BIOEFFICACY OF AZADIRACHTIN CONTENT OF NEEM FORMULATION AGAINST THREE MAJOR SUCKING PESTS OF TEA IN SUB HIMALAYAN TEA PLANTATION OF NORTH BENGAL, INDIA

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Abstract

This study was conducted to determine dose-mortality response of the three sucking pests of tea: *Helopeltis theivora* Waterhouse, *Scirtothrips dorsalis* Hood and *Empoasca flavescens* Fabricious, to neem formulation of varying azadirachtin content (300, 1 500, 3 000, 10 000 and 50 000 ppm) under field conditions. Of neem formulation tested, 50 000 ppm and 10 000 ppm had the lowest LC_{50} values, highest slopes and relative potencies for the three target pests. Among the three sucking pest species studied, *S. dorsalis* was highly susceptible to neem formulation of varying azadirachtin content as revealed by the lower LC_{50} values (0.09 – 0.20 ppm), highest slopes (1.99-2.54) and mean percent reduction (46.8-75.8%) followed by *E. flavescens* (0.12-0.39 ppm; 1.92-2.38; 46.3-64.5%) and *H. theivora* (0.16-2.27 ppm; 1.48-2.25; 34.8-54.1%). LC_{50} decreased as the azadirachtin content of neem formulation was increased. Pest damage (evaluated in terms of mean % reduction of pest population) was lessened as the concentration of azadirachtin (tested dose) increased. The present study, however, revealed the fact that azadirachtin concentration is the determining factor in terms of its bioactivity, i.e., in controlling the pest. The bioactivity of azadirachtin concentration may vary from insect to insect but in tea, using of 50 000 ppm azadirachtin is ideal for managing the three major sucking pests.

Keywords: *Azadirachta indica* A. Juss; bioactivity; field conditions; mortality; population count; *Helopeltis theivora*; *Scirtothrips dorsalis*; *Empoasca flavescens*

INTRODUCTION

Tea, a global beverage, is grown on over 2.71 million ha in more than 34 countries across Asia, Africa, Latin America, and Oceania to produce 3.22 million metric tons of made tea annually (Hazarika et al., 2009). The demand for contaminant-free tea and the need to sustain productivity and quality have led to a movement toward organic tea cultivation by India in 1985 (Gurusubramanian et al., 2008). Recently, Gurusubramanian et al. (2009) reviewed work on botanicals and their uses in tea pest management. Among botanicals, neem, in various formulations, is recommended and registered against tea pests (Sarmah et al., 2006) and is widely used in several countries around the world today either singly in integrated pest management or in conjunction with synthetic pesticides (Isman, 2006).

The neem tree (*Azadirachta indica* A. Juss), from the Meliaceae (mahogany) family, known as margosa or Indian lilac, has long been recognized for its properties both against insects and in improving human health.

The tree is now grown in most tropical and subtropical areas of the world for shade, for reforestation programmes and in plantations for the production of compounds which have toxic, antifeedant and repellent properties against insects (Mordue and Nisbet, 2000).

Azadirachtin, a complex tetranortri-terpenoid limonoid from the neem plant, is the main component responsible for antifeedant, growth inhibitory, growth regulatory and toxic effects on insects (Koul, 1992; Mordue and Blackwell, 1993, Aerts and Mordue, 1997, Koul et al., 2004).

The amount of azadirachtin in the neem oils varies widely and is highly correlated with its bioactivity (Isman et al., 1990). This is also true with the neem formulations available in the market showing a variation of 0.03-6.5% in their azadirachtin content (Kumar et al., 2003). Now the question arises as to whether formulations of higher azadirachtin content are superior to formulations of lower azadirachtin content.

The present study was aimed to determine the insecticidal potential of neem formulation of varying azadirachtin content (300, 1500, 3000, 10,000 and 50,000 ppm) against three major sucking pests of tea, tea mosquito bug: *Helopeltis theivora,* (Hemiptera: Miridae), tea thrips: *Scirtothrips dorsalis,* (Thysanoptera: Thripidae) and green fly: *Empoasca flavescens* (Homoptera: Jassidae) under natural field conditions of a tea plantation in India.

MATERIALS AND METHODS

Preparation of neem formulation

Neem formulations (Biogold, liquid formulation; date of manufacture – February, 2008; batch no. 1429/02/2008 – 2009; produced by crushing dried seeds under pressure referred to as expeller oil) of varying azadirachtin content (300, 1 500, 3 000, 10 000, and 50 000 ppm) were obtained from Biogold India Ltd., Kolkata and further analysed for azadirachtin content through HPLC at Entomology Research Institute, Loyola College, Chennai for accuracy (Isman et al., 1990). Overall recovery of azadirachtin from spiked samples averaged 86% and their bioefficacy between 75-90% under laboratory conditions (Gurusubramanian et al., 2009). From each such neem formulation five doses were prepared (at 0.50, 0.33, 0.20, 0.10 and 0.066 %) and tested against *H. theivora*, *S. dorsalis* and *E. flavescens*.

Field evaluation of varying formulations of azadirachtin

Separate field experiments for three sucking tea pests were conducted during first flushing season (April – May 2007 for tea mosquito bug and thrips; April – May 2008 for green fly) of tea in section 32 at North Bengal University experimental station, University of North Bengal, Siliguri, Darjeeling, India**.** Treatment dose (0.50, 0.33, 0.20, 0.10 and 0.066 %) of the five varying formulations of neem with varying azadirachtin content (300, 1 500, 3 000, 10 000 and 50 000 ppm) was determined on the basis of the results of laboratory evaluation carried out against the tea pests (Sarmah et al., 2006; Roy et al., 2010).

Initially a survey was carried out in section 32 to ensure plots with heavy infestation of target species for the trial. Pest density was determined using standard direct count method (Rahman et al., 2005). Mixed Assam seed jat young tea plants (15 years old) growing under the shade trees, *Albizia chinensis* and *Albizia lebbek* were used for the experiments in the current study. Randomized block design with three replications was used. The pattern of plantation was single hedge type, i.e. distance between the tea bushes was maintained at 65 cm and distance between two parallel rows of bushes was maintained at 100 cm. Standard cultivation practices for this location were used to maintain the plants. One hundred bushes comprised each block (6.5 × 9.0 m) for each treatment of varying formulations of azadirachtin content. Each treated block in the experiment was separated by two buffer rows or guard row (1.3 m) to avoid cross contamination. The experiments were conducted at 28 ± 3 °C, 75 ± 5% relative humidity and a photoperiod of 14:10 (L:D) h.

Indirect population count was conducted in 30 young shoots (feeding injury by the nymphs and adults was used to assess) for tea mosquito bugs and direct population count was conducted in lower surface of leaves up to 5 and a bud in the case of green fly (nymphs and adults); and 3 and a bud for thrips (nymphs and adults), respectively. After selection of the plots, pretreatment count was taken in the respective plots and two rounds of foliar spray were given at a 15-day interval with precalibrated knapsack sprayer with spray fluid of 400 l ha^{-1}. Pest density was recorded a day before application for both experimental and control tea plots. Post treatment observations were taken in four consecutive weeks. Percent reduction was calculated for the three pests using the formula: %Reduction = 100 – [C1*T2/C2*T1] * 100 where C1, C2 are pre-treatment, post-treatment pest density in control whereas T1, T2 are pre-treatment, post-treatment pest density in experimental plots respectively (Rahman et al., 2005; Dua et al., 2009).

Statistical analysis

Median lethal concentrations (LC_{50}) were computed from per cent reduction data of the three pests through probit regression analysis (Finney, 1971). The slopes, lethal concentrations in ppm (LC_{50}), were calculated. Relative potencies (RP) are used to compare the degree of effectiveness of azadirachtin content of neem formulation against a standard, 300 ppm azadirachtin content. A relative potency >1 indicates the formulation was more toxic to tea target pests than was 300 ppm azadirachtin content. The two-way ANOVA was carried out on the mean percent reduction data of pest population to justify the difference between critical difference of mean ($P < 0.05$) and coefficient of variation (%) in terms of azadirachtin content, concentration and their interactions for taking statistical decisions.

RESULTS

Dose mortality response

Tea mosquito bug, thrips and jassids dose-mortality responses to the tested neem formulations are summarized in Tables 1-3. Lowest LC_{50} values of 0.09-0.23 ppm and 0.12-0.38 ppm for thrips; 0.12-0.24 ppm and 0.14-0.25 ppm for jassids; and 0.16-0.38 ppm and 0.18-0.51 ppm for tea mosquito bug were observed in neem formulations of higher azadirachtin content i.e. 50 000

Table 1: Comparison of median lethal concentration (LC_{50}) values, slopes, chi square, and relative potency (RP) of neem formulations of varying azadirachtin content against *H. theivora* under field conditions

Azadirachtin content (ppm)	Round of spray	Observation (week)	LC_{50}	Slope	SE	Chi square*	RP
300	I	First	2.0797	1.5069	0.0568	4.80	1.00
1 500			0.8221	1.7153	0.0336	10.42	2.52
3 000			0.5123	1.8453	0.0251	13.94	4.05
10 000			0.4535	1.8821	0.0237	15.49	4.58
50 000			0.3218	1.9939	0.0230	20.33	6.46
300	I	Second	2.2727	1.4896	0.0612	4.60	1.00
1 500			1.0265	1.6603	0.0438	8.69	2.21
3 000			0.6441	1.7800	0.0297	12.85	3.52
10 000			0.5198	1.8410	0.0251	13.65	4.37
50 000			0.3847	1.9341	0.0220	17.85	5.90
300	II	Third	0.3382	1.9769	0.0202	16.83	1.00
1 500			0.2905	2.0299	0.0189	19.33	1.16
3 000			0.2410	2.0989	0.0177	21.16	1.40
10 000			0.2186	2.1369	0.0172	20.69	1.54
50 000			0.1861	2.2028	0.0164	23.36	1.81
300	II	Fourth	0.2926	2.0272	0.0190	18.53	1.00
1 500			0.2596	2.0708	0.0182	20.12	1.12
3 000			0.2055	2.1618	0.0171	19.10	1.42
10 000			0.1886	2.1972	0.0165	23.82	1.55
50 000			0.1645	2.2559	0.0163	22.68	1.77

* degrees of freedom – 4; values significant at $P < 0.05$ level.

Table 2: Comparison of median lethal concentration (LC_{50}) values, slopes, chi square, and relative potency (RP) of neem formulations of varying azadirachtin content against *S. dorsalis* under field conditions

Azadirachtin content (ppm)	Round of spray	Observation (week)	LC_{50}	Slope	SE	Chi square*	RP
300	I	First	0.2677	2.0595	0.0184	17.78	1.00
1 500			0.1935	2.1865	0.0169	20.29	1.38
3 000			0.2137	2.1460	0.0173	18.35	1.25
10 000			0.2233	2.1285	0.0173	22.88	1.19
50 000			0.1594	2.2700	0.0159	24.95	1.67
300	I	Second	0.3208	1.9949	0.0197	17.11	1.00
1 500			0.2695	2.0571	0.0184	16.71	1.19
3 000			0.2794	2.0439	0.0187	15.57	1.14
10 000			0.2811	2.0417	0.0187	20.31	1.14
50 000			0.2326	2.1126	0.0208	24.14	1.37
300	II	Third	0.2102	2.1525	0.0169	23.54	1.00
1 500			0.1619	2.2630	0.0159	25.36	1.29
3 000			0.1573	2.2761	0.0167	19.83	1.33
10 000			0.1485	2.3020	0.0160	23.87	1.41
50 000			0.1152	2.4253	0.0153	27.98	1.82
300	II	Fourth	0.2087	2.1555	0.0171	20.15	1.00
1 500			0.1384	2.3350	0.0154	27.82	1.50
3 000			0.1451	2.3290	0.0167	19.71	1.43
10 000			0.1261	2.3800	0.0157	25.35	1.65
50 000			0.0922	2.5440	0.0162	26.07	2.26

* degrees of freedom – 4; values significant at $P < 0.05$ level.

Table 3: Comparison of median lethal concentration (LC_{50}) values, slopes, chi square, and relative potency (RP) of neem formulations of varying azadirachtin content against *E. flavescens* under field conditions

Azadirachtin content	Round of spray	Observation (week)	LC_{50}	Slope	SE	Chi square*	RP
300	I	First	0.2972	2.0217	0.0192	19.30	1.00
1 500			0.2455	2.0918	0.0179	24.47	1.21
3 000			0.2561	2.0760	0.0181	20.56	1.16
10 000			0.2185	2.1372	0.0175	17.58	1.36
50 000			0.1948	2.1837	0.0171	18.39	1.52
300	I	Second	0.3999	1.9216	0.0218	14.75	1.00
1 500			0.2900	2.0304	0.0224	18.51	1.37
3 000			0.3207	1.9950	0.0199	18.29	0.08
10 000			0.2501	2.0849	0.0182	13.93	1.59
50 000			0.2405	2.0998	0.0178	18.35	1.66
300	II	Third	0.1970	2.1791	0.0167	22.86	1.00
1 500			0.1590	2.2710	0.0155	29.38	1.23
3 000			0.1376	2.3378	0.0156	25.87	1.43
10 000			0.1427	2.3206	0.0162	22.56	1.38
50 000			0.1244	2.3866	0.0164	22.08	1.58
300	II	Fourth	0.2097	2.1535	0.0169	23.04	1.00
1 500			0.1681	2.2464	0.0157	28.15	1.24
3 000			0.1630	2.2600	0.0155	29.35	1.28
10 000			0.1604	2.2671	0.0168	19.06	1.30
50 000			0.1315	2.3594	0.0152	28.85	1.59

* degrees of freedom – 4; values significant at $P < 0.05$ level.

ppm and 10 000 ppm, respectively. In contrast, highest LC_{50} values were observed in lower azadirachtin content neem formulations for all the target pests (0.13-2.27 ppm) (Tables 1-3). LC_{50} values of the neem formulations for the sucking pests were gradually decreased from week 1 to week 4 after application. The LC_{50} decreased as the azadirachtin content of neem formulation was increased.

The slopes of the dose-mortality curves for neem formulation of varying azadirachtin content were 1.45-2.25, 1.92-2.38 and 1.99-2.54 for tea mosquito bug, jassids and thrips, respectively. Higher slope value was observed in thrips, followed by jassids and tea mosquito bug. Azadirachtin content of neem formulation and their bioactivity were considered significantly different because their 95% fiducial limit of the LC_{50} did not overlap. Chi-square values ranged from 10.18–30.45 and were significant at $P < 0.05$ level, showing a heterogenous response of three sucking pests towards neem formulations. Relative potency of the neem formulations of varying azadirachtin content was compared with 300 ppm azadirachtin content and found that neem formulations of 50 000 and 10 000 ppm azadirachtin content had the highest relative potencies.These were the most efficacious materials tested against the three sucking pests (Tables 1-3).

Relationship between bioactivity of azadirachtin content and pest damage

The damage of tea mosquito bug, thrips and jassids (evaluated in terms of mean % reduction of pest population) in relation to azadirachtin content are summarized in Tables 4, 6 and 8. In neem formulation of higher azadirachtin content, percent reduction of *H. theivora* nymphs and adults was 24.2-32.42%, 21.82-28.00%, 44.48-50.78% and 49.72-54.1 % on weeks 1-4 post application, respectively, while < 22% and < 46% reduction were observed up to weeks 2 and 4 in lower azadirachtin content neem formulations. In case of thrips, 35.9-55.2% and 58.0-758 % reduction of nymphs and adults were noted up to weeks 2 and 4 post application in higher azadirachtin content neem formulation, while in lower azadirachtin content neem formulations there was 32.7-60.8% pest density reduction up to week 4. An effective control of nymphs and adults of jassids in neem formulations of higher azadirachtin content was observed with 39.9-64.6% reduction till week 4, whereas 27.9-61.1% reduction was recorded in lower azadirachtin content neem formulations up to week 4 (Tables 4, 6, 8).

The highest mean percent reduction was observed in thrips (46.8-75.8 %) followed by jassids (46.3-64.5

Table 4: Relationship between bioactivity of varying azadirachtin content and mean percent reduction of *H. theivora* population under field conditions

Tested dose (%)	Round of spray	Observation (week)	Mean percent reduction of *H. theivora* population Neem formulation – azadirachtin (ppm) 300	1 500	3 000	10 000	50 000
0.066	I	First	3.4	10.8	16.9	18.9	26.2
0.10			4.6	12.6	18.9	19.8	29.8
0.20			7.8	14.4	21.4	24.6	32.4
0.33			8.9	17.4	24.6	27.9	36.8
0.50			8.6	17.8	27.8	29.8	36.9
			6.66	14.6	21.92	24.2	32.42
0.066	I	Second	3.2	8.6	12.8	16.8	21.3
0.10			4.1	10.9	14.1	19.2	24.6
0.20			6.8	12.2	18.6	21.4	29.3
0.33			7.4	13.6	19.4	23.4	31.6
0.50			6.8	12.6	21.4	28.3	33.2
			5.66	11.58	17.26	21.82	28.0
0.066	II	Third	22.5	26.5	28.5	35.0	40.5
0.10			26.6	30.2	38.4	38.8	46.4
0.20			29.4	34.6	42.6	44.4	51.4
0.33			38.2	38.9	47.0	49.6	56.2
0.50			41.4	44.2	50.2	54.6	59.4
			31.62	34.88	41.34	44.48	50.78
0.066	II	Fourth	26.4	29.8	32.4	38.9	42.4
0.10			29.5	34.6	42.6	46.4	45.3
0.20			33.6	39.0	45.8	48.6	56.6
0.33			39.5	41.4	50.6	57.2	60.8
0.50			45.4	47.7	59.6	57.5	65.4
			34.8	38.5	46.2	49.72	54.1

Table 5: Two way ANOVA for insecticidal activity of different azadirachtin contents, concentrations and their interactions against *Helopeltis theivora*

Factor	I Week			II Week			III Week			IV Week		
	SEM (±)	CD (0.05)	CV (%)	SEM (±)	CD (0.05)	CV (%)	SEM (±)	CD (0.05)	CV (%)	SEM (±)	CD (0.05)	CV (%)
A	1.64	1.47	8.77	1.56	1.00	7.08	1.99	0.56	5.45	1.35	1.36	3.66
B	1.43	0.79	5.39	1.67	1.00	8.08	1.42	0.62	6.89	1.22	1.10	3.39
A × B	1.12	1.24	6.54	1.28	1.48	4.56	1.87	0.25	7.74	1.66	1.69	4.68

Factor A – azadirachtin content; Factor B – Concentration; Interaction A × B – azadirachtin content and concentration; SEM – Standard Error of the Mean; CD – Critical Difference ($P < 0.05$); CV – Coefficient of Variation (%)

%) and finally by tea mosquito bug (34.8-54.1 %) after week 4 of application. Mean percent reduction of the pest population was increased as the concentration of azadirachtin [tested dose] increased indicating increased bioactivity of azadirachtin content (Tables 4, 6, 8).

All the tested neem formulations affected the three sucking pests and were significantly ($P < 0.01$) different from each other. Significant differences in mean percent reduction of pest populations between azadirachtin content (CD-0.56-14.05 ; CV- 3.66-42.08%), tested dose(CD-0.62-14.17 ; CV-3.39-42.39%) and their interactions (CD-1.24-16.32 ; CV-4.56-24.64) were found from week 1 to 4 after application, respectively (Tables 5, 7, 9). Further, lower CV values were observed during week 4 compared with week 1 in all the three field trials.

DISCUSSION

The dose-mortality relationship of an insect to a toxin is typically expressed as an LC_{50} value, which is the toxin concentration required to kill 50% of the popula-

Table 6: Relationship between bioactivity of varying azadirachtin content and mean percent reduction of *S. dorsalis* population under field

Tested dose (%)	Round of spray	Observation (week)	Mean percent reduction of *S. dorsalis* population – Neem formulation – Azadirachtin (ppm)				
			300	1500	3000	10000	50000
0.066	I	First	18.5	32.6	29.7	38.8	51.0
0.10			35.0	43.7	35.2	44.0	42.5
0.20			45.1	47.1	47.0	43.2	55.7
0.33			44.7	57.6	56.4	43.4	63.1
0.50			49.8	61.2	59.4	50.6	63.8
			38.6	48.4	45.5	44.0	55.2
0.066	I	Second	19.7	24.3	22.5	31.2	41.2
0.10			28.1	31.6	25.5	37.8	32.1
0.20			34.3	39.0	38.0	34.0	47.6
0.33			38.7	43.7	48.5	33.1	39.4
0.50			42.9	51.1	51.3	43.8	39.5
			32.7	37.9	37.1	35.9	39.9
0.066	II	Third	32.0	38.9	40.8	46.9	61.2
0.10			41.1	54.8	53.0	55.1	64.5
0.20			49.6	58.2	54.5	57.8	68.9
0.33			52.7	62.0	60.0	62.1	70.8
0.50			52.8	63.3	69.4	68.4	73.8
			45.6	55.4	55.5	58.0	67.8
0.066	II	Fourth	39.7	44.4	42.3	56.4	66.4
0.10			26.2	60.4	58.0	63.3	72.4
0.20			55.3	64.7	58.2	59.7	77.0
0.33			55.3	67.8	62.1	69.5	81.5
0.50			57.6	67.1	72.9	73.3	81.9
			46.8	60.8	58.7	64.4	75.8

Table 7: Two way ANOVA for insecticidal activity of different azadirachtin contents, concentrations and their interactions against *Scirtothrips dorsalis*

Factor	I Week			II Week			III Week			IV Week		
	SEM (±)	CD (0.05)	CV (%)	SEM (±)	CD (0.05)	CV (%)	SEM (±)	CD (0.05)	CV (%)	SEM (±)	CD (0.05)	CV (%)
A	2.45	13.49	34.52	1.44	8.10	27.03	1.56	8.61	18.10	2.14	14.05	17.74
B	1.97	7.26	21.20	1.28	11.13	42.39	1.88	6.40	15.35	2.02	14.17	11.95
A × B	1.49	9.67	18.97	1.67	15.47	22.14	1.24	5.64	14.22	2.56	16.32	12.87

For legend, see Table 5.

tion in a specified period. The lower the LC_{50} value, the greater is the toxicity. Neem formulation of 10 000 ppm and 50 000 ppm had the lowest LC_{50} values, suggesting that they were more toxic to three sucking pests than 300 – 3 000 ppm with the highest LC_{50} values (Tables 1-3). However, the LC_{50} alone does not reveal an accurate picture of the total insecticidal effect.

Our results clearly indicate that the LC_{50} values decrease with increasing azadirachtin concentration in neem formulation. This is in agreement with the findings of Kumar et al. (2003). Neem formulations provide three great advantages over neem seed kernel extract as 1) they reduce the rate of loss of azadirachtin manifolds, 2) can be bought off-the-shelf and used with greater ease than the seeds, and 3) their likely rapid action on the insects that might consequently reduce the rate of crop loss (Isman, 2006).

The present study suggests a possible relationship between bioactivity of varying azadirachtin content and pest damage. Among five varying formulations of azadirachtin only 50 000 ppm resulted in the maximum (54.1- 75.8%) control of three sucking pests at

Table 8: Relationship between bioactivity of varying azadirachtin content and mean percent reduction of *E. flavescens* population under field conditions

Tested dose (%)	Round of spray	Observation (week)	Mean percent reduction of *E. flavescens* population				
			Neem formulation – Azadirachtin (ppm)				
			300	1 500	3 000	10 000	50 000
0.066	I	First	19.0	36.3	28.3	38.6	33.3
0.10			38.0	32.0	32.3	30.0	42.0
0.20			35.0	40.0	45.3	45.6	47.0
0.33			38.6	50.3	44.6	50.3	56.3
0.50			42.3	44.3	48.3	59.0	63.3
			34.5	40.5	39.7	44.7	42.38
0.066	I	Second	9.0	29.3	20.3	32.6	24.6
0.10			30.0	25.6	25.3	30.0	36.0
0.20			30.6	34.6	38.0	34.3	42.3
0.33			33.6	43.6	39.3	45.0	50.0
0.50			36.6	38.6	41.0	58.0	53.6
			27.96	34.34	32.78	39.98	41.3
0.066	II	Third	41.0	52.6	51.0	55.6	56.0
0.10			47.3	49.6	59.0	53.3	57.3
0.20			37.0	54.0	65.3	58.0	65.0
0.33			54.6	62.0	61.0	60.0	68.3
0.50			56.6	58.6	69.3	71.0	75.6
			47.3	55.36	61.1	59.5	64.6
0.066	II	Fourth	39.3	53.0	48.3	46.6	74.3
0.10			44.3	53.0	55.3	39.0	57.3
0.20			44.3	57.0	55.6	56.3	60.3
0.33			49.6	46.3	56.6	62.6	62.6
0.50			54.3	57.6	58.3	70.3	68.3
			46.36	53.3	54.82	54.96	64.56

higher concentration (0.5%). As the pests have sucking type of mouth parts, a chance of ingesting toxicologically active neem (azadirachtin) from leaf surface is much less. This may be the principal reason for a limited control of *H. theivora* with neem formulations alone. Such observations are validated by the findings of Lowery and Isman (1995) and Martinez and van Embden (2001).

The slope of the dose-mortality curve is a measure of variability in response to treatment within the insect population tested. As the value of the slope increases, mortality associated with changes in concentration increases. Conversely, as the value of slope decreases, less change in mortality is seen per unit change in concentration of the mortality agent. Neem formulation of varying azadirachtin content have slopes of 2.54 or less, whereas insecticides, such as DDT, have slopes of 5.5 or greater (Metcalf and Luckman, 1994). Insect pathogens that produce toxins, such as *B. thuringiensis,* are usually characterized by intermediate slopes of 2.58 (Burges and Thomson, 1971). The slopes of the dose-mortality curves for neem formulation of 50 000, 10 000, 3 000, 1 500 and 300 ppm ranged from 1.48 to 2.54 suggesting that these formulations have some toxicity to the sucking tea pests (Tables 1-3).

In the present study, application of neem formulation of various azadirachtin content at varying doses to tea plants provided > 50-75.8% reduction of three tea pests after week 4 with two rounds of foliar application (Tables 4, 6, 8). Sarmah et al. (2006) and Roy et al. (2010) demonstrated the insecticidal activity of neem formulations of varying azadirachtin content against tea mosquito bug, thrips and jassids under laboratory conditions with LC_{50} value of 0.09-0.24 ppm, 0.07-0.16 ppm and 0.07-0.18 ppm, respectively. But under field conditions after week 4, LC_{50} value of the neem formulations of varying azadirachtin content varied to 0.16-0.29 ppm for tea mosquito bug, 0.09-0.20 ppm for thrips and 0.13-0.20 ppm for jassids (Tables 1-3). In the present study, neem oil formulation was found effective to control tea sucking pests under natural field conditions and more than >50 – 75.8% reduction of thrips, jassids and tea mosquito bug was observed up to four weeks post application.

Neem derivatives have been regarded as the most promising and effective as feeding poisons for nymphs

Table 9: Two way ANOVA for insecticidal activity of different azadirachtin contents, concentrations and their interactions against *Empoasca flavescens*

Factor	I Week			II Week			III Week			IV Week		
	SEM (±)	CD (0.05)	CV (%)	SEM (±)	CD (0.05)	CV (%)	SEM (±)	CD (0.05)	CV (%)	SEM (±)	CD (0.05)	CV (%)
A	2.01	8.60	24.55	2.45	12.51	42.08	2.09	6.39	13.21	1.47	7.96	14.26
B	1.89	8.30	27.04	2.67	8.08	31.04	1.54	6.12	14.44	1.62	8.23	13.36
A × B	1.97	10.22	19.64	1.98	9.47	24.64	1.68	8.64	17.41	1.55	9.47	17.01

For legend, see Table 5.

or larvae of several phytophagous insects; lepidopterous larvae, especially, are very susceptible (Ghatak et al., 2008, Pavela et al., 2009). This property makes neem suitable for use in pest management programs because non-target pests are spared (Belmain et al., 2000, Perera et al., 2000, Raguraman and Singh, 2000, Simmonds et al., 2000). The neem insecticides have no immediate knockdown effect on pests, but reduce feeding and death occurs within several days (Rizwan et al., 2009). Schmutterer (1990) concluded that a foliar spray application of most commercial neem formulations persist 5–7 days under field conditions. The present findings corroborate the foregoing observation, indicated by the mean percent reduction of pest population density that had a decreasing trend during the 2nd week of observation. However, later the reduction in pest density had been increased during weeks 3 and 4 post application (Tables 4, 6, 8). Half-life of the active compound is considered important as it facilitates persistence of the residue and thus the pesticide's efficacy (Akhtar et al., 2008). Kinetic studies of field degradation of azadirachtin showed that the mechanism of disappearance was related to photodegradation as azadirachtin and related compounds are very sensitive to sunlight (Nisar et al., 2009). The compounds persist for seven to twelve days (Rahman et al., 2007). Even though breakdown of azadirachtin occurs in UV light, its metabolites (dihydroazadirachtin) may still remain bioactive for some time (Mordue and Blackwell, 1993). Our field trials were conducted during summer season (April-May) and to facilitate the persistence of azadirachtin two rounds of foliar spray were applied. Further, the three sucking pests lay their eggs inside the leaf and stem portions. It was noticed that foliar spraying of neem formulation did not kill the eggs during the first spray. But the second foliar spray after a 15-day interval had checked the nymphal population, which hatched from the eggs of target pests. This was evidenced in the 3rd week observation of percent reduction of pest density. Further, the evidence of lower CV values during the fourth week after application proved the effectiveness of the neem formulations of varying azadirachtin content (Tables 5, 7, 9).

Finally, the variation observed in the insecticidal property of neem formulation between azadirachtin content based on percent reduction of pest density and LC_{50} has a more important and immediate implication for pest management. According to the present recommendation on the use of neem for pest management, farmers are encouraged to use any neem formulation of varying azadirachtin content. Although as an alternative to chemical insecticide, any of the neem formulations could be used those with lowest LC_{50} values, and highest slopes and relative potencies would probably be most effective. The additional benefit from using neem formulation is the absence of any adverse impact on natural enemies and insect pollinators (Goektepe et al., 2004). The Central Insecticide Board (CIB) and tea board have also approved only 5% azadirachtin formulations (50 000 ppm) as pesticide. Based on this study, a clear recommendation can be made to the farmers to use higher azadirachtin content neem formulations, i.e., either 50 000 ppm or 10 000 ppm than low azadirachtin content neem formulation. The possibility of managing the sucking pests in tea is maximized, as the farmers become aware of its benefits as an eco-friendly botanical pesticide with no residue problems in tea.

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