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Relative efficacy of agricultural spray oil and azadirachtin against two-spotted spider mite (*Tetranychus urticae*) on cucumber (*Cucumis sativus*) under greenhouse and laboratory conditions

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ABSTRACT

Laboratory and greenhouse experiments were conducted on relative efficacy of agricultural spray oil and azadirachtin for the management of two-spotted spider mite *(Tetranychus urticae* on cucumber (*Cucumis sativus* L.). The treatments included the spray of agricultural spray oil, azadirachtin and combination of both at 0.1, 0.5, 1 and 2% under laboratory conditions and 0.1 and 0.5% under greenhouse conditions, replicated thrice. The laboratory experiment results indicated highest mortality of the mite after 24, 48 and 72 hr of spray in agricultural spray oil + azadirachtin (at 1 and 2%), followed by 2% agricultural spray oil alone in both the seasons. Greenhouse experiment results also indicated highest mortality of the mite on spraying agricultural spray oil + azadirachtin (0.5%) throughout the period, however, agricultural spray oil (0.5%) resulted in highest mortality only a day after spraying during the first season.

Key words: Agricultural spray oil, Azadirachtin, Cucumber, Greenhouse, Mites

Two-spotted spider mite *(Tetranychus urticae* Koch), the most common type of mite affecting crop plants, is a serious pest of cucumber in greenhouses. It is a polyphagous pest and is globally considered an important agricultural pest (Jeppson *et al.* 1975). It is particularly dominant in intensive, high yielding cropping systems and in green houses (Alzoubi and Cobonoglu, 2008). It affects crops by direct feeding, usually colonizing the lower side of the leaves primarily along the mid-ribs and lateral veins. When many mites are present, silken webs and white flakes of molted skin can be observed on the lower leaf surface. It causes severe damage, expressed as leaf wilting, losses of yield quantity and quality and even plant death. The introduction and spread of covered cultivation, along with newer agricultural practices enabled the spider mites to rise in very large populations. It causes significant economic losses in cucumber, tomato, pepper, and bean grown in greenhouses.

Many spider mites have become resistant to most of the commonly used pesticides. The loss of acaricidal efficacy as a result of resistant mite population is the major problem encountered in greenhouses (Alzoubi and Cobonoglu, 2008). They have evolved resistant to more than 80 acaricides to date, and resistance has been reported from more than 60 countries (Database from online). Spider mites impose a great expense on greenhouse growers worldwide in terms of damage and control cost and are, therefore, considered one of the most important pests of greenhouse production (Miresmailli *et al.* 2006). Considerable research efforts have been devoted to finding alternative strategies for suppression of *T. urticae*. Integrated Pest Management (IPM) is a holistic approach to the management of pests in green house. Now, IPM programmes using biorational insecticides are in progress in greenhouse production. Biorational insecticides are more acceptable than conventional insecticides because they are assumed to be active against pest populations and relatively innocuous to beneficial organisms. Biorational pesticides such as agricultural spray oils and azadirachtin have been shown to be effective against the most common interiorscape pests (Smith and Krischik, 2000).

Azadirachtin is a natural biorational pesticide derived from neem tree. It is growth regulating, antifeedant and repellent compound for phytophagous insects (Menke and Gerhard, 2009). It has been demonstrated that the antifeedant effects of azadirachtin on insects varies depending on the concentration and species of the target insects (Xie *et al.*

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1995). Among the plants possessing environment-friendly compounds, it is proving to be a valuable asset on account of its insecticidal properties (Abdullah *et al.* 2001). Agricultural spray oils are commercial by-product of petroleum industry. The oil is sprayed in the form of oil-in-water emulsion. The emulsion dissolves the waxy protective shield of the insect and the oil film envelops it thereby killing the insect by cutting off its air supply. Agricultural spray oil can effectively control red spider mite, pink mite and purple mites in tea plantations and it can be used in combination with common insecticides and pesticides under integrated pest management program (USPTO Patent Application 20080194704, Composition of agricultural spray oil).

Till now, no studies have been done on effectiveness of these biorational pesticides against mites on cucumber in greenhouse as well as laboratory conditions. Therefore, the aim of this study was to determine the efficacy of these two biorational pesticides- azadirachtin and agricultural spray oil, singly and in mixture, under greenhouse and laboratory conditions.

MATERIALS AND METHODS

The toxicity of commercial biorational pesticides (Table1) to the spider mite was tested in the laboratory of NCIPM, Pusa campus, New Delhi in 2009 (first crop season) and 2010 (second crop season). Newly grown leaves infested with mites were sampled from green houses of Centre for Protected Cultivation Technology (CPCT), IARI, New Delhi. The bioassay method was devised to test the toxicities of the two biorational pesticides, viz azadirachtin (Neembaan® from Sri Ram Solvents, Jaspur, US Nagar, Uttarakhand), agricultural spray oil (Agrospray® from Indian Oil Corporation Ltd) and mixture of both (azadirachtin and agricultural spray oil). The bioassay method was followed according to Amiri Besheli (2008) with slight modifications involving spraying of leaves in pesticide solution instead of dipping.

For the bioassay, leaves with actively feeding mites along with petioles were considered. To keep the leaves turgescent, each petiole was covered by wet cotton. Leaves were sprayed with each of the concentration of pesticides; spray of distilled water served as control. After spraying, the leaves were air dried for approximately 2 hrs and placed at the bottom of plastic petri dishes (9 cm diameter × 2.5 cm high) that were lined with wet filter paper and covered with a plastic lid. Four concentrations (Table 1) were used for each pesticide along with four replicates and control. The number of live and dead mites in each replicate was counted under a stereomicroscope before the treatment and 24, 48 and 72 hr after the treatment. From each treated leaf, population count was made randomly from 2.5 cm^2 leaf area. Mites were considered dead if their appendages did not move when prodded with a fine paintbrush.

The experiments were carried out in the greenhouse of CPCT, IARI, New Delhi during 2009 (first crop season) and 2010 (second crop season). The experiment was laid out in randomized block design with four treatments including control. The plot size was 1×3 m^2. 'Satis' cucumber (*Cucumis sativus* L.) was grown following the recommended package of practices. Two concentrations (0.1% and 0.5%) were used for each biorational pesticide along with three replicates and control. Two sprays were performed at an interval of 10 days.

Table 1 Effect of biorational pesticides on the mortality of *T. urticae* in laboratory (first crop season)

Treatment	Concentration (%)	Per cent mortality of mite population after treatment*		
		24 hr	48 hr	72 hr
Agricultural spray oil	0.1	46.60^{f} (43.05)	59.30^{d} (50.36)	56.80^{d} (48.91)
	0.5	56.60^{e} (48.79)	42.30^{ed} (40.57)	85.90^{b} (67.94)
	1.0	86.60^{b} (68.53)	83.80^{b} (66.27)	81.80^{b} (64.75)
	2.0	90.00^{b} (71.56)	98.60^{a} (83.20)	100.00^{a} (90.00)
Azadirachtin	0.1	32.50^{h} (34.76)	36.00^{d} (36.87)	31.60^{d} (34.20)
	0.5	43.00^{g} (40.98)	49.60^{e} (44.77)	40.00^{e} (39.23)
	1.0	70.30^{d} (56.98)	72.00^{c} (58.05)	45.50^{e} (42.42)
	2.0	79.30^{c} (62.94)	98.60^{a} (83.20)	99.00^{a} (84.26)
Agricultural spray oil + azadirachtin	0.1	40.30^{g} (39.41)	44.00^{e} (41.55)	56.00^{d} (48.45)
	0.5	42.00^{g} (40.40)	49.60^{e} (44.77)	68.20^{c} (55.67)
	1.0	100.00^{a} (90.00)	100.00^{a} (90.00)	100.00^{a} (90.00)
	2.0	100.00^{a} (90.00)	100.00^{a} (90.00)	100.00^{a} (90.00)
Control	0.0	5.00^{i} (12.92)	4.30^{f} (11.97)	3.20^{f} (10.31)
SEm±		2.54	3.59	3.03
CD (*P*=0.05)		5.25	7.83	6.61

P= 0.05

*Mean of three replicates; Figures in parentheses are arcsine transformed values; In a column, 'means' followed by a common letter do not differ significantly at P d"0.05 by Duncan`s Multiple Range test

Spraying was done onto leaves two weeks after natural pest infestation based on pest load on the crop when the population exceeded recommended treatment thresholds, ie 25–30 mites/ leaf (John and Dorie 1997).

Methodology of Rachana (2004) was followed to record the incidence of mites. Three plants were randomly selected from each plot and tagged. From each tagged plant, population count was made from 1 sq inch leaf area of three leaves (top, middle, and bottom) and average population was recorded. The observations were recorded before the spray as well as 1, 2, 3, 7 and 10 days after each spray. Mites not showing any movement when lightly touched with a brush were considered dead.

Results were expressed as percent mortality with correction for untreated (control) mortality using Abbott's formula (Abbott 1925), transformed to arcsine to homogenize the variances. The data were subjected to ANOVA tests.

RESULTS AND DISCUSSION

Two biopesticides, viz agrospray and azadirachtin and their mixtures were evaluated against mites. Analysis of variance shows that the pesticide application had significant effect on the mortality of *T. urticae*, proving thereby, that the application of pesticides individually or in combination, on an average, was more effective in reducing the mite population as compared to control (Tables 1, 2, 3, 4). The trend of relative efficacy of various treatments has been described below.

Efficacy of biorational pesticides on mites in the laboratory

The efficacy of biorational pesticides was observed in the laboratory. After 24 hr of treatment, mixture of agricultural spray oil + azadirachtin at 1% and 2% each were found to inflict highest mite mortality (100%), followed by agricultural spray oil at 2% (90% mortality) which was however at par with agrospray at 1% affording 86.6% mortality (Table 1). The mite mortality increased in most of the treatments after 48 hrs of application in both the seasons (Table 1 and Table 2). After 72 hrs of treatment also all treatments were significantly effective in comparison to 3.2% and 2.6% mortality of untreated control in first crop season and second crop season, respectively. But it was observed in first crop season that in all lower concentration treatments (0.1% and 0.5%) of all pesticides, toxicity was more or less similar and gave approximately 50% mortality. From all these observations, the result of laboratory experiments indicated that the combination of agrospray + azadirachtin at 1% and 2% were most effective, closely followed by agricultural spray oil at 2% alone. Combination of biopesticides showed synergistic activity i.e. the efficacy of the mixture was more as compared to either of the individual components alone. Reddy *et al.* (2001) also observed good results in combination of biopesticides against pod borer in pigeonpea. Dabi *et al.* (1998) also got increased efficacy of *Bt* in combination with insecticides against *H. armigera* and stated that the same was due to synergistic effect. Minimum reduction of the mite population was observed in azadirachtin treatments.

Efficacy of biorational pesticides on mites in the greenhouse

As lower concentration (0.1% and 0.5%) treatments are effective in controlling mite population in the laboratory, an experiment was set to determine the toxicity of these pesticides in lower concentration in greenhouse also.

The results indicated a significant reduction in the mite

Table 2 Effect of biorational pesticides on the mortality of *T. urticae* in laboratory (second crop season)

Treatment	Concentration (%)	Per cent mortality of mite population after treatment*		
		24 hr	48 hr	72 hr
Agricultural spray oil	0.1	20.00 g (26.56)	29.00 f (32.58)	25.00 f (30.00)
	0.5	32.50 f (34.76)	49.60 d (44.77)	42.20 d (40.51)
	1.0	40.00 de (39.23)	83.80 b (66.27)	56.80 c (48.91)
	2.0	98.60 a (83.20)	99.00 a (84.26)	98.60 a (83.20)
Azadirachtin	0.1	18.60 g (25.55)	26.33 f (30.85)	20.00 f (26.56)
	0.5	31.60 f (34.2)	36.00e (36.87)	33.83 e (35.55)
	1.0	33.60 ef (35.43)	49.67d (44.77)	44.00 d (41.55)
	2.0	56.60 c (48.79)	70.33c (56.98)	59.30 c (50.36)
Agricultural spray oil + azadirachtin	0.1	33.00 ef (35.06)	45.53d (42.42)	33.60 e (35.43)
	0.5	81.80 b (64.75)	86.67b (68.53)	85.90 b (67.94)
	1.0	95.00 a (77.08)	100 a (90.00)	100 a (90.00)
	2.0	100 a (90.00)	100 a (90.00)	100 a (90.00)
Control	0.0	1.80 h (7.71)	4.33 g (11.97)	2.67 g (9.28)
SEm±		3.38	2.61	3.13
CD (*P*=0.05)		7.36	5.68	6.81

*Mean of three replicates; Figures in parentheses are arcsine transformed values; In a column, 'means' followed by a common letter do not differ significantly at P d"0.05 by Duncan`s Multiple Range test population. All these treatments recording 36.00% to 90.00% mite mortality which proved significantly superior to control after 1 day of treatment in both the crop seasons (Tables 3, 4). After 1 day of application, among the treatments in first crop season (Table 3), agricultural spray oil at 0.5% inflicted the highest per cent mortality (83.2%) of the mites, followed by combination of agricultural spray oil + azadirachtin at 0.5% (58.23%). In second crop season (Table 4), combination of agricultural spray oil + azadirachtin at 0.5% inflicted the highest percent mortality (90%) of the mites, followed by agricultural spray oil at 0.5% (85.9%). After 2, 3, 7, and 10 days of treatments, results of the both seasons indicated that combination of agricultural spray oil + azadirachtin at 0.5% inflicted highest mortality of the mites throughout the period as compared to other treatments, followed by combination of agricultural spray oil + azadirachtin at 0.1% and agricultural spray oil at 0.5% alone. After 3 days of treatments, all the pesticides showed slightly reduced efficacy, but were significantly superior in comparison to 2.0% and 2.41% mortality of untreated control in the first crop and the second crop season, respectively. The petroleum oil spray residues reduced infestation of some insects by preventing oviposition and its effect depended on concentration of oil and time of spraying (Amiri Besheli 2008). Petroleum oil alone or combined with a microbial agent as emulsifier have a synergistic and less harmful effect for the environment and are recommended for use in IPM programmes (Khyami and Ateyyat 2002). Moreover, the agricultural spray oil does not increase pesticide resistance because its mode of action is mechanical, not chemical. In this experiment, treatments of agricultural spray oil got the second position in effectiveness in reducing mite population. Though it is long lasting, it has no residual killing action, but the coating it makes on leaves and stems can protect against transmission of some plant viruses and fungi. The comparatively low toxicity of the treatments of agricultural spray oil alone in this study may be due to different factors including cuticle properties, ambient temperature and the molecular size and volume of oil molecule.

Treatments of azadirachtin were observed to be least effective in this experiment in both the seasons. It appears that the azadirachtin might not be effective in suppressing the population of the mite at low concentration. Better control might be achieved by increasing its concentration. Neem

Table 3 Effect of biorational pesticides on the mortality of *T. urticae* in greenhouse (first crop season)

Treatment	Concentration	% mortality of mite population after treatment*				
	(%)	1day	2 days	3 days	7 days	10 days
Agricultural spray oil	0.1	45.06 ^{bcd} (42.13)	42.20^{c} (40.41)	23.33^{bc} (28.86)	25.00^{b} (30.00)	19.60^{b} (26.28)
	0.5	83.20^{a} (65.80)	70.03^{b} (56.79)	48.60^{a} (44.20)	31.60^{a} (34.20)	29.00^{a} (32.58)
Azadirachtin	0.1	36.33^{de} (35.06)	38.00^{c} (38.06)	22.30^{c} (9.98)	20.00^{c} (26.56)	20.00^{b} (26.56)
	0.5	44.20^{bcdc} (41.67)	53.83^{c} (47.18)	24.00^{bc} (29.33)	22.00^{bc} (27.97)	18.60^{b} (25.55)
Agricultural spray oil+	0.1	57.10^{bc} (49.02)	52.97^{c} (46.66)	32^{b} (34.45)	26.30^{b} (30.85)	33.00^{a} (35.06)
azadirachtin	0.5	58.23^{b} (49.66)	90.10^{a} (71.66)	49.30^{a} (44.60)	33.6^{a} (35.43)	29.3^{a} (32.77)
Control	0	3.76^{f} (11.09)	2.67^{d} (9.28)	2.00^{d} (8.13)	1.8 0^{d} (7.71)	1.43^{c} (6.80)
SEm±		6.66	10.49	3.99	2.23	2.68
CD (P=0.05)		14.51	22.86	8.69	4.86	5.84

*Data based on mean of two sprays and three replicates each; Figures in parentheses are arcsine transformed values; In a column, 'means' followed by a common letter do not differ significantly at $P \leq 0.05$ by Duncan`s Multiple Range test.

Table 4 Effects of biorational pesticides on the mortality of mites in greenhouse (second crop season)

Treatment	Concentration	% mortality of mite population after treatment*				
	(%)	1day	2 days	3 days	7 days	10 days
Agricultural spray oil	0.1	56.6^{b} (48.79)	57.1 ^{cd} (49.02)	24^{e} (29.33)	19.6^{e} (26.28)	18.6^{c} (25.55)
	0.5	85.9^{a} (67.94)	86.6^{ab} (68.53)	48.6^{c} (44.20)	23.33^{d} (28.86)	25^{b} (30.0)
Azadirachtin	0.1	36.00^{d} (36.87)	33.83^{e} (35.55)	22.3^{e} (9.98)	20de (26.56)	18.6^{c} (25.55)
	0.5	45.6^{c} (42.48)	55.00^{de} (47.87)	32^{d} (34.45)	29^{c} (32.58)	22^{bc} (27.97)
Agricultural spray oil+	0.1	59.3^{b} (50.36)	70.03^{abc} (56.79)	49.3^{b} (44.60)	40^{b} (39.23)	31.6^{a} (34.2)
azadirachtin	0.5	90^{a} (71.56)	92.20^{a} (73.78)	59.3^{a} (50.36)	45.5^{a} (42.42)	33^{a} (35.06)
Control	0	2^{e} (8.13)	3.0^{f} (9.98)	2.41^{f} (8.91)	1.8^{f} (7.71)	3.2^{d} (10.31)
SEm±		2.58	10.48	3.41	2.20	2.21
CD (P=0.05)		5.63	22.85	7.43	4.79	4.82

P= 0.05; *Data based on mean of two sprays and three replicates each; Figures in parentheses are arcsine transformed values; In a column, 'means' followed by a common letter do not differ significantly at $P \leq 0.05$ by Duncan`s Multiple Range test derivatives have been reported to provide broad spectrum control of over 200 species of phytophagous insects (Ascher 1993). Coudriet *et al.* (1985) observed oviposition deterrence and mortality of *Bemisia tabaci* after treatment with neem seed extract under greenhouse conditions. Past research did not find direct toxic effects of formulations of azadirachtin on mites. Earlier studies have reported the repellent and feeding deterrent effects of neem materials against a wide range of insect and mite pests (Dimetry *et al.* 1993). Banken and Stark (1998) found that a commercial product containing azadirachtin did not cause mortality of adults of coccinellid species, but it reduced oviposition by these beetles and significantly delayed larval development. The best documented effects of azadirachtin involve insect growth regulation and feeding deterrence (Banken and Stark 1998, Koul *et al.* 1990). Cote *et al.* (2002) also reported that neem products may be a useful part of Integrated Pest Management programmes, however, its short residual toxicity may not suppress large population of two spotted spider mite. In conclusion, the present study showed that combination of azadirachtin and agricultural spray oil in increased concentration (1 and 2%) could be recommended for effective control of mite, but under heavy infestation, use of synthetic insecticides is necessary to prevent reinfestation by the mite.

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