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**Review Article**

# The potentiality of botanicals and their products as an alternative to chemical insecticides to sandflies (Diptera: Psychodidae): A review

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ABSTRACT

Use of chemical pesticides is the current method for controlling sandflies. However, resistance is being developed in sandflies against the insecticide of choice that is DDT (dichlorodiphenyl trichloroethane). Botanicals have potential to act as an alternative to chemical insecticides as the crude extracts and active molecules of some plants show insecticidal effect to sandflies. This will lead to safe, easy and environment friendly method for control of sandflies. Therefore, information regarding botanicals acting as alternative to chemical insecticide against sandflies assumes importance in the context of development of resistance to insecticides as well as to prevent environment from contamination. This review deals with some plants and their products having repellent and insecticidal effect to sandflies in India and abroad. Different methods of extraction and their bioassay on sandflies have been emphasized in the text. Various extracts of some plants like *Ricinus communis* (Euphorbiaceae), *Solanum jasminoides* (Solanaceae), *Bougainvillea glabra* (Nyctaginaceae), *Capparis spinosa* (Capparidaceae), *Acalypha fruticosa* (Euphorbiaceae) and *Tagetes minuta* (Asteraceae) had shown repellent/insecticidal effect on sandflies. This review will be useful in conducting the research work to find out botanicals of Indian context having insecticidal effect on sandflies.

**Key words** Biopesticides; insecticide; plant extract; repellent; sandflies

## INTRODUCTION

Plants were evolved over 400 million years ago and to defend themselves from insect attack, they have developed protection mechanisms such as repellents and even insecticidal effects[1]. They degrade rapidly and, therefore, are considered safer to the environment than the common synthetic chemicals. However, as with any pesticide, plant-based products must be used properly[2–4]. The indiscriminate use of chemical pesticides has given rise to many well-known and serious problems, including genetic resistance of pest species, toxic residues in stored products, increasing costs of application, hazards of handling etc. The problems caused by pesticides and their residues have increased the need for effective biodegradable pesticides with greater selectivity. Therefore, alternative strategies like use of traditional plant-based pest control agents are being explored. Plant-based insecticides tend to have a broad-spectrum activity, are safe and relatively specific in their mode of action and easy to process and use. They also tend to be safe for higher animals and environment[5]. Plant-based insecticides can often be easily produced by farmers and small-scale industries.

Crude plant extracts and inorganic larvicides were largely used as natural insecticides before the organic laboratory-synthesized insecticides became available in the 1940s[6–8]. Applications of phytochemicals in mosquito control were in use since the 1920s[9], but the discovery of synthetic insecticides such as DDT in 1939 side tracked the application of phytochemicals in mosquito control programme. After facing several problems due to injudicious and over application of synthetic insecticides in nature, re-focus on phytochemicals that are easily biodegradable and have no ill-effects on non-target organisms was appreciated. Since then, the search for new bioactive compounds from the plant kingdom and an effort to determine its structure and commercial production has been initiated. At present phytochemicals make up to 1% of world's pesticide market[10]. Several groups of phytochemicals such as alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported previously for their insecticidal activities[11]. Plant extracts such as pyrethrum, nicotine and rotenone were among the first compounds used to control insects of medical and agricultural importance[12–13]. Pyrethrins, a complex of esters extracted from flowers of *Chrysanthemum cinerariefolium* (Compositae), are still used to enhance commercial preparations of household insecticides[14]. Nicotine extracts from *Nicotiana glauca* and its nicotinoid derivatives are choice molecules for the manufacture of new insecticides. Rotenone and rotenoids, isoflavanoids found in several genera of tropical leguminosae plants such as *Derris* (Papillionaceae), *Antonia* (Loganiaceae) and *Lonchocarpus* (Fabaceae), were shown to have insecticidal properties against *Lutzomyia longipalpis* Lutz and Neiva, the vector for *Leishmania chagasi* in Brazil[15]. Essential lemon oil was found to be 70% protective against sandfly bites[16]. A concentration of 2% neem oil mixed in coconut or mustard oil provided 100% protection against *P. argentipes* throughout the night in field conditions[17]. Pyrethrin esters were found to be effective repellents against *P. argentipes*, the vector of Indian kala-azar[18].

Secondary compounds in *Tagetes* are effective deterrents of numerous organisms including insect pests through different mechanisms[19–22]. Dried plants can be hung indoors as an insect repellent[23]. Crude extracts from *T. minuta* aerial parts have been found effective against mosquito larvae[24] with $LC_{50}$ and $LC_{90}$ of 1.5 and 1 mg/l, respectively[24]. Repellent activity of *Tagetes* species were reported against *Anopheles gambiae*, the vector of malaria[25]. *Tagetes* species also showed insecticidal activity against stored product pests[26]. The potential of 100 ppm of *T. minuta* essential oil against head lice *Pediculus humanus capitis* (Phthiraptera: Pediculidae) was evaluated denoting toxicity of the essential oil[27]. Terpenes were responsible for the toxic effects reported in dipterans in *T. minuta*[28]. Brown[23] reported that dried plants can be hung indoors as insect repellents. *T. minuta* was found having larvicidal effect against *Aedes aegypti* larvae at 10 ppm[29]. The terpene and ocimenone in *Tagetes* were found as larvicidal only at higher concentrations than the whole oil. The discovery of insecticide activity of phototoxins present in *Asteraceae* species has stimulated the interest in this plant family as part of the search for new plant derived insecticides[7]. Although, *T. minuta* is perceived to have insecticidal activities, its action against phlebo-tomine sandflies has not been evaluated. The plant has been used extensively for its medicinal value, food, fodder and repellent activities against insects[30].

Distilled leaf extracts of *Tarchonanthus camphoratus* yielded compounds with insecticidal activities. Wild animals have been seen rubbing against the plant to keep off biting insects[31–32]. More than 2000 other plant species are catalogued as having insecticidal properties[1, 33–35]. There is no doubt that botanical insecticides are an interesting alternative to insect pest control, and on the other hand only a few of the >250,000 plant species on our planet have been properly evaluated for this purpose[1, 36–38]. When synthetic insecticides appeared in the 1940s some people thought that botanical insecticides would disappear forever but problems like environmental contamination, residues in food and feed, and pest resistance brought them from back to the fore.

In fact, plants like neem (*Azadirachta indica* J., Meliaceae), have shown excellent results[10, 39–40] and there are already commercial products in the market made from it. Many other plants like *Ricinus communis* (Euphorbiaceae), *Solanum jasminoides* (Solanaceae), *Bougainvillea glabra* (Nyctaginaceae) and *Capparis spinosa*[41–42] had also shown to act as future alternative for the control of sandflies. DDT as used now a days, has been found not much effective because the sandflies are showing resistance to it in some regions of Bihar, India[43]. The studies have been mostly conducted outside India and since the flora and climatic conditions of India are different, hence, extensive studies need to be conducted on endemic flora of India showing similar effects on sandflies to control leishmaniasis and also many new diseases arising due to this vector. The botanicals can also be tested on other vectors for controlling the spread of many diseases. It can also be tested for crop improvement and control of plant pathogens.

The rapid growth of knowledge of natural products with biological activities towards pests now provides an option for treatment, a clearer understanding of biochemical mechanisms, and a basis for biorational approaches to the design of pest control agents. Compounds that modify insect behaviour are also valuable for pest control because they are normally not toxic to the target insect or to the environment.

Unlike conventional insecticides which are based on a single active ingredient, plant derived insecticides comprise botanical blends of chemical compounds which act concertedly on both behavioural and physiological processes. Thus, there is a very little chance of pests developing resistance to such substances. Identifying bioinsecticides that are efficient, as well as being suitable and adaptive to ecological conditions, is imperative for continued effective vector control management. The botanicals have widespread insecticidal properties and will obviously work as a new weapon in the arsenal of synthetic insecticides and in future may act as suitable alternative products to fight against mosquito-borne diseases[44]. Reports showed that methanol extract of *Acalypha alnifolia* leaf has larvicidal effect against *An. stephensi, Ae. aegypti* and *Culex quinquefasciatus*[45]. The berry of *Solanum villosum* (chloroform: methanol::1:1) extract showed insecticidal and larvicidal effect against *Ae. aegypti*[46] and larvicidal effect against *An. subpictus*[47]. The larvicidal activity of *Cestrum diurnum* leaf (chloroform: methanol:: 1:1) was also reported against all instar larvae of *Cx. quinquefasciatus*[48]. The active ingredient of *C. diurnum* leaf (chloroform: methanol::1:1) acting as larvicide was determined against I, II, III and IV instar larvae of *An. stephensi,* respectively[49].

In view of the latest demand of the era to find alternatives of chemical pesticides, this review was done for conducting future studies with reference to Indian flora and climate especially in Bihar, an endemic region of leishmaniasis. Plants so far studied showing insecticidal or repellent effect to sandflies are *Ricinus communis* (Euphorbiaceae)[41–42], *Solanum jasminoides* (Solanaceae), *Bougainvillea glabra* (Nyctaginaceae)[41], *Capparis spinosa* (Capparidaceae)[41–42], *Solanum luteum* (Solanaceae), *Malva nicaeensis* (Malvaceae)[42], *Tagetes minuta* Linnaeus (Asteraceae), *Acalypha fruticosa* Forssk (Euphorbiaceae), *Tarchonanthus camphoratus* L. (Compositae)[50–52], *Eucalyptus staigeriana, E. citriodora, E. globulus*[53], *Myrtus communis* (Myrtaceae)[54], *Antonia ovate* and *Derris amazonica*[15]. Studies showed that essential lemon oil protects human skin against sandfly bites[16]. Protection against *P. argentipes* was also observed with 2% neem oil mixed in coconut or mustard oil[17].

*Extraction of plant products*

There are several processes for plant extraction like hydro distillation, steam distillation, hydro diffusion, enfleurage, maceration, liquid carbon dioxide extraction, etc. of which in majority, different solvent extraction methods were used including aroma principles[50–51]. Application of these processes, singly or in combination, depends upon the nature of the material and of the essential oil or absolute to be recovered. Methanol extract was found to give more mortality rates of insects in most of the cases as compared to ethyl acetate[50]. Almost 50% of the cost is rendered for the extraction of essential oil from the plant material. Essential oils are obtained by distillation, usually with water[54] or steam or as in the case of citrus fruits, by a mechanical process. Concretes are odorous concentrates obtained from fresh plant material of low resinous content by extraction with a volatile non-aqueous solvent[51], followed by the removal of the solvent by evaporation at moderate temperatures and under partial vacuum. Concretes are usually waxy solids. Absolutes are highly concentrated perfumery materials obtained from concretes by repeated extraction with ethyl alcohol followed by chilling of extract (to precipitate waxes and non-odorous matter), filtration or centrifugation of the remaining alcohol solution and finally removal of most of the alcohol by evaporation at moderate temperatures and under partial vacuum. Absolutes are usually liquids and entirely soluble in alcohol. Spice oleoresins are obtained from dried spices by extraction with a volatile non-aqueous solvent, followed by removal of the solvent by evaporation under partial vacuum. Oleoresins contain the aroma and flavour of the spice (including any non-volatile principles, unlike spice essential oils) in a concentrated form and are usually viscous liquids or semi-solid materials. They should be distinguished from spice aqua resins, which have closely related applications but which are extracted with aqueous alcohol rather than with volatile solvents.

Of primary consideration is the type of solvent used since polar solvents will extract polar molecules and non-polar solvents will extract non-polar molecules. The purpose of a general screening for bioactivity is to extract as many potentially active constituents as possible. This is achieved by using solvents ranging from water, the most polar with a polarity index (P) of 10.2 to chloroform (relatively non-polar; P =4.1) and hexane (non-polar P = 0.1) including a number of intermediary solvents such as ethyl alcohols and then, filtered and dried out by evaporating at their boiling point[15, 50–51]. Screening of botanical material is if attempted incomplete, the solvents for phytochemical extractions should be carefully selected because different solvent types can significantly affect the potency of extracted plant compounds[55]. A converse relationship is said to exist between extract effectiveness and solvent polarity where efficacy increases with decreasing polarity[37]. This is not consistent due to differences between the characteristics of active chemicals among plants. Berry and Rodriguez[56], suggested the use of different solvents based on the type of molecules targeted for extraction. Petroleum ether (P = 0.1) appears to have been the solvent of choice for some time. The crude extract described above contains a complex mixture of biocidal active compounds. If an exceptionally low lethal concentration is detected, the extract may be fractionated in order to locate the particular chemical constituent causing the lethal effect. The purpose of fractionation is to produce several simple mixtures of compounds, to reduce the number of compounds which may be identified in further analyses. Once a fraction has proved to be effective, compounds can be extracted to isolate the active ingredients. Some compounds loose efficacy when separated since many synergistic relations potentially exist in botanical preparations which may promote killing activity.

*Bioassay on Phlebotomus spp.*

For the bioassay of sandflies for the aroma from plant parts sandflies <24 h old are introduced into a plastic box (35 × 30 × 15 cm) with a layer of plaster of paris at the bottom and a suitable net cover. A sleeve of cloth fitted to a 10 cm hole in one of the walls is used for the daily exchange of diet plant-branches. Branches are offered in an Erlenmeyer flask as described earlier and water is offered *ad libitum.* One control series receive only water and the other one receives water and 20% sucrose solution on wet cotton-wool swabs daily. The daily mortality of flies is recorded[41]. For the solid extracts obtained, different concentrations of solution are prepared. One or 2 ml of the solutions are blotted on filter papers, which are dried overnight and placed into jars where adult sandflies are aspirated. Males and females may be assayed separately for knowing the effect on them separately[51]. Female sandflies can also be aspirated into vials where they are fed on a mixture of the plant extracts and sucrose solution prepared in a ratio of 1:1[52]. Different treatments with different concentrations are performed along with two negative controls, distilled water and Tween 80 (3%), and a positive control, cypermethrin (0.196 mg/ml). The tests are carried out in plastic pots internally coated with sterile plaster and filled with a substrate made of rabbit feces and crushed cassava leaves. The eggs, larvae and adults are sprayed with the oils. The hatched larvae counted for 10 consecutive days and observed until pupation. Insect mortality is observed after 24, 48 and 72 h. Sucrose-extracts feeding technique is also another method of bioassay in sandflies.

The tube bioassay experiments are conducted in the laboratory using newly emerged laboratory bred *P. argentipes* 3-day old fed on 10% glucose solution and 50 µl of crude extracts of plant samples were blotted on 1.31 $cm^2$ area of Whatman filter paper. The filter paper is dried at 40°C and placed in a tube. About 20 sandflies are aspirated in the tube and kept overnight for bioassay. Knockdown is observed after 30 min and mortality is recorded after 24 h. The same protocol is applied to negative control, control and positive control experiments in which sandflies are aspirated into tubes containing filter papers blotted with distilled water, solvent used for extraction, and deltamethrin, respectively and dried in the same condition as for the extracts[57].

*Larval bioassay*

The I, II, III and IV instar larvae series are prepared in triplicates in each vial. One gram of larval food prepared from a fungal growth obtained from rabbit chow is mixed with each extract solution and allowed to dry overnight under shade. The vials are appropriately marked for each plant extract. Small amounts of the prepared dry food-extract mixtures are then sprinkled into the vials each day. The four triplicate series of larvae are used for each plant extract. Larvae that fed on larval food mixed with distilled water and dried under the same conditions as the treatments are used as controls. Larvae are also fed on plain powdered plant parts and without any larval food mixture. Those that fed on larval food alone formed the control group. Larvae are monitored daily and mortality is recorded for analysis. Therefore, at least 120 larvae are assayed for each plant extract. Mean lethal dosage designated $LD_{50}$ is determined daily[50]. The solid extract obtained can be diluted in water at different concentrations to make solutions and blotted on filter paper placed at the bottom of cylindrical glass tubes containing sandflies. For each plant extract and dilution, two series of triplicates with male and female specimens of *L. longipalpis* are used. Mortality is recorded every 2 h during 72 h of exposure[15].

## DISCUSSION

Studies conducted on laboratory reared colonies originated with flies from Jordan Valley and Kfar Adumim, a village approximately 15 km east of Jerusalem with branches of *Ricinus communis* (Euphorbiaceae), *Solanum jasminoides* (Solanaceae), *Bougainvillea glabra* (Nyctaginaceae) and *Capparis spinosa* showed that one night of feeding on branches of *Solanum jasminoides, Ricinus communis,* or *B. glabra* drastically shortened the life span of the sandflies (*Phlebotomus papatasi*)[41]. In the region endemic for *L. major* in yards abounding with vector sandflies, the number of *P. papatasi* trapped near hedges of *B. glabra* was eight times less (62 *versus* 502 flies trapped) than that of the control sites[41], therefore, *B. glabra* affords local protection against sandfly bites and decreases the risk of leishmaniasis[41]. Feeding on *Ricinus communis, Capparis spinosa* and *Solanum luteum* caused >50% mortality and deformation of parasites in 88, 55, and 46% of the infections, respectively[42], *Malva nicaeensis* and the honeydew of *Icerya purchasi* produced thriving parasitaemias[42]. The extracts of *Tagetes minuta* Linnaeus (Asteraceae), *Acalypha fruticosa* Forssk (Euphorbiaceae) and *Tarchonanthus camphoratus* L. (Compositae) prepared from floral and foliar parts of the plants collected from Baringo district in the Rift Valley Province of Kenya were also found to be insecticidal to adult flies[51]. Study carried out at the Kenya Medical Research Institute's Centre for Biotechnology Research and Development (CBRD), Nairobi, Kenya has shown that the crude extracts from dried aerial parts of *T. camphoratus*, *A. fruticosa* and *T. minuta* have also been found to reduce the fecundity of *P. duboscqi* significantly ($p$ <0.05) and vectorial capacity of sandflies[52]. The essential oils of *Eucalyptus* spp. *E. staigeriana, E. citriodora* and *E. globulus* were effective against egg, larval and adult phases of *L. longipalpis*. The eucalyptus essential oils constitute alternative natural products for the control of *L. longipalpis* since the median effective concentration ($EC_{50}$) values revealed relevant action as compared with other natural products[53].

Crude extracts from *T. minuta* aerial parts showed $LC_{50}$ and $LC_{90}$ of 1.5 and 1 mg/l, respectively to mosquito larvae[24]. The potential of 100 ppm of *T. minuta* essential oil against head lice *Pediculus humanus capitis* (Phthiraptera: Pediculidae) exhibited lethal time ($LT_{50}$) of 16.4 ± 1.62 min denoting toxicity of the essential oil[27]. In *T. minuta* oil, essential terpenes were responsible for the toxic effects reported in dipterans[28]. The n-hexane, dichloromethane, ethyl acetate and methanol extracts of *T. minuta* and *A. fruticosa* extracts in sugar baits bioassays showed significant mortality ($p$< 0.05) in both males and females and had comparable $LD_{50}$ values[50]. The insecticidal action of Myrtle oil was also observed during the study, the mortality after exposing to repellents was only observed when sand flies exposed to high doses of Myrtle oil. The highest mortality rate was 62.2% at dosages of 1 mg/sq cm[54]. Preliminary assays indicated that *Antonia ovata* and *Derris amazonica* displayed significant insecticide effect against *L. longipalpis*[15]. Application of essential lemon oil to human skin was found 70% protective against sandfly bites[16]. Bioassays have revealed that 2% neem oil mixed in coconut or mustard oil offered 100% protection against *P. argentipes*[17].

The methanol extract of *Acalypha alnifolia* leaf showed larvicidal effect against *An. stephensi, Ae. aegypti* and *Cx. quinquefasciatus* species at 24 h exposure with $LC_{50}$ value of 125.73, 127.98 and 128.55 ppm, respectively[45]. The berry of *S. villosum* (chloroform : methanol::1:1) extract exhibited $LC_{50}$ value of 5.97 ppm at 72 h of bioassay against *Ae. aegypti*[46] whereas, the $LC_{50}$ values of leaf extract were between 24.20 and 33.73 ppm after 24 h and between 23.47 and 30.63 ppm after 48 h of exposure against all instars of *An. subpictus*[47]. The larvicidal activity of *C. diurnum* leaf (chloroform: methanol::1:1) was reported with $LC_{50}$ value of 0.29, 0.35, 0.57 and 0.65%, respectively in all instar larvae of *Cx. quinquefasciatus*[48] and the $LC_{50}$ value of the active ingredient was determined as 0.70, 0.89, 0.90 and 1.03 mg/100 ml for I, II, III and IV instar larvae of *An. stephensi*, respectively in 24 h[49]. Studies on methanol, ethyl acetate and petroleum ether extract of *Lantana camara* Linn. showed them to be effective against sandfly (*P. argentipes*) whereas methanol extract was found effective against diamondback moth (*Plutella xylostella)* and red spider mites (*Tetranychus urticae*). Methanol extract was 87.5% effective against *P. argentipes* and 100% against *P. xylostella* and *T. urticae*. The antifeedancy of *P. xylostella* was observed to be 83% with methanol extract of *L. camara*[57]. Therefore, the local flora of India can also be tested for these effects on sandflies.

## CONCLUSION

The available literature showed that there is immense potential for plants and their extracts to act as an alternative for chemical pesticides but more studies are to be carried out on the Indian species as they may show different effect and even the same species might show some different results due to varying climatic conditions of India. There are more plants that need to be tested against sandflies depending upon their availability which have shown insecticidal effect on other insects. Different extraction methods may be employed for different plant parts for testing the efficacy on sandflies because the effect depends on method of extraction. The plant extracts obtained from different methods can be evaluated for cost-effectiveness.

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