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Full Length Research Paper

Efficacy of Syringa (*Melia Azedarach* L.) extracts on eggs, nymphs and adult red spider mites, *Tetranychus* spp. (Acari: Tetranychidae) on tomatoes

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This study evaluated the effect of Syringa (*Melia azedarach*) fruit and seed extracts (SSE) on red spider mite (*Tetranychus* spp.) eggs, nymphs and adults. Bioassay investigations were carried at the Vegetable and Ornamental Plant Institute (VOPI) outside Pretoria in South Africa using different concentrations (0.1, 1, 10, 20, 50, 75 and 100%) of SSE. Mortalities were measured at 24, 48 and 72 h after treatment and compared to the effects of the synthetic acaricides: Abamectin, chlorfenapyr and protenofos. A completely randomized design (CRD) was used with 12 treatments. Analysis of variance (ANOVA) was used to test for effects of treatments. Differences in treatment means were identified using Fisher's protected t-test least significant difference (LSD) at the 1% level of significance. Data were analysed using the statistical program GenStat (2003). The result of the analyses revealed that the efficacy of SSE and commercial synthetic acaricides increased with exposure time. Concentration of 50% and above SSE was as effective against red spider mite (RSM) adults, eggs and nymphs as the synthetic acaricides.

Key words: Syringa fruit and seed extracts, *Melia azedarach*, red spider mites, acaricidal activity, tomatoes.

INTRODUCTION

Red spider mites, *Tetranychus* spp. (Acari: Tetranychidae) attack several cultivated crops such as maize, tobacco, cotton, beans, eggplant, pepper, tomatoes, cucurbits, and many flowers such as carnations, chrysanthemums, cymbidiums, gladioli, marigold and roses (Guo et al., 1998; Tadmore et al., 1999; Bolland and Vella, 2000; Batta, 2003; Knapp et al., 2003). They are usually found in pockets on the undersides of leaves. Red spider mites can complete their life cycle from egg to larva, nymph and adult in one to two weeks under favourable conditions (Bolland and Valla, 2000; Biswas et al*.,* 2004). Adults and nymphs suck sap which causes the upper surface of the leaf to become stippled with little dots that are signs of feeding punctures (Goff, 1986; Lu and Wang, 2005). Infested leaves eventually become bleached, discoloured, and covered with silken threads spun by the nymphs and adults (Figure 1). Heavy infestations under hot, dry conditions can lead to leaf drop (Knapp et al*.*, 2003). This drastically reduces the crop yield (Hill, 1983; Visser, 2005; Bok et al*.*, 2006), necessitating the application of acaricides.

Commercially available synthetic acaricides are usually expensive, and may BE needed to be imported for use by farmers. They also tend to have detrimental effects on the environment and can be hazardous to humans. These negative effects have resulted in an increasing interest for natural plant-based pesticides which are assumed to be safer than the synthetic pesticides (Yanar et al*.,* 2011).

Extracts of Neem (*Azadirachta indica* A. Juss) (Family:

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Figure 1. Showing a silken web spun by the adult mites on tomato leaves.

Meliaceae) seeds have been used for many years as an insecticide since it contains Azadirachtin, a nortriterpinoid belonging to the limonoids (Ware and Whitacre, 2004), which exhibits insect growth regulatory effects preventing immature stages of insects from moulting (Broughton et al., 1986; Stoll, 1992; Mordue and Blackwell, 1993; Thacker, 2002). It is known to disrupt insect moulting by antagonising the moulting hormones, ecdysone (Ware and Whitacre, 2004, Weinzierl and Henn, 1991; Abou-Fakhr et al., 2001). Syringa (*Melia azedarach* L.), which also belongs to the mahogany family (Meliaceae), is known to have pesticidal properties (Lee et al., 1987; Schmutterer, 1990, 1995; Stoll, 1992; Mansour et al., 2004, Srinivasan 2012). Aqueous extracts of Syringa fruits and seeds (SSE) have been used to control insect pests in cotton, tea, cucumbers and strawberries (Gupta and Sharma, 1997; Abou-Fakhr Hammad et al., 2000; Güncan et al., 2006; Yanar et al., 2011). In addition to containing small amounts of Azadirachtin (Mwandila, 2009), the seed extracts contain many limonoids such as meliacin and meliacarpin (tetranortriterpenoids) (Chung Huang et al., 1996; Carpinella et al*.,* 2003). These compounds are chemically related to Azadirachtin (Juan and Sans, 2000; Langeland and Burks, 2005) and may have insect growth regulatory action and affect fecundity, moulting, pupation and adult emergence (Ascher et al., 1995; Sarmah et al., 2009; Roy and Mukhopadhyay, 2012).

Although SSEs have been shown to be effective insecticides, research on their acaricidal properties is a relatively recent phenomenon (Stoll, 1992; Yanar et al., 2011; 2012; Roy and Mukhopadhyaya, 2012). Most research has either tested the efficacy of different concentrations of SSE on adult mites or the ovicidal activity of single strength extracts. In this paper, the acaricidal efficacy of different strengths of SSEs on the adults, nymphs and eggs of red spider mites (RSM), *Tetranychus* spp*.* (Tetranychidae) on tomatoes are tested for three exposure times. The efficacy of SSEs is compared to results obtained from three commercial available synthetic acaricides commonly used against the pest in southern Africa.

MATERIALS AND METHODS

The study was carried out at the Vegetable and Ornamental Production Institute (VOPI) of the Agriculture Research Council (ARC), at Roodeplaat (25°36´ S. and 28°36´E), Pretoria, South Africa. Tomato plants of the variety *Money Maker* were grown in the greenhouse at the Roodeplaat Research Station. Leaves from these plants were used in the bioassays using the leaf–dip method (Efil et al., 2005). Ripe Syringa fruits were collected from trees growing in the vicinity of VOPI. A 500 g sample of dried Syringa fruits and seeds were crushed to fine powder using a rotary blender and dried for 24 h at room temperature. Extraction was carried out according to Warthen et al. (1984). The powder was weighed and 1000 ml of 96% methanol was added and shaken for three hours using a magnetic stirring vibrating shaker in a beaker. The mixture was left in the shaker over night, followed by filtration using Whitman filter paper No 40. The filtrate was poured into a round bottom flask and concentrated to 500 ml for three hours on a rotary vacuum evaporator at 40°C. The Copping's (2001) water method was used to prepare the stock solution. For example, assuming 100 ml of the concentrated extracts were required for the preparation of the stock solutions, 99, 90, 80 and 70 ml distilled water was added to prepare a 1, 10, 20 or 30% Syringa stock solution, respectively. Seven concentrations (0.1, 1, 10, 20, 50, 75 and 100%) of SSE were prepared for the treatments.

The efficacy of these seven SSE on RSM were compared with that of three commercially obtained synthetic acaricides, Abamectin, chlorphenapyr and prophenofos, prepared according to the recommended concentrations (0.6, 0.4 and 0.3 ml/L) on the labels, respectively. Two control treatments (one with distilled water and the other with no treatment) were included in the experiments. For each treatment, nine tomato leaves were immersed for thirty seconds into each of the concentrations of SSE or an acaricides or distilled water. The leaves were allowed to dry for thirty minutes on a filter paper at room temperature. Nine treated tomato leaf discs were placed onto 3 ml water agar within each Petri dish for each experiment. Red spider mites were collected from infested leaves of tomatoes and eggplants in the green house at VOPI.

Similar procedures were used for all three experiments - with experiment 1 used for testing of the efficacy of SSE on adult mites; experiment 2 was used to test nymph susceptibility to SSE and commercial acaricides, and experiment 3, the effect of treatments on the viability of red spider mite eggs. Each experiment was carried out in a completely randomized design with 12 treatments (7 SSE concentrations, 3 synthetic acaricides and 2 controls) in three replicates.

For the first experiment, four adult mites were transferred onto each of the three leaves using a pencil brush and left to feed on the tomato leaves. The same procedure was followed for experiments 2 and 3 but transferring 3 nymphs and 6 eggs to each tomato leaf disc, respectively. Experiments 1, 2 and 3 were replicated three times. The average temperature in the laboratory was kept at 22 ± 4°C and the average relative humidity was 54 ± 2% as suggested by Meyer (1987) and Collyer (1998) for optimum mite growth. Counting of the dead adults, dead nymphs and hatched mite eggs was carried out under a microscope at 40x magnifications, 24, 48 and 72 h after application of treatments.

Table 1. Mean percentage mortality of red spider mite adults caused by Syringa seed extracts and synthetic acaricides (untransformed means) 24, 48 and 72 h after treatment.

Treatment	Percent mortality at 24 h	Percent mortality at 48 h	Percent mortality at 72 h
Control - no treatment	(0.0)^{e}	(0.0)^{e}	(0.0)^{e}
Control - distilled water	(0.0)^{e}	(0.0)^{e}	(0.0)^{e}
M. azedarach - 0.1	31.4 (27.8)^{d}	46.6 (52.8)^{d}	48.3 (55.6)^{c}
M. azedarach - 1	31.5 (27.8) ^{d}	50.0 (58.3)^{cd}	76.4 (91.7)^{b}
M. azedarach - 10	46.6 (52.8)^{cd}	60.0 (75.0)^{bcd}	84.4 (97.2)^{ab}
M. azedarach - 20	49.9 (58.3)^{cd}	70.2 (83.3)^{abc}	90.0 (100.0)^{a}
M. azedarach - 50	49.9 (58.3)^{cd}	73.9 (88.9)^{ab}	90.0 (100.0)^{a}
M. azedarach - 75	62.0 (77.8) ^{bc}	74.4 (88.9)^{ab}	90.0(100.0)^{a}
M. azedarach - 100	84.4 (97.2)^{a}	90.0 (100.0)^{a}	90.0 (100.0)^{a}
Abamectin: 0.6 ml/L	90.0 (100.0)^{a}	90.0 (100.0)^{a}	90.0 (100.0)^{a}
Chlorfenapyr 0.4 ml/L	78.2 (88.9)^{ab}	90.0 (100.0)^{a}	90.0 (100.0)^{a}
Profenofos 3 ml/L	41.8 (44.4)^{d}	90.0 (100.0)^{a}	90.0 (100.0)^{a}
SEM	4.87	5.40	2.89
F probability	<0.001	<0.001	<0.001
LSD (1%)	19.28	21.34	11.44
CV%	17.9	15.2	7.2

SEM is the standard error of the mean. LSD is the t-test least significant difference at the 1% level. Means within columns followed by the same lower case letter did not differ significantly at the 1% level. CV% is the coefficient of variation of each treatment and experiment. Angular transformation used to normalise percentages is in parentheses.

The data obtained were analysed using Analysis of Variance (ANOVA). The Least significant difference (LSD) was used to differentiate between means at the 1% level of significance (Snedecor and Cochran, 1980) using the GenStat statistical programme (2003). Means (± SEM) of the untransformed data are reported. Data in the brackets reflect angular transformations. These were used to normalize the percentage means on the left side of the column. The figures in parenthesis were used for the analyses.

RESULTS AND DISCUSSION

Table 1 indicates that a 0.1% SSE killed almost a third of the adult red spider mites after a 24 h exposure. Mortality caused by Syringa extracts of concentrations 0.1 to 50% were similar (LSD, P < 0.1%) and those with concentrations of 10 to 75% were also similar. The synthetic acaricide chlorfenapyr caused similar levels of mortality as SSE of 75% concentration. Adult RSM mortality at 48 h after treatment showed that treatments with 20% SSE were as effective as application of the three synthetic acaricides, Abamectin, chlorfenapyr and profenofos while the 1% SSE treatment killed more than 90% adult mites after a 72 h period of exposure and the 100% SSE caused total mortality of the adults at 48 h after treatment. The results showed that 20% SSE were as effective as the three synthetic acaricides and that 100% SSE caused total mortality after 48 h.

Table 2 shows that 50, 75 and 100% SSE concentrations achieved 100% mortality of RSM nymphs at 48 and 72 h while lower concentrations caused significantly (LSD, p<0.001) lower mortality levels at 48 h. Results in Table 2 also show that the level of nymph mortality achieved by as low as 0.1% SSE concentrations are significantly (LSD, p<0.001%) higher than mortalities on untreated (control) leaf discs for all the three periods (24, 48 and 72 h). Similar levels of RSM nymphs were killed by all SSE concentrations and the three synthetic acaricides when the mite nymphs were exposed for 72 h.

Table 3 shows that none of the treatments caused egg mortality when the leaf discs were assessed 24 or 48 h after application of treatments. However, inhibition of egg hatching was evident 72 h after application. Results in Table 3 show that while only 22.2% of eggs failed to hatch on the untreated control leaf discs, inhibition of egg hatching was significantly (LSD, p <0.001%) higher on leaf discs treated with all SSE concentrations as well as the synthetic acaricides. At 48 h of egg exposure to the treatments, the level of inhibition by the 10, 20, 50, 75 and 100% SSE concentrations was more than 90% and similar to that by Abamectin and chlorfenapyr. The level of inhibition (mortality) caused by the 10% SSE concentration at 72 h was 4x that on untreated control leaf discs. Syringa extracts are known to have acaricidal, repellent, antifeedant and growth inhibition effects on Red Spider Mites (RSM) (Stoll, 1992). The finding that the 100% SSE concentration was as effective as the recommended commercially obtained synthetic acaricides

Table 2. Mean percentage mortality of red spider mite nymphs on SSE, abamectin, chlorfenapyr and profenofos treated tomato leaf discs (untransformed means) at 24, 48 and 72 h after application.

Treatment	Percent dead at 24 h	Percent dead at 48 h	Percent dead at 72 h
Control - no treatment	0.00 (0.00)^{e}	0.00(0.00)^{e}	0.00 (0.00)^{b}
Control - distilled water	0.00 (0.00)^{e}	0.00(0.00)^{e}	0.00(0.00)^{b}
M. azedarach - 0.1	43.35 (47.22)^{d}	60.21(75.00)^{cd}	76.38(91.67)^{a}
M. azedarach - 1	48.20 (55.56)^{d}	54.84(66.67)^{d}	76.38(91.67)^{a}
M. azedarach - 10	48.20 (55.56)^{d}	56.49(69.44)^{d}	71.97(86.11)^{a}
M. azedarach - 20	60.21 (75.00)^{c}	63.94(80.56)^{bc}	73.94(88.89)^{a}
M. azedarach - 50	68.34 (86.11)^{bc}	90.00(100.00)^{a}	90.00(100.00)^{a}
M. azedarach - 75	70.78 (88.89)^{b}	90.00(100.00)^{a}	90.00(100.00)^{a}
M. azedarach - 100	84.41 (97.22)^{a}	90.00(100.00)^{a}	90.00(100.00)^{a}
Abamectin : 0.6 ml/L	90.00 (100.00)^{a}	90.00(100.00)^{a}	90.00(100.00)^{a}
Chlorfenapyr 0.4 ml/L	90.00 (100.0) ^{a}	90.00 (100.0) ^{a}	90.00 (100.0) ^{a}
Profenofos 3 ml/L	61.97 (77.78)^{bc}	68.34(86.11)^{b}	84.41(97.22^{a}
SEM	2.60	1.63	4.85
F probability	<0.001	<0.001	<0.001
LSD (1%)	10.27	6.46	19.17
CV%	8.1	4.5	12.1

SEM is the standard error of the mean. LSD is the t-test least significant difference at the 1% level. Means within columns followed by the same lower case letter did not differ significantly at the 1% level. CV% is the coefficient of variation of each treatment and experiment. Angular transformation used to normalise percentages is in parentheses.

(Abamectin, chlorfenapyr and profenofos) confirms the findings by Stoll (1992). This applies to the effectiveness of SSE against adult red spider mites from a period as short as 24 h (Table 1). This indicates that SSEs can be a potential alternative for use by resource poor farmers if applied at high concentration. Results in Table 1 also show that although lower concentrations of SSE (0.1 and 1) caused significantly (LSD, $p<0.001$) lower mortalities of RSM adults than the synthetic acaricides, the level of mortality they achieved is significantly (LSD, $p<0.001$) higher than the natural mortality in the untreated controls even at 72 h. Mwandila (2009) found that the active ingredient, Azadirachtin, was found in low amounts in SSE. This might explain why longer periods (in excess of 24 h) of exposure to SSE treatments increased adult RSM mortality. A longer period of exposure enabled the mites to collect the required lethal dose as they moved and fed on the treated leaf discs. Since adult mites do not moult, the high mortality caused by SSE concentrations at 24, 48 or 72 h exposure was probably due to other modes of action, such as repellent or antifeedant action, rather than the antagonistic action against the moulting hormone, ecdysone. RSM nymphs undergo moulting during their growth and development. The high nymph mortality caused by the 100% SSE from a short period such as 24 h could be a result of repellent, antifeedant as well as antagonistic action of the Azadirachtin in SSEs against the moulting hormone. Unlike the results with RSM adults, the mortality of nymphs on leaf discs treated with 50 and 75% SSE concentrations after 48 h of exposure also caused 100% pest mortality. The high nymph mortalities at 48 and 72 h of exposure caused by low concentrations of SSE could be because in addition to repellent and antifeedant action, the Azadirachtin in the SSEs also affected the nymphs through antagonizing ecdysone. The finding that similar and significantly (LSD, $p<0.001\%$) greater levels of inhibition of egg hatching occurred in all SSE concentrations than in the untreated (control) treatments shows that SSEs were highly ovicidal, even at very low concentrations. This is probably because, being smaller in size, the lethal dose of the egg was relatively lower than that of nymphs and adults.

Conclusion

The efficacy of SSE extracts increased with the concentration and exposure time of the treatment. At 48 h exposure, a 50% SSE was as effective on eggs, nymphs and adults as the synthetic acaricides recommended for use against RSM in southern Africa. At 72 h exposure time, there was no significant difference in the effects of 10% SSE and the synthetic acaricides. A 100% Syringa extract is as effective against RSM adults, nymphs and eggs as the commercially available synthetic acaricides, Abamectin and chlorfenapyr, regardless of exposure time after treatment. Although lower concentrations of SSE had considerable acaricidal activity on different stages of the life cycle and different exposure times, it appears that a 50% extract would be the most effective to be applied under field conditions.

The ability of Syringa extracts to inhibit hatching of RSM

Table 3. Mean percentage syringa number of RSM eggs hatched (untransformed means) at 48 and 72 h.

Treatment	Percent eggs hatched at 48 h	Percent egg mortality at 48 h	Percent eggs hatched at 72 h	Percent egg mortality at 72 h
Control - no treatment	24.09 (16.67)^{ab}	83.3	62.62 (77.78)^{ab}	22.2
Control - distilled water	31.34 (27.78)^{a}	72.2	71.52 (83.33)^{a}	28.5
M. azedarach - 0.1	19.07 (11.11)^{ab}	88.9	38.34 (38.89)^{ab}	61.1
M. azedarach - 1	26.28 (20.37)^{ab}	79.6	31.66 (29.63)^{bc}	70.4
M. azedarach - 10	0.00 (0.00)^{c}	100	8.03 (5.56)^{c}	94.4
M. azedarach - 20	4.54 (1.85)^{bc}	98.2	11.75 (11.11)^{c}	88.9
M. azedarach - 50	4.54 (1.85)^{bc}	98.2	11.03 (5.56)^{c}	94.4
M. azedarach - 75	4.54 (1.85)^{bc}	98.2	13.63 (5.56)^{c}	94.4
M. azedarach - 100	0.00 (0.00)^{c}	98.2	4.54 (1.85)^{c}	98.2
Abamectin : 0.6 ml/L	10.60 (9.26)^{ab}	90.8	12.86 (12.96)^{c}	87.2
Chlorfenapyr 0.4 ml/L	4.54 (1.85)^{bc}	98..2	11.03 (5.56)^{c}	94.4
Profenofos 3 ml/L	19.54 (18.52)^{abc}	81.5	21.49 (20.37)^{c}	79.6
SEM	6.00		8.91	
F probability liter	<0.001		<0.001	
LSD (1%)	23.74		35.23	
CV%	83.7		62.0	

SEM is the standard error of the mean. LSD is the t-test least significant difference at the 1% level. Means within columns followed by the same lower case letter did not differ significantly at the 1% level. CV% is the coefficient of variation of each treatment and experiment. Angular transformation used to normalise percentages is in parentheses.

eggs, and cause high nymph and adult mortality makes this botanical an effective acaricide for use in the control of this pest by resource poor farmers. Southern African countries could decrease costs and limit importation of expensive acaricides by promoting use of SSEs. The introduction of home-made Syringa extracts as organic agricultural pesticides would be an innovation that would benefit many subsistence tomato producers who may be unable to afford expensive synthetic acaricides that are available commercially.

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