

STUDIES ON THE INSECTICIDAL AND REPELLENT PROPERTIES OF THE SEED EXTRACT OF *TEPHROSIA PURPUREA* (LINN.) PERS

B. N. SAXENA, D. N. DUBEY & (MRS) A. L. NAIR

Field Laboratory, Tezpur

(Received 19 July 1972; revised 16 March 1973)

Laboratory and field trials were conducted to find out the insecticidal and repellent properties of petroleum ether extract of the seeds of *Tephrosia purpurea*. In laboratory trials contact toxicity of the extract was assessed against land leeches, houseflies, mosquitoes, rice weevil and flour beetle. In field trials, the repellency of the extract was assessed against land leeches, mosquitoes and *Simulium* flies.

In laboratory trials, the dosage required for 100 per cent mortality was 0.0005 gm/cm² for land leeches, 0.0157 gm/cm² for houseflies; 0.00785 gm/cm² for mosquitoes, 0.0157 gm/cm² for rice weevil and 0.011775 gm/cm² for flour beetle. In field trials, the extract was found to be repellent against land leeches for 5 hours, mosquitoes for 4 hours and *Simulium* flies for 5 hours.

Plants of wild indigo (Sarphonka), *Tephrosia purpurea* (Linn.) Pers. grow abundantly in Assam and Arunachal Pradesh. The roots of these plants were known to be poisonous to fishes in French Guiana, though no such reports are available from India^{1, 2, 3, 4}. The extract of plant body (except root) has also been found to possess antiprotozoal property against *Entamoeba histolytica* and has a gross effect on the nervous system⁵. The chemical constituents of this plant include tephrosin, deguelin, isotephrosin, rotenone and rutin^{6, 7}. The seeds of *T. purpurea* contain sufficient quantity of oil, which has been found to contain, three crystalline compounds⁸. It was, therefore, considered worthwhile to study the insecticidal and repellent properties of the extract of these seeds.

MATERIALS AND METHODS

Selection of the Solvent

Crushed seeds of *T. purpurea* were extracted in the Soxhlet extractor with acetone, benzene, chloroform, ethyl alcohol, methyl alcohol, petroleum ether (40°—60° b.p.) and 1 : 1 mixture of ether and ethyl alcohol. The extracts, thus obtained, were tested for their contact toxicity against 4 to 5 days old laboratory bred female houseflies, *Musca domestica* Linn. Filter papers (11 cm. dia.) were treated with 1 ml of the extract, and the flies were exposed over them for 4 hours. There were 2 replicates for each extract and each replicate had 20 flies. The number of flies knocked down after the exposure period was counted and the mortality was recorded after 24 hours. The yields of the extract with different solvents and their toxicity to houseflies are given in Table 1. Since, the extract obtained with petroleum ether (40°—60° b.p.) was found most effective, it was used for further studies. The extract was in the form of thick oil, yellowish brown in colour.

TABLE I

COMPARATIVE ASSESSMENT OF INSECTICIDAL ACTIVITY OF THE EXTRACTS OBTAINED WITH DIFFERENT SOLVENTS AGAINST HOUSEFLIES

Solvent	Yield of extract (%)	Knock down (%)	Mortality (%)	Order of effectiveness
Petroleum ether (40°—60° b.p.)	11.3	100.0	100.0	I
Benzene	10.4	82.0	95.0	II
1 : 1 mixture of ether and ethyl alcohol	21.4	90.0	92.0	III
Acetone	11.0	93.0	88.5	IV
Ethyl alcohol	24.5	88.0	84.5	V
Chloroform	12.6	66.0	80.0	VI
Methyl alcohol	14.5	Nil	Nil	VII

Contact Toxicity Against Land Leeches

The contact toxicity of petroleum ether extract against land leeches was assessed by dry film technique. Filter papers (11 cm. dia.) were treated with different dosages of the petroleum ether extract. Two to three cm. long specimens of the land leech, *Haemadipsa sylvestris* Blanchard obtained from the laboratory culture, were exposed to treated filter papers. There were 2 replicates for each dosage and each replicate had 10 leeches. Observations were taken on per cent mortality and the duration after which the leeches died. The results are given in Table 2.

Contact Toxicity Against Insects

4 to 5 day old female houseflies (*Musca domestica* Linn.), 40 to 60 hour old female mosquitoes (*Aedes aegypti* Linn.), one to ten week old rice weevil (*Sitophilus oryzae* Linn.) and flour beetle (*Tribolium castaneum* Herbst) obtained from the laboratory bred cultures, were exposed to filter papers treated with different dosages of the extract by dry film technique⁹. There were 2 replicates for each dosage. The number of insects exposed in each replicate was 20 for houseflies and mosquitoes and 100 for rice weevil and flour beetle. The exposure period for flies and mosquitoes was 4 hours and for rice weevil and flour beetle 24 hours. The mortality was recorded after 24 hours. The results are given in Table 3.

TABLE 2
CONTACT TOXICITY AGAINST LAND LEECHES

Dosage (gm/cm ²)	Mortality (%)	Duration after which leeches died		
		Min. (hr)	Max. (hr)	Av. (hr)
0.0005	100.0	2½	5¼	4
0.0001	90.0	3½	5½	4½
0.00005	80.0	3½	5¼	4¼
0.00001	70.0	4¼	5¼	4¾
0.000005	50.0	4¼	5¼	4¾

TABLE 3
CONTACT TOXICITY AGAINST HOUSEFLIES, MOSQUITOES, RICE WEEVIL AND FLOUR BEETLE

Dosage (gm/cm ²)	Houseflies		Mosquitoes		Rice Weevil	Flour Beetle
	Knock down (%)	Mortality (%)	Knock down (%)	Mortality (%)	Mortality (%)	Mortality (%)
0.0157000	100.0	100.0	Not done	Not done	Not done	Not done
0.0117750	Not done	Not done	Not done	Not done	Not done	100.0
0.0078500	76.9	96.1	100.0	100.0	100.0	57.5
0.0039250	61.0	61.0	80.0	80.0	75.5	5.0
0.0031400	42.5	42.5	75.0	77.5	Not done	Not done
0.0019625	Not done	Not done	Not done	Not done	39.6	Nil
0.0015700	„	„	52.5	60.0	Not done	Not done
0.0007850	„	„	37.5	47.5	28.6	Nil
0.0004710	„	„	Not done	Not done	21.0	Nil
0.0001570	„	„	20.0	27.5	Not done	Not done

FIELD REPELLENCY TRIALS

Field trials were conducted to find out the repellent property of the extract against the land leeches, mosquitoes, and *Simulium* flies. DEET (*N, N*-diethyl-*m*-toluamide) and DMP (Di-methyl phthalate) were taken as reference repellents.

Sites of the Trials

Trials against the land leeches were conducted in Sonai—Rupai Reserve forests in between Rekhabari and Foothills in Darrang District of Assam. Trials against mosquitoes were conducted in Tezpur town in the premises of the Laboratory and the trials against *Simulium* flies were conducted in Doimara and Khelong in Kameng Frontier Division of Arunachal Pradesh.

Test Subjects

All the three field trials were conducted with Army jawans in their summer-uniform consisting of black boots, OG woollen socks, OG drill trousers, OG shirts of cellular cloth and barrets/turbans.

Standardisation of Dosage

Before conducting the field trials, the dosage of the repellents to be applied was standardised in the Laboratory. Repellents were applied in different quantities on both the legs below knee joint, forearms upto elbow and neck, so as to get a uniform smear. A quantity of 4 ml was found to be the optimum quantity to give a uniform smear on the legs upto knee, forearms upto elbow and neck, about one ml being sufficient for one leg and a little less than one ml for each forearm.

Susceptibility Test

Work carried out earlier in this Laboratory had shown that certain individuals are not attacked by leeches, mosquitoes or *Simulium* flies. To eliminate such individuals, susceptibility tests were conducted. These susceptibility tests were carried out at the places where actual repellency trials were conducted. The trial subjects, unprotected, were exposed to the attack of leeches, mosquitoes or *Simulium* flies. 20 out of 73 were not attacked by leeches, 6 out of 30 were not attacked by mosquitoes and 4 out of 27 were not attacked by *Simulium* flies. All those who were not attacked were dropped from the repellency trials.

Repellency Trials

Trials were conducted on three days and 3 to 6 replicates were taken on each day, depending upon the number of subjects. For trials against the leeches, the subjects, after application of repellents, put on their trousers, socks and boots. They then walked through the forests along with the control ones. The subjects were divided in 4 batches according to a randomised plan. The batches moved out together through the forests. The number of leeches climbing or biting were recorded when perceived or at hourly intervals. The duration after which first bite occurred in a replicate of repellent was taken as the minimum period of effectiveness, the duration after which first bite occurred in the last replicate was taken as the maximum period of effectiveness. The average duration of effectiveness was calculated on the basis of all the replicates of a repellent. During the period of the trials, the temperature ranged between 27.2°C to 33.3°C on first day, 24.4°C to 33.3°C on second day and 26.1°C to 32.8°C on third day. The relative humidity varied

from 71 to 91 per cent on first day, 78 to 100 per cent on second day and 74 to 95 per cent on third day. The results are given in Table 4.

The subjects for mosquito trial, after application of repellents, were divided in 4 batches according to a randomised plan. They then sat at a distance of about 30 cm. from one another. The observations were taken as in the case of land leeches. The duration of trials on first and third day was from 2030 hours to 0400 hours and on second day from 2000 hours to 0400 hours. During this period, the temperature ranged between 21.5°C and 24.0°C on first day, 21.5°C and 26.0°C on second day and 22.0°C and 27.0°C on third day. The results are given in Table 4.

In the trials against *Simulium* flies, the trial subjects after application of repellents sat still according to a randomised plan, at a place having maximum activity of *Simulium* flies. The subjects were divided into 4 batches. The observations were taken as in the case of land leeches. The trials were conducted on two days from 0800 hours to 1700 hours. The fly activity was maximum during the period of trials. The temperature on the days of trials ranged between 20.0°C to 23.0°C on first day and between 21.5°C to 24.0°C on second day. The results are given in Table 4.

RESULTS AND DISCUSSION

Yield of Extract

The maximum yield was obtained with ethyl alcohol (24.5%), followed by 1 : 1 mixture of ether and ethyl alcohol (21.4%), methyl alcohol (14.5%), chloroform (12.6%), petroleum ether (11.3%), acetone (11.0%) and benzene (10.4%). But, the insecticidal activity of these extracts had no relationship with their yield.

The comparative assessment of activity of the extracts is given in Table 1. The extract obtained with

TABLE 4

FIELD TRIALS ON REPELLENCY AGAINST LAND LEECHES, MOSQUITOES AND *Simulium* FLIES

Repellent	Day	Land Leeches				Mosquitoes				<i>Simulium</i> Flies			
		Trial period (hr)	Effectiveness			Trial period (hr)	Effectiveness			Trial period (hr)	Effectiveness		
			Min. (hr)	Max. (hr)	Av. (hr)		Min. (hr)	Max. (hr)	Av. (hr)		Min. (hr)	Max. (hr)	Av. (hr)
<i>Tephrosia</i> Extract	First	5	5	5	5	7½	3	4¾	3¾	9	3	5½	5
	Second	6	4½	4¾	4¾	8	3½	5	4½	9	4	6	5
	Third	6	4½	5	5	7½	3¼	4		Not done	Not done	Not done	Not done
DEET*	First	5	5	5	5	7½	4½	6	5½	9	5	6¾	6
	Second	6	4½	6	5	8	6½	8	7	9	2¼	8	6
	Third	6	3½	5¾	5	7½	5	7½	6¾	Not done	Not done	Not done	Not done
DMP†	First	5	5	5	5	7½	1¾	6	4½	9	4¾	7¼	6½
	Second	6	2½	3¼	3	8	4½	5	4¾	9	6½	7¾	6¾
	Third	6	3½	4¾	4	7½	4½	6	5½	Not done	Not done	Not done	Not done

*N,N-diethyl-m-toluamide

†Di-methyl phthalate

petroleum ether produced 100% mortality of houseflies, followed by benzene (95.0%), 1 : 1 mixture of ether and alcohol (92.0%), acetone (88.5%), ethyl alcohol (84.5%) and chloroform (80.0%). Extract obtained with methyl alcohol did not show insecticidal activity. Therefore, extract obtained with petroleum ether (40°—60° b.p.) was used for further studies.

Contact Toxicity against Land Leeches

The results in Table 2 show that a dosage of 0.0005 gm/cm² produces 100 per cent mortality of land leeches in about 4 hours. There is not much difference in the duration with lower dosages, but the percentage of mortality shows a gradation with the descending dosages.

Contact Toxicity against Insects

The results on contact toxicity given in Table 3 show that the dosage of 0.0157 gm/cm² produces 100 per cent mortality of houseflies. The mortality percentage at lower dosages shows a gradation with the decreasing dosage. In case of mosquitoes, 100 per cent mortality was obtained at the dosage of 0.00785 gm/cm² and gradual reduction in mortality percentage was obtained with the reduction in the dosages. The rice weevil was found to be more susceptible than the flour beetle. In case of rice weevil, 100 per cent mortality was obtained at the dosage of 0.00785 gm/cm² as compared to 57.5 per cent of flour beetle at the same dosage. The lower dosages showed a gradation of mortality in case of rice weevil, but no mortality was obtained in case of flour beetle at those dosages.

Comparative Repellency of Tephrosia against Land Leeches, Mosquitoes and Simulium Flies

The results of field trials against land leeches (Table 4) show that the extract was effective for about 5 hours which may be compared with *DEET*. *DMP* was effective for 4 hours. The control subjects had leech bites throughout the period of the trials, indicating the activity of leeches. The activity of land leeches depends very much on the weather condition. On the first day, the weather became sunny and hot after 1100 hours and leeches were not active at that time even in the thickest part of the forest. On the second day, there was heavy rain after the commencement of the trial and leech bites were very few. However, the number of leech bites increased after the rains stopped. On the third day, there were light showers for about an hour after the commencement of the trials and leech bites were less. But, after the rains, bites were much more than on the earlier two days. The leeches were found to be active when the soil is moist or during light drizzle and are not active during heavy rains or in hot weather.

The extract was found to be effective against mosquitoes for about 4 hours, which was almost similar to *DMP* (See Table 4). *DEET* was effective for about 6 hours. The bites received by control subjects showed the activity of mosquitoes during the period of the trials.

Results of field trials against *Simulium* flies, show that the extract was effective for 5 hours, while *DEET* and *DMP* were effective for 6 hours (See Table 4). The control subjects had *Simulium* bites throughout the period of the trials.

Simulium flies are very sensitive to variation in temperature and sunlight. In the morning they were more active on higher ground where the sun was visible. The flies again became active on the higher ground in the afternoon. But, at noon, when the day was hot, the flies were active only near the stream. The flies completely disappeared after sun-set.

REFERENCES

1. CLARK, *Science*, **71** (1930), 396.
2. CLARK, *Science*, **73** (1931), 17.
3. CLARK & CLABORN, *J. Amer. Chem. Soc.*, **54** (1932), 4454.
4. CLARK & BANERJEE, *Proc. Chem. Soc.*, **25** (1910), 16.
5. BHAKUNI, D. S., DHAR, M. K., DHAR, M. M., DHAWAN, B. N. & MEHROTRA, B. N., *Indian. J. Exptl. Bio.* **7** (1969), 250-262.

6. CHOPRA, R. N., CHOPRA, I. C., HANDA, K. L. & KAPUR, L. D., "Indigenous Drugs of India—I", (U. N. Dhar & Sons Pvt. Ltd., Calcutta), 1958, p. 589.
7. CHOPRA, I. C. & HANDA, K. L., "Review of Research on Indian Medicinal and Allied Plants—I", (Indian Council of Agricultural Research, New Delhi) 1965, p. 55-56.
8. SUBBA RAO, N. V., *Curr. Sci.*, **25** (1956), 396.
9. WAL, Y. C., PERTI, S. L. & DAMODAR, P., *Indian J. Mal.* **16** (1962), 129.