JBiopest 9(2):204-210 (2016)

Bioefficacy of neem kernel aqueous extract (NKAE) against tea red spider mite, *Oligonychus coffeae*, Nietner and its effect on *Stethorus gilvifrons* Mulsant, a potential predator of red spider mite

Mridul Sarmah

ABSTRACT

Neem kernel aqueous extract (NKAE) was evaluated at different concentrations (1-10%) against red spider mite, *Oligonychus coffeae* under laboratory and field conditions. Also the impact of NKAE on survival of *Stethorus gilvifrons*, a potential predator of red spider mite was studied. Parameters assessed were ovicidal and acaricidal activities, adult emergence, oviposition deterrent in case of red spider mite and larval & adult mortality and adult emergence for the predator. Significant ovicidal activity was exhibited at higher concentrations (6-10%). Nymphs were more susceptible to NKAE than adults. Higher concentrations (6-10%) showed 53-95% mortality of mite population under laboratory conditions. LC_{50} values of NKAE for nymph and adult red spider mite were found to be 47.73 mg/ml and 66.02mg/ml respectively after 24h of treatment. Field evaluation however exhibited 43-69% reduction of mite population at 6-10% concentrations. Egg laying by tea red spider mite on the NKAE treated tea leaf surface was significantly decreased (1.16-1.50 eggs/female/day) than control (3.83 eggs/female/day). Significant reduction in adult emergence (20.0-56.7%) and increased duration of total developmental period (1.00-4.33 days) were noticed at higher concentrations (4-10%) only. However, the application of NKAE on different life stages of *S. gilvifrons* showed no adverse effect in respect of their growth and development.

MS History: 22.09.2016 (Received)-24.10.2016 (Revised) - 28.11.2016 (Accepted)

Key words: NKAE, bioefficacy, red spider mite, *Stethorus gilvifrons*, tea.

Citation: Mridul Sarmah. 2016. Bioefficacy of Neem Kernel Aqueous extract (NKAE) against tea red spider mite, *Oligonychus coffeae*, Nietner and its effect on *Stethorus gilvifrons*, Mulsant, a potential predator of red spider mite. *Journal of Biopesticides*, 9 (2): 204-210.

INTRODUCTION

The tea, *Camellia sinensis* (L) O. Kuntze is one of the most important beverage and commercial crops covering an area of 5.64 lakh hectare in India with an annual production of 1197 million kg made tea during 2014-2015. Tea being a perennial crop and is grown extensively as mono crop, it is susceptible to various insect and mite pests of which red spider mite, *Oligonychus coffeae* (Tetranychidae: Acarina) is the most predominant. Approximately 15-20 per cent crop is lost annually due to the attack of red spider (Banerjee, 1966). During the last several decades, the management of red spider mite in tea plantation was predominantly by the use of synthetic chemicals which caused various problems like pest resistance, pest outbreak, environmental pollution, problems of pesticide residues on made tea etc. Realizing the hazards caused by these xenobiotics there is an urgent need to search for some safer alternatives to manage the pests of tea. Among the safer alternatives botanical pesticides may play an important role in this aspect. Pertinent to above, there was a trend to shift from chemocentric approaches of pest management to organic management approaches and various works have tried to see the effectiveness of botanicals against the mite as well as insect pests complex of tea Botanical pesticides are safe to beneficial organisms like parasitoids and predators. Natural defenses of plants against herbivores consist almost always of mixtures of closely related compounds, rather than a single toxicant alone. The crude plant extracts contain the complex mixture of active

constituents and being a complex mixture of different bioactive molecules there is less likely to develop pest resistance. Hence in the present study aqueous extract of neem seed kernel was tried out against different stages of red spider mite and also their impact on growth and development of *Stethorus gilvifrons,* a potential coccinellid predator of red spider mite.

MATERIALS AND METHODS

The study was carried out at the Entomology department, Tocklai Tea Research Institute, Tea Research Association, Jorhat (26.74^0N, 94.20^0E) 2012-2013.

Rearing of red spider mite

Mite culture: Initial population of red spider mite was collected during February from the Borbhetta experimental tea estate, Tea Research Association, Jorhat, Assam, India. The collected mites were shifted to fresh mature leaves of genotype TV1 and allowed them to lay eggs for 24 hrs. Those eggs were maintained by following the method as described by Helle and Sabelis (1985) with modification. This experimental set up was kept under laboratory conditions at the temperature of 27 ± 2^o C, 70-80% RH and a 14 L: 10 D photoperiod. The adult female mites of the second generation, reared for 48 hrs were used.

Maintenance of Predator culture

Pupae of *Stethorus gilvifrons* were collected from the Borbhetta experimental tea estate, TRA, Jorhat, Assam and were kept in glass tubes (1.5″ × 8.0″) under laboratory conditions. After emergence, adults were supplied with laboratory reared red spider mites to maintain the culture as per the method described by Sarmah (1999).

Preparation of neem kernel aqueous extract

Neem seed kernels (NSK) were ground to fine powder with the help of mixer grinder. The powdered materials were passed through 60 mesh sieve. Then 10 grams of NSK powder were dissolved in 100 ml of water and allowed to stand overnight. Next day the solution was filtered by using cheese cloth. The filtrate was then considered as a stock solution for preparing different concentrations.

Laboratory bioassay against adult red spider mite

Ten healthy adult female red spider mites (48 hrs old) were released on 2.5 cm diameter leaf disc of variety TV1 from the culture maintained in the laboratory. Final count of mite population was taken after one hour (after proper settlement of mite). Different concentrations of NKAE (1, 2, 4, 6, 8 and 10 %) were sprayed on both the surfaces of leaf separately using glass atomizer. Number of live red spider mites was then counted after 24 hrs, 48 hrs, 72 hrs, 96 hrs and one week after treatment. Each treatment was replicated five times. Per cent mortality was calculated using the following formula:

Per cent mortality= Pre-treatment population – post treatment population / Pre treatment population × 100

Ovicidal test

To assess the ovicidal action of NKAE, ten gravid females were released on 2.5cm diameter leaf disc of variety TV1 from the culture for oviposition. Leaf discs were kept in petri dish padded with water soaked cotton. After 18 hrs the introduced mites were removed with the help of fine camel hair. The eggs laid on tea leaves were counted under microscope as pre-treatment count up to 20 eggs and tea leaves containing more than 20 eggs were removed cautiously by using fine needle. 100 eggs were considered for each ovicidal treatment of the NKAE and observed for five times (20 eggs/observation). After counting, the eggs are subjected to spraying with different treatments as 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 % w/v by using glass atomizer. The exposure of eggs to different treatments was scheduled separately. The control eggs (100 numbers) were also segregated as above manner and treated with distilled water. Hatchability was determined for both treated and control batches of eggs for a period of 15 days after oviposition. Those eggs that did not hatch after this period were regarded as non-viable (Sarmah *et al.,* 1999). Per cent reduction in hatchability was calculated by using the following formula:

Per cent reduction in hatchability= No. of unhatched eggs/treatment/Total No. of eggs/treatment x 100

Adult emergence and total duration of red spider mite

The larvae hatched from the treated eggs of mites with different concentration of NKAE (from the ovicidal experiment) were shifted to 2.5cm diameter leaf discs of variety TV1 and observed up to adult emergence under laboratory conditions. Ten larvae were shifted to each leaf disc (one replication) and there were three replications per treatment.

Oviposition behaviour of red spider mite

Female red spider mites (48 hrs old) were introduced on 2.5 cm diameter leaf disc of variety TV1 treated with 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 % NKAE separately and water was sprayed in case of control. Number of eggs laid on the treated and untreated surface per female per day was recorded up to six days. Each treatment was replicated five times.

NKAE impact on the different stages of *S. gilvifrons*

Larvae, pupae and adults of laboratory maintained *S. gilvifrons* were exposed to different concentrations of NKAE by directly spraying with a glass atomizer under laboratory conditions. Observations in terms of mortality, adult emergence and duration of each stage were taken separately. Observations were made for five times.

Field bioassay test

A field trial was conducted at Borbhetta tea estate to evaluate the efficacy of different concentrations of NKAE (1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 % w/v) against red spider mite along with unsprayed control. Mixed Assam tea clones TV 1, 18, 20, 22, 23, 24, 25 and 26 were selected for the study by following Randomized Block Design with three replications. Each plot (3.6 m× 7.5 m) in the experiment was separated by two buffer rows of non-experimental tea bushes. Thirty bushes per replication were considered for each treatment. Plots (3.6 m× 7.5 m) with uniform infestation of red spider mite were chosen for this study. After selecting the plots, pre-treatment count was taken in the respective plots and two rounds of foliar spray were given at 7-day interval with hand operated Knapsak sprayer @ 400 L/ha. Post treatment observations were taken for four weeks after treatment at weekly interval. Observations on mite population were made on both adaxial and abaxial side of the thirty randomly collected mature leaves per replication for each treatment of NKAE along with unsprayed control by following mangling method adopted by Das (1960). Population of mites per 90 leaves per treatment was calculated by using the following formula:

% Reduction = Pre-treatment Population Count - Post treatment Population Count/Pre-treatment Population Count × 100.

RESULTS AND DISCUSSIONS

The efficacy of NKAE at different concentrations was evaluated against both nymphal and adult red spider mite and the results are presented in Table 1 and 2. All the tested concentrations of NKAE except 1% were found to be superior over control against nymphs and adults of red spider mite. The LC_{50} values of NKAE for nymphs and adults of red spider mite were found to be 47.73 mg/ml and 66.02 mg/ml respectively after 24 hrs of treatment. Perusal of data presented in Table 1 and 2 reveals that nymphs were more susceptible than adults. The toxic effect of NKAE was concentration and time dependant. NKAE @ 1% could not produce any toxicity against both nymphs and adults of red spider mite; however 2% NKAE exhibited 12-21% mortality after 24 hrs of treatment. At 10% concentration NKAE exhibited 72 hrs and 57% mortality of nymphs and adults red spider mite respectively after 24 hrs of treatment. But after 72 hrs of treatment the same was enhanced to the tune of 95% and 92% (Table 1 and 2). Similar results were also obtained by Radhakrishnan and Prabhakaran (2014) and reported 86% mortality of adults after 96h of exposure to 5% NKAE. They also observed 25% egg mortality of red spider mite after spraying NKAE at 5% concentration. Similarly Mourao *et al.* (2004) evaluated the toxicity of extracts of oil cake, seeds and leaves of *Azadirachta indica* against coffee red mite *Oligonychus ilicis* and estimated the LC_{50}

Table 1. Bio-efficacy of NKAE against adult red spider mite (RSM)

Treatment	Conc. (%)	N[a] (Adults)	Mortality [b] (%)		Lethal concentration [c]		
			24h	72h	LC_{50}	LC_{95}	b
NKAE	10	30.0±2.12	57.3±3.00e	92.0±3.73f			
	8	29.6±2.07	54.7±4.56d	82.0±4.75e			
	6	30.0±2.74	53.3±3.81d	78.3±3.78d	66.02	526.1	1.83
	4	29.4±1.14	40.8±3.61c	71.4±3.99c	mg/mL	mg/mL	
	2	29.6±1.52	12.1±3.63b	25.0±1.60b			
	1	29.8±1.30	0.0±0.00a	0.0±0.00a			
Control	-	29.6±1.52	0.0±0.00a	0.0±0.00a			
F- value			184.462	222.212			
P- value			0.001	0.001			

[a] Number (mean ± S.D) of adult red spider mite before application, [b] Percentage (mean ± S.D.) of mortality calculated as ratio between numbers of dead adult RSM after application of treatment and numbers of adult RSM before application of treatment, [c] Lethal concentration (for 50% or 95% mortality) in mg/mL, b regression coefficient. Means followed in the same column by the same letter are not significantly different ($P \leq 0.05$).

Table 2. Bio-efficacy of NKAE against nymphs of red spider mite.

Treatment	Conc. (%)	N[a] (Nymphs)	Mortality [b] (%)		Lethal concentration [c]		
			24 hrs	72 hrs	LC_{50}	LC_{95}	b
NKAE	10	29.4±2.88	72.5±6.32f	95.2±2.05g			
	8	30.0±1.87	67.1±7.22e	87.2±3.67f			
	6	29.6±2.70	62.8±6.02d	82.2±3.57e	47.73	265.09	2.22
	4	29.8±2.39	47.5±5.04c	75.1±3.86d	mg/mL	mg/mL	
	2	30.0±2.92	21.6±6.78b	31.6±6.72c			
	1	29.4±1.95	2.1±1.89a	10.7±3.98b			
Control	-	29.8±3.11	0.0±0.00a	0.0±0.00a			
F- value			74.581	120.169			
P- value			0.001	0.001			

[a] Number (mean ± S.D) of nymphs before application, [b] Percentage (mean ± S.D.) of mortality calculated as ratio between numbers of dead nymphs after application of treatment and numbers of nymphs before application of treatment, [c] Lethal concentration (for 50% or 95% mortality) in mg/mL, b regression coefficient. Means followed in the same column by the same letter are not significantly different ($P \leq 0.05$).

values at 0.02, 15.9 and 121.4 mg/ml respectively which substantiate our results. The ovicidal effect of NKAE on the eggs of red spider mite after exposing them to different concentrations is graphically depicted in fig.1. Significant reduction in egg hatchability was exhibited only at 4% & above concentrations of NKAE. Further, after the exposure of NKAE to eggs, the hatched larvae were carefully sifted from different treatments and released into fresh TV1 mature leaves to observe their post embryonic development in terms of total duration (from egg to adult) and per cent adult emergence (Table 3). The total developmental period was prolonged by 1-4-day at 4-10% NKAE in comparison with untreated control. The adult emergence was also affected significantly at higher concentrations (4-10%) only. The lowest adult emergence was recorded at 10% NKAE, whereas 1 and 2% NKAE showed no any effect on adult emergence and was at par with control (Table 3). Coudrict *et al.* (1985) also observed that neem seed extract prolonged larval development and induced larval mortality of *Bemisia tabaci* on cotton foliage. Similarly Narasimhan *et al*. (2005) observed that desacetylsalannobutyrolactone isolated from the kernel of *A. Indica* affected the growth and development of tobacco cutworm,

Spodoptera litura and prolonged the larval duration and caused larval mortality. Furthermore NKAE at all tested concentrations exhibited pronounced anti-ovipositional properties against mites. The fecundity/day was significantly reduced in all the NKAE treated leaf surfaces from 1.16 – 1.66 eggs/female/day compared to 3.83 eggs/female/day in untreated leaf surface (Table 4).

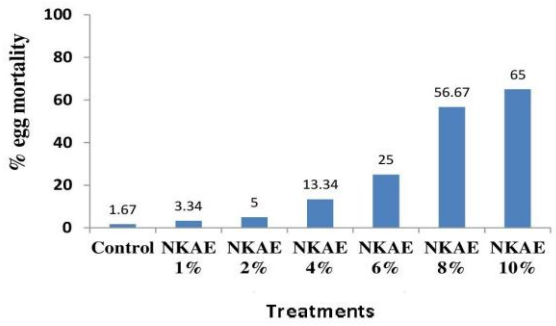

Fig. 1. Ovicidal activity of NKAE on the eggs of red spider mite.

Pasini *et al.* (2003) determined the efficacy of commercially-formulated neem oil against red mite, *O. yothersi*, infesting Paraguay tea (*Ilex paraguariensis*). The formulation efficiently controlled adult red mite, by affecting the fecundity but did not inhibit oviposition. In contrast with Pasini's observation, NKAE inhibits the oviposition rate of *O. coffeae*.

Table 3. Effect of NKAE on adult emergence (Mean ±SD) of Red spider mite.

Concentration (%)	Adult emergence (%)	Total duration (days) (Mean ± SD)
10.0	43.33±5.77f	23.66±1.24
8.0	53.33±11.54e	22.00±0.81
6.0	66.66±11.54d	21.33±0.94
4.0	80.00±10c	20.33±0.94
2.0	93.33±5.77 b	19.33±0.47
1.0	96.66±5.77a	19.66±0.47
0.0	96.66±5.77a	19.33±0.94
F- value	19.867	
P- value	0.001	

[a]Percentage (mean ± S.D.) of adult emergence calculated as ratio between numbers of adult emerged and numbers of larvae. Means followed by the same letter are not significantly different ($P \leq 0.05$).

Table 4. Effect of NKAE on oviposition behaviour of red spider mite. (Mean ± SD)

Concentration (%)	No. of eggs laid/female/day[a]
10.0	1.16±0.68
8.0	1.50±0.50
6.0	1.50±0.50
4.0	1.66±0.74
2.0	1.33±0.47
1.0	1.33±0.47
Control (Untreated)	3.83±0.68
CD (P=0.01)	1.05
CV (%)	37.84

[a] Average of six days

Our findings are in agreement with Yasodha and Natarajan (2007) who studied the oviposition deterrence and ovicidal action of certain wild *Solanum* spp., kernels of *A. indica* and dried powder of *Acorus calamus* against *Leucinodes orbonalis* and reported strong oviposition deterrent as well as ovicidal action by 5% NSKE in combination with *A. Calamus*. The data on the bioefficacy of NKAE under field conditions against red spider mite are summarized in Table 5. After the first spray significant reduction of mite population was recorded only at 4% and above concentrations of NKAE indicating that the mortality of mites was concentration dependent. After application of second round of treatments the reduction of mite population was further enhanced to the tune of 60.2–69.8 % at 4-10% concentrations. However, in the subsequent third and fourth weeks there was slight decline in the efficacy of NKAE. Concentrations below 4% did not show any encouraging effect under field condition (Table 5). Sudoi (1998) observed that direct application of 2.5% Neem Seed Oil on *O. coffeae* significantly reduced their survival after 24 hrs and act as a contact poison on *O. coffeae* indicating their potentiality in the control of *O. coffeae* in tea nurseries and in the field. Kwaifa *et al.* (2015) from Nigeria reported that though synthetic insecticide conferred more protection on okra against flea beetles, neem kernel extract also significantly reduced the incidence of beetles on okra in the field by conferring different levels of protection to the leaves, flowers and pods of okra.

Table 5. Field bio-efficacy of NKAE on tea Red spider mite (RSM)

Treatments	Per cent reduction of RSM population after			
	1st week	2nd week	3rd week	4th week
NKAE @1%	9.0a	16.6a	13.7a	14.3b
NKAE @2%	13.5a	19.6ab	19.6ab	17.0b
NKAE @4%	38.9b	60.2c	59.0c	56.7c
NKAE @6%	43.0b	66.7c	64.1c	63.5c
NKAE @8%	43.4b	67.3c	66.0c	65.0c
NKAE @10%	49.6b	69.8c	68.1c	67.6c
Control (untreated)	0.0a	- 0.9a	2.4a	1.7a
F- value	46.586	97.268	169.326	202.393
P- value	0.001	0.001	0.001	0.001

Means followed in the same column by the same letter are not significantly different ($P \leq 0.05$).

Different stages of predatory coccinellid, *S. gilvifrons* were exposed to different concentrations of NKAE to see the effect on growth and development of the predator. Study revealed that exposure to NKAE did not cause mortality to all the stages of the predator. Comparatively at higher concentrations (8 and 10%) NKAE extended the larval duration of the predator by two days compared to untreated control (Table 6).

Table 6. Effect of NKAE on *S. gilvifrons* larval and adult mortality (%), pula emergence (%), duration of larva, pula and adult (days)

Concentration (%)	Larva		Pupa		Adult	
	Mortality	Duration	Emergence	Duration	Mortality	Duration
10.0	0	9.5-12.9	100.0	3.0-5.7	0.0	Adult
8.0	0	10.0-	100.0	2.8-6.0	0.0	longevity
6.0	0	13.2	100.0	3.2-6.0	0.0	was
4.0	0	8.5-11.0	100.0	3.0-5.5	0.0	observed
2.0	0	8.0-10.5	100.0	3.5-6.2	0.0	for 30
1.0	0	7.0-10.2	100.0	3.0-6.0	0.0	days
Control (water)	0	6.8-9.0	100.0	3.0-6.0	0.0	

Schmutterer (1995) reported that, NKAE did not cause any appreciable effect to coccinellid predator, *S. gilvifrons* presumably due to low concentrations of AzaA in NKAE. This fact was further substantiated by Ingawale *et al.* (2005) where he observed no any adverse effect on the population level of different lady bird beetles following the application of 5% NKAE under field conditions.

From this investigation it was vivid that the NKAE was very effective against red spider mite exhibiting strong acaricidal, ovicidal, anti-ovipositional, and growth inhibitory action. Study also revealed that NKAE up to 10% concentration is completely safe to the cocinellid predator, *S. gilvifrons.* Hence NKAE can effectively be utilized for the management of red spider mite in tea plantations and can be explored in organic IPM which will substantially reduce the insecticide load in tea.

ACKNOWLEDGEMENT

The author is thankful to the Director, Tocklai Tea Research Institute, Tea Research Association, Jorhat, Assam for providing necessary facilities, constant encouragement and valuable suggestions.

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Mridul Sarmah
North Bengal Regional R and D Centre, Tea Research Association, Nagrakata 735 225, West Bengal, India
Contact N0. *:* + 91 8145904501
Email: mridulmm_24@yahoo.co.in