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SHORT COMMUNICATION

Strong larvicidal activity of three species of *Spilanthes* (Akarkara) against malaria (*Anopheles stephensi* Liston, *Anopheles culicifacies*, species C) and filaria vector (*Culex quinquefasciatus* Say)

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Abstract A system for biocontrol of malaria and filarial mosquito vectors has been developed using herbal extracts of three *Spilanthes* species, *S. acmella* L.var *oleraceae* Clarke, *S. calva* L. and *S. paniculata* Wall ex DC. Cent percent mortalities was achieved against the late third/early fourth instar larvae of *A. stephensi* Liston, *A. culicifacies* species C and *C. quinquefasciatus* Say using crude hexane extract obtained from flower heads of *Spilanthes* spp. Of the three plant species, *S. acmella* extract proved to be the most effective in inducing complete lethality at minimum doses, the respective LC50 and LC90 values being 4.57 and 7.83 (*A. stephensi*), 0.87 and 1.92 (*A. culicifacies*) and 3.11and 8.89 ppm (*C. quinquefasciatus*). This was followed by *S. calva* and *S. paniculata* extracts, respectively. This is the first report of achieving cent percent lethality against these mosquito larvae using minimal doses of plant extracts from this or any other plant species.

Introduction

Malaria and filariasis are the two most serious parasite-borne diseases of the tropical regions. These diseases are essentially transmitted by *Anopheles* and *Culex* mosquitoes. In India, malaria is transmitted by nine anopheline vector species of which six are of primary importance (Raghavendra and Subbarao 2002a). The primary vectors include *A. culicifacies*, *A. stephensi*, *A. fluatilis*, *A. minimus*, *A. gyrus* and *A. sundicus*. These species are responsible for transmission in specific ecotypes. Of the six primary vector species, *A. culicifacies* is squarely responsible for transmission of about 60–70% malaria in rural plains and peri-urban areas. The control of malaria in India is per se the control of this species. The control of this species is further complicated, as it exists as a complex of five species reported so far namely, A, B, C, D and E, which show different distribution and behaviour.

Filariasis is transmitted by *Culex quinquefasciatus* say (bancroftii filariasis) and *Mansonia* species (Brugien filariasis). For the control of these vectors, dichlorodiphenyl trichloroethane, organophosphates and pyrethroids are being used as adulticides/larvicides for the last several decades in the National Vector Borne Disease Control programme in India, depending on the requirement of the given disease control programme. Continuous use of these chemical insecticides have led to the control failures and disease resurgence owing mainly to the development of resistance in the vectors (Raghavendra and Subbarao 2002b). Hence, a renewed interest has been shown by various workers world over to the feasibility of use of environment friendly and generally least toxic herbal extracts from plants.

In the light of the above prospects, we have conducted investigations on three species of a traditional medicinal plant *Spilanthes* species, commonly known as Akarkara (Hindi), maratti mogga (Telugu) or piccaraza (Assamese; Anonymous 1989). A member of the family Asteraceae, the genus is

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widely distributed in the tropics and sub-tropics. In India, the plants have been found growing in the northern and southern hills and plateaus. There are around five species of *Spilanthes* reported so far (Anonymous 1989) growing in India: *S. acmella* Murr., *S. acmella* L. var *oleraceae* clarke, *S. calva* L., *S. paniculata* L. and *S. mauritiana* L. Of these, *S. calva* L., *S. mauritiana* L. and *S. paniculata* L. are found more commonly, whereas *S. acmella* Murr. and *S. acmella* L. var *oleraceae* Clarke are rare in occurrence. The genus is attributed with immense medicinal (Borges-Del-Castillo et al. 1984; Anonymous 1989), antimicrobial (Fabry et al. 1996, 1998; Rai et al. 2004), larvicidal (Pendse et al. 1945) and insecticidal properties (Jondiko 1986; Ramsewak et al. 1999) because of the presence of several bioactive compounds, which includes Spilanthol and a group of other isobutylamides. The flower heads and root part of the plant have been known to be especially rich in the active principle content (Nayak 2002). Although, some fragmentary studies have been carried out on the larvicidal/insecticidal activity using the flower head extract of *S. acmella* Murr. against *Anopheles* spp.(Pendse et al. 1945), *A. stephensi* Liston and *C. quinquefasciatus* (Saraf and Dixit 2002), *S. mauritiana* against *Aedes* (Jondiko 1986) and *S. oleraceae* against *Helicoverpa zea* (Ramsewak et al. 1999), but to date, none of the aforesaid work reports the extensive analysis of bioefficacy and comparative analysis of these species (for selection of elite species) against mosquito vectors to achieve 100% mortality with minimum doses. However, some recent reports have appeared on the larvicidal activity of plant extracts of other species, e.g. *Vitex* (Kannathasan et al. 2007), *Azadirachta indica* (Siddiqui et al. 2003) and *Feronia limonia* (Rahman et al. 2000), but their lethal doses (LC50 and LC90) were very high. In this paper, we report extensive investigations of the larvicidal efficacy of the crude extracts of three *Spilanthes* species tested against *A. stephensi* Liston, *A. culicifacies* species C and *C. quinquefasciatus* Say mosquito larvae, where 100% mortality was achieved with abysmally much lower doses.

Materials and methods

Plant material

Seeds of *S. acmella* L. var *oleraceae* Clarke, *S. calva* L. and *S. paniculata* Wall ex DC were collected from plateaus of Northern India and were subsequently sown in the field beds of the Botanical Garden, Department of Botany, University of Delhi. The flower heads from the respective species were collected for preparation of the extract, once they reached maturity. The flower heads were thoroughly washed under running tap water. The flowers were dried under shade, and the dry material was ground into a fine powder using pestle and mortar. Thereafter, the materials were filtered through muslin cloth and were subsequently extracted twice with hexane (20 ml/g of the dry weight of the material) overnight with continuous stirring over a magnetic stirrer. The extract concentrate was further allowed to evaporate to dryness in a vaccum. The yield of a solvent-free gummy extract relative to the dry starting material was recorded. Different dilutions of the extracts were made (50, 25, 12.5, 6.25, 3.125 and 1.5625 ppm) through serial dilution in autoclaved sterile water mixed with triton (10 μl/l of water).

Larvicidal assay

Bioassays were conducted essentially following the standard World Health Organization (1981) larval susceptibility test method at the National Institute of Malaria Research, Delhi, on laboratory-reared late third/early fourth instar larvae of *A. stephensi* Liston, *A. culicifacies* species C and *C. quinquefasciatus*. Tests were carried out in four replicates (25 larvae/replicate) with two controls run simultaneously. Results were scored after 24 h of continuous exposure to the test solution and were expressed as percent mortality. Pure water and hexane without the dissolved plant extract served as the positive control, whereas pure water alone served as the control. Where needed, the mortality in the test replicate was correlated with control mortality using Abott's formulae. LC50, LC90 and 95% confidence limit (upper–lower) were calculated by using probit analysis (Finney 1971).

Results and discussion

The hexane extracts obtained from the flower heads of the three species of "Akarkara" namely, *S. acmella* L. var *oleraceae* Clarke, *S. calva* L. and *S. paniculata* Wall ex DC tried in different dilutions (50, 25,12.5, 6.25, 3.125 and 1.5625 ppm) have shown variable mortality against all the vectors (*A. stephensi* Liston, *A. culicifacies* and *C. quinquefasciatus*) exposed to the test solution (Fig. 1a–c). Table 1 reveals that the lethal doses (LC50 and LC90) required for *A. stephensi* Liston larvae were 4.57 and 7.83 (*S. acmella* L.var *oleraceae* Clarke), 5.10 and 8.46 (*S. calva* L.) and 5.09 and 13.55 ppm (*S. paniculata*.), respectively. It was observed that at higher doses, the larvae turned immobile within an hour of exposure period.

It is interesting to note that for *A. culicifacies*, a significantly lower dose (of the plant extracts) was needed for inducing cent percent mortality. The respective doses (LC50 and LC90) in this case are 0.87 and 1.92 (*S. acmella* L. var *oleraceae*), 0.92 and 1.99 (*S. calva* L.) and 3.23 and 7.10 ppm (*S. paniculata*). This is the first report of

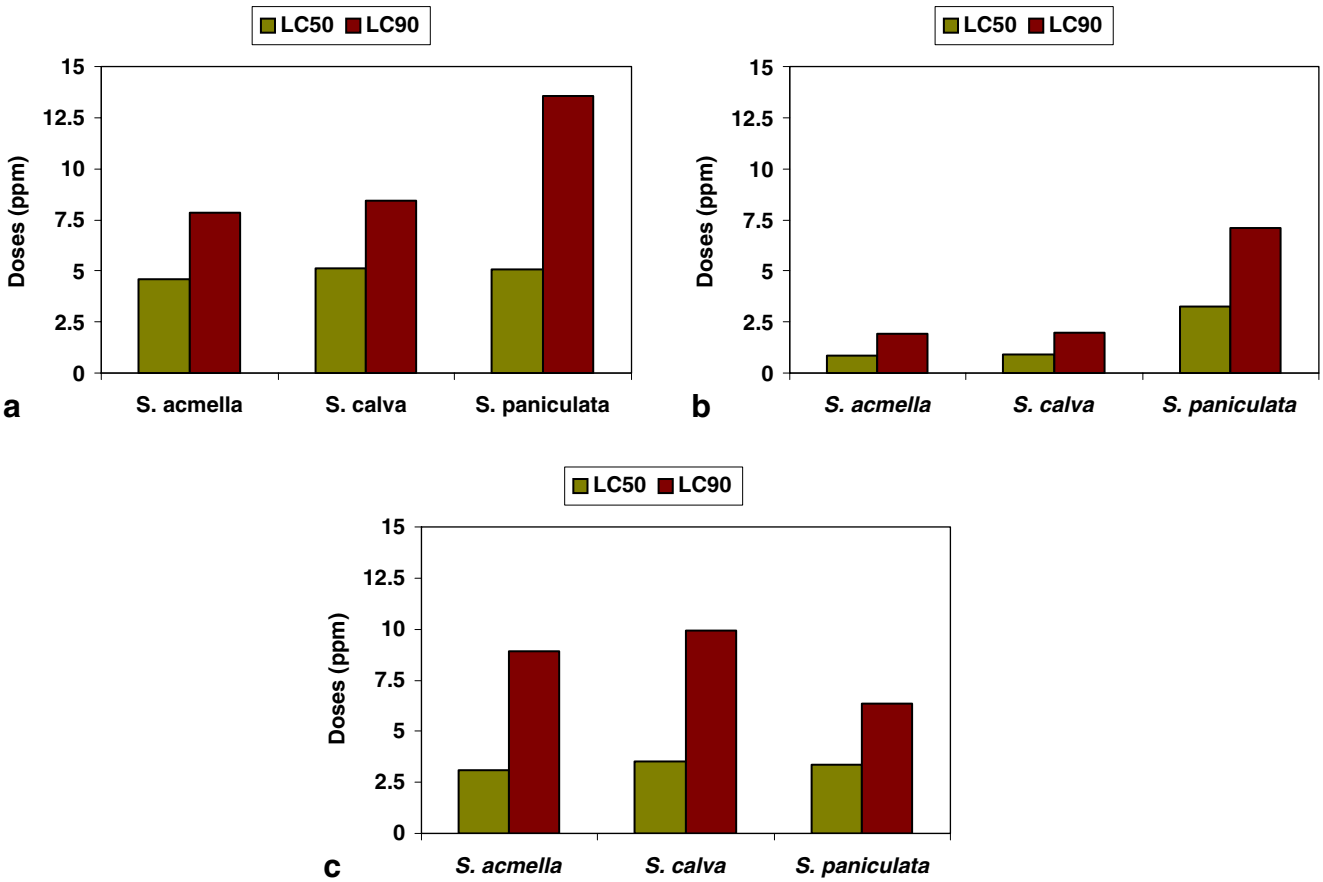

Fig. 1 **a**, **b**, **c** Graph showing relative dose dependent mortality of mosquito vectors **a** *Anopheles stephensi*, **b** *Anopheles culicifacies* and **c** *Culex quinquefasciatus*

larvicidal activity from *Spilanthes* extracts against *A. culicifacies* (species C) mosquito larvae.

Incidentally, in case of *C. quinquefasciatus*, the lethal doses (LC50 and LC90) were 3.11 and 8.89 (*S. acmella* L. var *oleraceae*), 3.54 and 9.92 (*S. calva* L.) and 3.36 and 6.33 ppm (*S. paniculata*), respectively. This is in sharp contrast to the earlier report of Pitasawat et al. (1998) where the LC50 value was 61.43 ppm from the extracts of *S. acmella* L. This may be due to the variation in the species/locality of the source material, as the synthesis of bioactive

Table 1 Bioefficacy of crude extract of *Spilanthes* species on Late III/Early IV instar larvae of *Anopheles stephensi* Liston, *Anopheles culicifacies* species C and *Culex quinquefasciatus* Say

Species number	Plant species	Mosquito species	LC50 (ppm)[a]	LC90 (ppm)[b]	χ^2 (*df*=4)[c]	FL (LC50) (lower–upper)[d]	FL (LC90) (lower–upper)
1	*Spilanthes acmella* L. var *oleraceae* Clarke	*Anopheles stephensi* Liston	4.57	7.83	2.64	4.22–4.95	7.04–8.98
		Anopheles culicifacies species C	0.87	1.92	2.04	0.38–1.16	1.62–2.39
		Culex quinquefasciatus Say	3.11	8.89	15.62	2.01–4.34	6.02–20.51
2	*Spilanthes calva* L.	*Anopheles stephensi* Liston	5.10	8.46	1.05	4.71–5.51	7.63–9.67
		Anopheles culicifacies species C	0.92	1.99	2.26	0.46–1.19	1.70–2.48
		Culex quinquefasciatus Say	3.54	9.92	4.39	3.13–3.96	8.47–12.11
3	*Spilanthes paniculata* Wall ex DC	*Anopheles stephensi* Liston	5.09	13.55	19.42	3.41–7.26	9.12–30.01
		Anopheles culicifacies species C	3.23	7.10	9.76	2.52–4.03	5.46–11.21
		Culex quinquefasciatus Say	3.36	6.33	11.44	2.66–4.18	4.95–10.14

[a] *LC50* Lethal concentration that kills 50% of the exposed larvae
[b] *LC90* Lethal concentration that kills 90% of the exposed larvae
[c] χ^2 Chi-square, *df* degree of freedom
[d] *FL* Fiducial limits

compounds is regulated by different stress factors, such as soil temperature, soil type, pressure and overall climate of the area (Sukumar et al. 1991).

Nevertheless, this system has proved to be much more effective over other plant systems reported earlier (Rahman et al. 2000; Siddiqui et al. 2003; Kannathasan et al. 2007), where significantly higher doses of plant extracts were required for achieving 100% lethality against *C. quinquefasciatus* (LC50=41.41 and 129.24 ppm) and *A. stephensi* (LC50=16 and 79.58 ppm) larvae from *Vitex*, *Azadirachta* and *Feronia* plant extracts, respectively. However, during current investigations, a minimum of 4.57-, 0.87- and 3.11-ppm (LC50) doses proved effective rendering this as the most potential system for biocontrol of such vectors.

Further field trials are in progress for making this technology viable on a large scale to eradicate this recurrent problem. Besides, the active crude extract has already been separated into different fractions through column chromatography. Their bioassays, further isolation (through thin-layer chromatography [TLC] and preparatory TLC) and characterization of the larvicidal compounds (through nuclear magnetic resonance and infrared) is in progress.

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