Effect of neem kernel aqueous extract (NKAE) on growth and development of red slug caterpillar, *Eterusia magnifica* butl in tea in North-East India, India

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ABSTRACT

Aqueous extract of neem seed kernel (NKAE) was tested in laboratory conditions to evaluate its effects on larval weight, larval duration, mortality percent, adult emergence percent and antifeedant activity against red slug caterpillar, *Eterusia magnifica* (Lepidoptera: Zygaenidae).Different concentrations of NKAE as 2,4,6,8 and 10% were used separately to evaluate the effects of NKAE. NKAE was found to be effective and concentration dependant against *Eterusia magnifica*. Larval weight was concentration dependant and decreased with the increase of NKAE concentrations. Antifeedant activity was in ascending order with increase in concentrations. The highest leaf area consumed were recorded at 2% concentration in fifth instar as 1158.6±254.79 sq cm and it was lowest at 8% concentration in first instar larva as 92.2±26.04 sq cm. The leaf area protection of NKAE was recorded as 48.09+12.61-61.59+11.28% in first, 22.74+13.59-54.31+14.16% in second, 60.05+11.94-87.5+2.98% in third, 33.9+10.79-65.82+15.71% in 4th and 30.32+10.2-64.48+19.35% in 5th instar larva at 2-10% concentrations respectively. Preference index obtained for NKAE in all tested concentrations indicated its deterrence against feeding of red slug caterpillar. The larval duration recorded maximum as 5.8±0.45, 6.6±0.55, 7.6±0.55, 7.6±0.55 and 13.6+2.1 days at 8 percent concentrations over control as 4.4±0.55, 5.4±0.54, 5.6±0.55, 6.6±0.55 and 10.6±1.7 days in 1st, 2nd, 3rd, 4th and 5th instar larva respectively. The larval mortality was maximum after 72 hrs of treatment at all the concentrations and recorded 12.92±0.02-98.23±2.1% in 1st, 9.07±0.4 - 39.4±2.2% in 2nd, 7.53±0.7 – 32.63 ±0.8% in 3rd and 2.59±1.0 – 11.9±0.6% in 4th instar at 2, 4, 6, 8 and 10 percent concentrations of NKAE respectively. Adult emergence percent was reduced to 0-35.63±1.7 in male and 0-29.4±1.1 percent in female at 8-2 percent concentrations as against 99.1±0.6 and 97.6±0.5 respectively in the untreated control.

Key words: Tea, Neem Kernel Aqueous Extract, *Eterusia magnifica* (Red Slug Caterpillar), Antifeedant

INTRODUCTION

Tea, *Camellia sinensis* (L.) O. Kuntz is one of the most important commercial crop covering an area of 5.07 lakh hectare in India producing annually 8.54 million kg made tea. Among the various constraints of tea production in North-East India, the problem of insect pests is of major importance. More than one hundred species of arthropod pests are known to attack tea in North-East India (Das, 1965). In North East India, tea plant is colonized by a complex of insect species including the tea mosquito bug, red, pink, and purple mites, thrips, termites, red slug caterpillar, looper caterpillar, green leaf hopper and so on. Among the chewing pests, *Eterusia magnifica* (Lepidoptera: Zygaenidae) also known as red slug caterpillar is widely distributed and is considered as one of the productivity barriers in teas which caused periodical menace mostly during the flushing period and attaining peak during March-July (Das, 1965). To control this pest various efforts have been made by using conventional synthetic pesticides. The annual consumption of insecticides in North-East India alone ranged from 7.35 to 16.75 kg/ha (Barbora and Biswas, 1996). Applications of such huge quantity of pesticides cause various problems such as resistance problem, deleterious effects on natural enemies, up setting of ecological balances and accumulation of pesticide residue on made tea. Considering ill effects caused by large scale and indiscriminate usage of pesticides, presently thrust has been given on bio-rational approaches to develop an effective management strategy for this periodical serious pest. Among the natural insecticides investigated in recent years, the constituents of neem tree (*Azadirachta indica* Juss: Meliaceae) have shown great promise in controlling numerous (nearly over 200 species) insect pests of cultivated crop plants (Mithyantha and Shankar, 1999; Vyas and Mistry, 1999). Neem *(A. indica)* is claimed to be

effective against a variety of insects, nematodes, bacteria, fungi and viral diseases and is reported to be effective against over 300 pest species (Mithyantha and Sankar, 1999). In recent years, several reviews have been published which out line the use of *A. indica* as a botanical insecticide (Jacobson, 1989; Koul *et al*., 1990; Schmutterer, 1990; Ascher, 1993; Casey Scalar, 1998). The compounds from neem have a number of properties useful for insect pest management. These include toxicity, repellence, feeding and oviposition deterrence, insect's growth regulatory activity etc. (Schmutterer, 1990 and Koul, 2004). The mode of action of Azadirachtin, the principal insecticidal constituents of neem oil has only been elucidated (Mordue and Blackwell, 1993). Within the Azadirachtin molecule, the decalin fragment is responsible for the insect growth regulation and developmental effects, while the hydroxy furan fragment causes the antifeedant effect more widely observed among the target species (Aldhous, 1992).

Neem extracts including Azadirachtin are known to deter feeding in *Schistocerca gregaria* (Pradhan *et al*., 1962; Butterworth and Morgan, 1971). Saxena *et al*. (1981) reported repellency and feeding deterrency of neem oil to brown plant hopper. Although, good numbers of references on record about neem and neem-based formulation have been tried to control various insect pests over the world (Vyas and Mistry, 1999; Dhawan, 1999; Dhawan and Simwat, 1999; Dhaliwal *et al.,* 1999; Singh *et al.,* 1999). But no systematic work has been reported on effect of NKAE against red slug caterpillar in North-East India. Hence an attempt has been made to study the effectiveness and utilization of NKAE against red slug caterpillar.

MATERIALS AND METHODS

Preparation of neem kernel aqueous extract: The collected 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 materials were dissolved in 100 ml of water and kept it for over night. The dissolved NSK materials were filtered through Whatmen filter paper. The filtered liquid portion was taken as a stock solution for preparing different concentrations.

Antifeedant activity of NKAE against Red slug caterpillar: Fresh mature leaves were collected from the field, washed thoroughly under tap water and dried under fan for ten minutes. Before spraying the NKAE, the area of the mature leaves were measured by using graph paper treatment wise. The leaves were then sprayed with different concentrations of the aqueous extract by using glass atomizer, allowed to dry under mild fan for about five minutes, and placed in a glass petridis (20 cm diameter) with moist filter paper at the bottom. One prestarved larva was released in each petri plate. Each treatment was replicated five times at room temperature. Actual leaf area consumed, percent area protection due to treatment over control and preference index were calculated (Kogan and Goedon, 1970) after 48 hours.

$$\text{Percent area protection} = \frac{\text{Area consumed in Control} - \text{Area consumed treatment}}{\text{Area consumed in control}} X100$$

$$\text{Preference index (PI)} = \frac{2\,X\,\text{feeding on test concentration}}{\text{Feeding on test concentration} + \text{feeding on control}}$$

Where PI > 1 - preference for test concentration
PI < 1 - deterrence against feeding
PI = 1 - feeding on test concentration equals control

Impact of NKAE on mortality of red slug caterpillar
Healthy mature tea leaves were subjected to different concentrations of NKAE by using glass atomizer and treated leaves were allowed to dry under mild fan about five minutes. On each treated leaf, one larva was released with the help of camlin hair brush from the culture and placed in a glass Petri plate (20 cm diameter) with moist filter paper at the bottom. Each treatment was replicated fifteen times. The individuals of red slug caterpillar were recorded for up to 72 hours at 24 hours of interval. Percent mortality was calculated by using the following formula-

$$\text{Percent mortality} = \frac{\text{Pre treatment population} - \text{post treatment population}}{\text{Pre treatment population}} x100$$

RESULTS AND DISCUSSION

The result presented in Table-1 indicated the effect of NKAE treated in different concentrations on larval weight of red slug caterpillar. NKAE had a great impact on larval weight of red slug caterpillar. Result showed that the larval weight was concentration dependant, larval weight decreased with the increase of NKAE concentrations. Coudrier *et al*. (1985) also observed that neem seed extract prolonged larval development and induced larval mortality of *Bienisia tabaci* on cotton foliage.

Antifeedant activities of NKAE against different larval instars of red slug caterpillar are summarized in Table-II. Antifeedant activity was in ascending order with increase in concentrations. The leaf area consumed was highest 1158.6±254.79 sq cm at 2% concentration in 5th instar and it was lowest at 8% concentration in 1st instar larva as 92.2±26.04 sq cm. similar experiment also initiated by Yoshida and Toscano (1994) and found that the relative consumption rate of *Heliothis virescence* larvae treated

Table 1. Effect of NKAE on larval weight of Red Slug Caterpillar

Treatments	Weight (gm) of larva in different days of observation (Mean ± SD)				
	1st	2nd	3rd	4th	5th
Control	0.007±0.005	0.023±0.007	0.143±0.06	0.703±0.13	1.587±0.14
NKAE (2%)	0.003±0.002	0.0031±0.0008	0.026±0.01	0.688±0.13	1.520±0.08
NKAE (4%)	0.002±0.001	0.003±0.0009	0.005±0.002	0.676±0.12	1.439±0.05
NKAE (6%)	0.002±0.001	0.002±0.0006	0.004±0.001	0.620±0.08	1.430±0.05
NKAE (8%)	0.001±0.0004	0.001±0.0004	0.003±0.001	0.615±0.23	1.419±0.05
CD (P = 0.05)	2.41	1.004	0.024	0.104	0.078

with azadirachtin was 25 % of the control. Percent leaf area protection was increased with the increase of NKAE concentrations. The antifeedant property to the tune of 87.5±2.98 percent over control was attained in 3rd instar larva at 8% concentration, while it was lowest as 22.74±13.59 percent in 2nd instar larva at 2% concentration. Preference index obtained for NKAE in all tested concentrations were below one (1), indicating its deterrence effect against feeding of red slug caterpillar. Kakoty *et al*. (1993) and Borthakur *et al*. (1992) tried various neem formulation and neem oil against Bunch and Psychid caterpillars in the laboratory shown promising antifeedant property, protecting leaf in the range of 62-100%. Grisakova *et al*. (2006) reported that cabbage leaf sprayed with neem seed karnel suspension reduced the damaged by *Pieris brassicae* larvae but failed to give any significant protection to castor plant leaves against the hairy caterpillar, *Amasacta albistriga*. Different insect species showed varying degrees of sensitivity to various neem extracts and compounds. Naumann and Isman (1995) found that neem seed oil extract have little or no effect on three species of noctoid moth; *Trichoplusiae ni*, *Peidroma saucia* and *Spodoptera litura*. According to Mordue and Blackwell (1993), order, Lepidoptera is the most sensitive to Azadirachtin's antifeedant effect (< 1-50 ppm), coleopteran, hemipteran and homopteran (100-600 ppm) being less sensitive.

The result presented in Table-3 indicated the larval duration of red slug caterpillar after treating with 2, 4, 6 and 8 percent concentration of NKAE. Result showed that the larval duration increased in each larval stage with the increase of NKAE concentrations. The larval duration recorded were 5.8±0.45, 6.6±0.55, 7.6±0.55, 7.6±0.55 and 13.6+2.1 days at 8 percent concentrations over control (4.4±0.55, 5.4±0.54, 5.6±0.55, 6.6±0.55 and 10.6±1.7 days) in 1st, 2nd, 3rd, 4th and 5th instar larva, respectively.

The results of mortality of NKAE in different larval instars of red slug caterpillar at 24, 48 and 72 hrs are summarized

Table 2. Anti-feedant activity of NKAE against different larval instars of Red Slug caterpillar

Developmental stages	Parameters	Treatments (Mean ± SD)					
		Control	2%	4%	6%	8%	CD(P=0.05)
1st	AC (sq.cm)	259.2±111.38	128±51.07	123.2±58.4	103.4±42.81	92.2±26.04	59.13
	LAP (%)	-	48.09±12.61	52.13±7.01	57.7±12.65	61.59±11.28	9.28
	PI	-	0.68±0.11	0.64±0.06	0.59±0.13	0.55±0.13	0.09
2nd	AC (sq.cm)	321+151.9	246.6+111.7	215.8+100.5	155.2+48.72	135.8+54.61	94.23
	LAP (%)	-	22.74+13.59	32.12+16.01	44.12+25.73	54.31+14.16	14.66
	PI	-	0.87+0.07	0.80+0.11	0.67+0.22	0.62+0.13	0.12
3rd	AC (sq.cm)	1660.4±500.8	641.2±185.3	497.8±496.7	239.4±70.09	208.2±79.4	307.44
	LAP (%)	-	60.05±11.94	74.15±19.59	85.11±3.75	87.5±2.98	9.78
	PI	-	0.56±0.11	0.38±0.24	0.26±0.06	0.24±0.06	0.37
4th	AC (sq.cm)	1500.8±669.9	1026.35±594.5	888.35±761.04	551.55±483.26	453±254.86	540.72
	LAP (%)	-	33.9±10.79	47.54±26.41	63.75±17.67	65.82±15.71	15.46
	PI	-	0.79±0.07	0.56±0.34	0.51±0.19	0.49±0.2	0.19
5th	AC (sq.cm)	1696±509.87	1158.6±254.79	1001.2±403.24	670±544.59	642±478.97	420.01
	LAP (%)	-	30.32±10.2	40.22±24.02	61.27±17.94	64.48±19.35	15.48
	PI	-	0.82±0.07	0.72±0.21	0.51±0.17	0.50±0.21	0.15

AC (sq.cm) = Area consumed (sq. cm), LAP (%) = Leaf area protection, PI= Preference index

Table 3. Effect of NKAE on larval duration of Red Slug caterpillar

Treatments	Duration of different larval stages (Days) (Mean ± SD)				
	1st	2nd	3rd	4th	5th
Control	4.4±0.55	5.4±0.54	5.6±0.55	6.6±0.55	10.6±1.7
NKAE (2%)	4.8±0.44	5.6±0.55	5.8±0.44	6.8±0.45	11.4±1.5
NKAE (4%)	5.2±0.44	5.8±0.45	6.6±0.55	6.8±0.45	12.4±1.5
NKAE (6%)	5.6±0.55	6.4±0.54	7.4±0.55	7.6±0.54	13.4±2.1
NKAE (8%)	5.8±0.45	6.6±0.55	7.6±0.55	7.6±0.55	13.6±2.1
CD (P = 0.05)	0.457	0.494	0.494	0.476	1.654

Table 4 (a). Effect of NKAE on percent larval mortality of Red Slug caterpillar after 24 hrs of observation

Treatments	Different stages (Mean ± SD)				
	1st	2nd	3rd	4th	5th
Control	0	0	0	0	0
NKAE (2%)	0	0	0	0	0
NKAE (4%)	0	0	0	0	0
NKAE (6%)	17.97±1.5	8.63±1.2	6.37±0.8	0	0
NKAE (8%)	28.93±1.5	1187±1.1	4.53±0.9	0	0
CD (P = 0.05)	1.19	0.92	0.68	-	-

Table 4(b). Effect of NKAE on percent larval mortality of Red Slug caterpillar after 48 hrs of observation

Treatments	Different stages (Mean ± SD)				
	1st	2nd	3rd	4th	5th
Control	0	0	0	0	0
NKAE (2%)	0	0	0	0	0
NKAE (4%)	29.07±1.3	9.97±0.5	8.4±0.92	0	0
NKAE (6%)	26.67±1.7	13.63±2.8	12.67±0.8	0	0
NKAE (8%)	37.67±1.7	22.13+3.0	7.56±0.9	2.45±0.6	0
CD (P = 0.05)	2.26	2.38	0.88	0.33	-

Table 4(c). Effect of NKAE on percent larval mortality of Red Slug caterpillar after 72 hrs of observation

Treatments	Different stages (Mean ± SD)				
	1st	2nd	3rd	4th	5th
Control	0	0	0	0	0
NKAE (2%)	12.92±0.2	9.07±0.4	7.53±0.7	2.59±1.0	0
NKAE (4%)	42.17±3.0	28.88+1.2	21.07±1.3	6.65±0.5	0
NKAE (6%)	61.97+1.9	37.17±3.1	27.73±1.9	8.6±0.8	1.8±0.4
NKAE (8%)	98.23±2.1	39.4±2.2	32.63±0.8	11.9±0.6	2.01±0.1
CD (P = 0.05)	2.36	2.31	1.43	0.92	0.22

in Table-4 (a), (b) and (c). The exposure of different larval instars at 2 and 4 percent NKAE did not show any mortality within 24 hrs. However, at 6 and 8 percent concentration of NKAE mortality recorded were 17.97±1.5, 28.93±1.5; 8.63±1.2, 11.87±1.1 and 6.37±0.8, 4.53±0.9 percent respectively in 1st, 2nd and 3rd instar larva. No mortality was recorded in 4th and 5th instar larva at any of the concentrations after 24 hrs of observation.

The exposure of different larval instars at 2 percent NKAE did not show any mortality after 48 hrs. However, at 4, 6 and 8 percent concentration of NKAE mortality recorded were 29.07±1.3, 26.67±1.7, 37.67±1.7; 9.97±0.50, 13.63±2.8, 22.13+3.0 and 8.4±0.92, 12.67±0.8 7.56+0.9 percent respectively in 1st, 2nd and 3rd instar larva and at 8 percent concentration mortality in 4th instar larva also was recorded (2.45±0.6%). The result showed no mortality in 4th instar

Table 5. Effect of NKAE on adult emergence percent of Red Slug caterpillar

Treatments	Adult emergence percentage (Mean ± SD)	
	Male	Female
Control	99.1±0.6	97.6±0.5
NKAE (2%)	35.63±1.7	29.4±1.1
NKAE (4%)	6.8±0.8	6.5±0.7
NKAE (6%)	6.2±0.3	0
NKAE (8%)	0	0
CD (P=0.05)	1.19	0.76

larva at 4 and 6 percent concentrations and in 5th instar larva at all the tested concentrations after 48 hrs of observation.

The result presented in Table-IV (c) revealed that the larval mortality was maximum after 72 hrs of treatment at all the concentrations. The larval mortality recorded were 12.92±0.2 - 98.23±2.1% in 1st, 9.07±0.4 - 39.4±2.2% in 2nd, 7.53±0.7 – 32.63 ±0.8% in 3rd and 2.59±1.0 – 11.9±0.6% in 4th instar at 2, 4, 6, 8 and 10 percent concentrations of NKAE respectively. The result indicated no mortality in 5th instar larva at 2 and 4 percent concentrations. At 6 and 8 percent concentration mortality were 1.8±0.4 and 2.01±0.1 percent respectively. Ascher, (1993) also observed the effect of NKAE as causing mal formation and mortality in a dose dependant manner.

The effect NKAE on percent adult emergence of red slug caterpillar is presented in Table-5. The results showed that the adult emergence significantly reduced to 0-35.63±1.7 percent in male and 0-29.4±1.1 percent in female at 8-2 percent concentrations as against 99.1±0.6 and 97.6±0.5 respectively in the untreated control.

CONCLUSION

The present study indicated that neem products (NKAE) can be effectively used for red slug caterpillar management in alternation with recommended insecticides. Moreover, this will not only restrict the over use of insecticides but can be effectively exploited by resource limited farmers particularly in rainfed areas. NKAE which is very effective against red slug caterpillar, can be prepared at farmer's level with simple technology and also it is cheap in cost. NKAE can be effecively used for control of red slug caterpillar if aimed at younger instars. To sum up, the NKAE has a good potential for controlling the red slug caterpillar infestation in tea plantations. There is a need to educate the farmers about side effects of toxic chemicals and benefits of adopting NKAE in a scientific manner.

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