0.30^{*a}	92.99±0.07^{*a}	89.17±0.11^{*,**a}	0.2202
Control	7.01±0.05^{**a}	0.0±0^{**a}	0.0±0^{**a}	5.73±0.06^{***a}	5.73±0.06^{***a}	0.2256
Dimethoate	8.28±0.06^{**a}	0.0±0^{**a}	0.0±0^{**a}	3.82±0.04^{***a}	9.55±0.05^{***a}	0.0403
Karate	81.53±0.15^{*ab}	95.54±0.07^{*a}	22.29±0.26^{**c}	50.32±0.18**bc	75.16±0.09^{**abc}	0.0004
Kelthane	100±0^{*a}	100±0^{*a}	100±0^{*a}	100±0^{*a}	100±0^{*a}	-
Omite	80.25±0.12^{*ab}	93.63±0.07^{*a}	75.8±0.25^{*ab}	97.45±0.05^{*a}	60.51±0.07^{**a}	0.0363
Polytrin	100±0^{*a}	78.98±0.21^{*a}	85.99±0.14^{*a}	88.54±0.18^{*a}	87.26±0.15^{***a}	0.6725
P-values	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	

*,**,***Means±SE down the columns are significantly different (P=0.05) [Analysis of variance (ANOVA) and Student-Newman-Keuls (SNK)]; ^{a,b,c}means±SE followed by different letters across the rows are significantly different (P=0.05) (ANOVA and SNK).

spider mites. This corroborates earlier findings, which reported widespread tolerance of *T. evansi* to this chemical at levels as high as 1000-fold in Zimbabwe (Blair, 1989). Similarly, in other spider mite species, 289.2 fold and 104.7 fold decrease in susceptibility to dimethoate by *Oligonychus pratensis* Banks and *T. urticae* respectively after only 10 cycles of exposure was reported (Yang *et al*., 2002). Resistance to another organophosphate (chlorpyrifos) by *T. evansi* was also reported in mites from Zimbabwe (Blair, 1989), Malawi and Southern France (Nyoni *et al*., 2011; Carvalho *et al.,* 2012).

The spider mites which had been maintained in ICIPE laboratory free of any chemicals also showed high levels of tolerance to dimethoate. Therefore, it is not clear whether resistance to some organophosphates is innate in this species or as a result of selection pressures. On the other hand, it is also possible that the ICIPE culture could have had prior exposure to chemicals before laboratory rearing. Yang *et al*. (2002) observed that even after three months without pesticide exposure, there was reduced susceptibility to dimethoate in the two-spotted spider mites (*T. urticae*). However, it is unknown whether this would be the case even after three years of continuous rearing without pesticide exposure as was observed in the current study. This observation is important as it could have serious ramifications for future pest control programs; especially in areas like Athi River and Kibwezi where the farmers perceive that mites have not acquired pest status yet they continue to use broad spectrum chemicals for routine pest management.

With the foregoing, it is possible to conclude, dimethoate should not be recommended for *T. evansi* control but instead, the specific acaricides Omite (propargite) and Kelthane (dicofol) should be used. From the findings of this study, farmer complaints of inability to control spider mites using the tested chemicals could be attributed to poor application methods due to insufficient knowledge of the pest behaviour. Therefore, there is need for farmer sensitization on proper application methods and acaricide rotation. There are acaricides that have been recently developed with new and complex modes of action that could be used together with other management options or in acaricide rotation strategies in order to delay resistance development. Gotoh *et al*. (2010) reported that bifenazate, cyenopyrafen, milbemectin, spirodiclofen and tebufenpyrad caused high toxicity to *T. evansi* from nine localities in the world. This means that these new products can also be incorporated into acaricide rotations taking into consideration their individual modes of action. The use of acaricides should be limited by adhering to IPM principles including cultural control practices to reduce *T. evansi* populations. In addition, other compatible strategies such as biological control using the phytoseiid mite *Phytoseiulus longipes* Evans (Furtado *et al*., 2007; Ferrero *et al*., 2007, 2011) as well as some strains of the entomopathogenic fungi *Metarhizium anisopliae* Metsch and *Beauveria bassiana* Balsamo (Bugeme *et al*., 2008; Maniania *et al*., 2008) could be considered as alternative control agents in order to minimize acaricide use.

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