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The role of botanicals as green pesticides in integrated mosquito management – A review

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Abstract
Mosquito borne diseases are prevalent in more than 100 countries across the world, infecting over 700,000,000 people every year globally. Prevention of man mosquito contact is indispensable for protection from mosquito borne diseases. There has been a paradigm shift towards botanicals to overcome the problems associated with the use of synthetic compounds in mosquito management. DDT and BHC dominated the insecticide market (80.1% in 1970) until 1983, when their production was prohibited owing to prevailing resistance in treated species, resurgence, residual problem, biomagnification, environmental and health hazards. The application of easily degradable plant compounds is considered to be one of the safest methods to control insect pests and vectors. The present article thus envisaged to review the current status of botanicals as green pesticides in Integrated Mosquito Management. As the role of botanicals as mosquitocides has been found to be promising in preliminary screenings, recent technological developments in isolation and standardization of herbal pesticides need to gear up.

Keywords: Botanicals, Chemical insecticides, Integrated Mosquito Management, Mosquitoes

1. Introduction
In almost all tropical and subtropical countries, mosquitoes (Diptera: Culicidae) serve as vectors of life threatening diseases such as malaria, filariasis, dengue, Japanese encephalitis etc. About 3000 species of mosquitoes have been recorded worldwide, out of which more than 100 species are reported to be capable of transmitting diseases to humans [1]. Mosquito borne diseases infect over 700,000,000 people every year globally, being prevalent in more than 100 countries across the world [2]. The data is alarming in Indian scenario with about 40,000,000 individuals affected by mosquito transmitted diseases every year [3]. WHO has declared mosquitoes as “public enemy number one”. Worldwide, malaria causes one to two million deaths annually. Lymphatic filariasis has been reported to affect at least 120 million people in 73 countries including Africa, India, Southeast Asia, and Pacific Islands. According to reports, global filariasis in India constitutes around 40 percent of global filariasis burden with the estimated annual economic loss of about 720 crores [2]. Japanese encephalitis accounts for the annual incidence of 30,000-50,000 with a mortality estimate of 10,000, respectively [4]. In developing countries such as India, the mosquito borne diseases not only cause high level of morbidity and mortality but also inflict great economic loss and social disruption.

2. Chemical control and resistance in vector mosquitoes
Over the centuries scientists are exploring various methods to combat threats from mosquito borne diseases and use of synthetic insecticides has been the major tool in mosquito control operations. However, the extensive and repeated use of synthetic organic insecticides such as organophosphates and organochlorines have led to disrupted natural biological control systems heading to resurgence and resistance in target species and destruction of nontarget beneficial fauna, in turn resulting in fostered environment and human health concern. Tikar *et al*. [5] have reported the development of insecticide resistance in *Culex quinquefasciatus* against temephos, fenthion, cypermethrin and cyhalothrin. The pesticide residues are known to exhibit biomagnification by entering into the ecosystem and circulating through food web.
The ill-effects of insecticide usage have thus necessitated the need for research and development on environmentally safe, bio-degradable and indigenous method for controlling mosquitoes.

Since early times even before the discovery of synthetic insecticides, many herbal products have been evaluated and used as natural insecticides. Botanicals such as *Chrysanthemum*, *Pyrethrum*, *Derris*, *Quassia*, *Nicotine*, *Hellebore*, *Azadirachtin*, *Turpentine* etc. have been reported to be used as plant based insecticides in the pre-DDT era [6]. However, the application of phytochemicals in mosquito control was side tracked by the discovery of synthetic insecticides particularly organochlorines, such as DDT in 1939, as they initially were successful in providing good control of vector species. After facing several problems due to injudicious and over application of synthetic insecticides in nature during mid 1970s and awareness published by Rachel Carson through her book Silent Spring [2], re-focus on easily biodegradable phytochemicals with no ill-effects on non-target organisms was appreciated. Since then, the search for new bioactive compounds from the plant kingdom was initiated. Efforts have been done to determine their structure and commercial production has been initiated as a part of Integrated Mosquito Management (IMM).

Culex, *Aedes*, and *Anopheles* genera constitute the major mosquito vectors. Soon after the introduction of DDT for mosquito control in 1946, the first cases of DDT resistance were recorded in *Aedes tritaeniorhynchus* and *Ae. solicitans* in 1947 [7]. Out of more than 100 mosquito species reported as resistant to one or more insecticides since then, about 50 are anophelines [8]. Organochlorines, organophosphates, carbamates, and pyrethroids constitute the major groups of insecticides used for malaria control, with the latter now taking increasing market share for both indoor residual spraying and large-scale insecticide-impregnated bednet programs. Other insecticide groups, such as the benzylphenyl ureas and Bti, have had limited use against mosquitoes. Resistance has tended to follow the switches of insecticides. Despite the lack of use of BHC/ dieldrin for many years, widespread resistance in mosquito populations has been recorded. The major vectors *An. culicifacies*, *An. stephensi*, *An. albimanus*, *An. arabiensis* and *An. sacharovi* have been reported to show Organophosphate (OP) resistance, either in the form of broad-spectrum OP resistance or malathion-specific resistance [9, 10, 11, 12, 13, 14]. Malathion resistance was reported to occur in *An. culicifacies* species B in Sri Lanka, while in species B and C in India [15]. In Sri Lanka Species B is developing pyrethroid resistance and has completely developed resistance against fenitrothion, independent of the malathion-specific resistance [16]. Organophosphorus insecticide resistance in all the major *Culex* vector species [17] while pyrethroid resistance in *Cx. quinquefasciatus* [18] are reported to be widespread. In addition, *An. albimanus*, *An. stephensi* and *An. gambiae* have shown pyrethroid resistance [19, 20, 21] while carbamate resistance has been noted in *An. sacharovi* and *An. albimanus* [22]. Widespread Pyrethroid resistance was observed in *Ae. aegypti* [23] along with OP and carbamate resistance [24]. Given the recent emphasis by the WHO and other organizations on the use of pyrethroid-impregnated bednets for malaria control, development of pyrethroid resistance in *An. gambiae* is of prime importance.

3. Integrated Mosquito Management- need of the hour

Integrated Mosquito Management (IMM) involves a combination of methods and strategies for maintaining low levels of mosquito vectors. It is a decision making process for the management of mosquito populations. The purpose of IMM is to provide protection against diseases transmitted by mosquitoes, maintain healthy environment through proper use and disposal of pesticides and improve the overall quality of life through practical and effective mosquito control strategies [2]. Destruction of vectors or intermediate hosts is one of the strategies of the WHO in combating tropical diseases. Controlling mosquitoes at the larval stage is more efficient and target specific of all the avenues of IMM because the mosquitoes are relatively immobile during the immature stage; remaining more concentrated than they are in the adult stage [25].

4. Botanicals as an essential component of IMM

Plants have co-evolved with insects that have equipped them with plethora of chemical defense, which can, in turn be used against insects [26]. Till date, more than 2000 plant species have been known to produce secondary metabolites of value in biological pest control programs and among these, products of some 344 species have been reported with significant activity against mosquitoes [27]. Larvicidal, adulticidal or repellent activities against different species of mosquitoes have been reported from members of the plant families Solanaceae, Asteraceae, Cladophoraceae, Labiatae, Miliaceae, Oocystaceae and Rutaceae [6]. The secondary metabolites present in plants constitute a defense system against insect/ pest attacks. The presence of compounds like phenolics, terpenoids and alkaloids present in plants, by acting as antifeedants, moulting hormones, oviposition deterrents, repellents, juvenile hormone mimics, growth inhibitors, antimoulting hormones as well as attractants, is held responsible for biological activity of plant extracts against target pest. Limonoids from Rutaceae particularly citrus have attracted greater apprehension due to their growth regulating activities [28]. Citrus seeds are available in large quantity as waste products of the citrus industry from which citrus including limonin, nomilin, obacunone, epilimonol and limonin diosphenol can readily be extracted. Citrus limonoids have been found to work as feeding deterrents and also induce toxicity. It has been determined in structure-activity studies of limonin that the furan ring and epoxide groups in the citrus limonoid structure are critical for the antifeedant activity of the limonoids. Limonoids lead to nutritional disruption inducing antifeedant effects that ultimately affect the egg laying process of insects [29].

4.1 Factors affecting the efficacy of botanical pesticides

The insecticidal effects of plant extracts depend upon the plant species, mosquito species, geographical varieties, plant parts used, extraction methodology adopted and the polarity of solvents used during extraction. Among these, the plant species and plant parts used significantly influence the efficacy of botanical mosquitocides. Different plant parts (leaves, roots, stem, fruits, fruit peel, seeds, rhizome, flowers, bark etc.) have been reported to be used as a source of botanicals in mosquito control with varying efficacy (Table 1). The larvicidal activity of five aromatic plant species was tested by Das *et al.*, 2007 [30] against *Ae. albopictus* and *Cx. quinquefasciatus* larvae and was found to vary according to plant species. Maximum efficacy was reported for methanol extract of *Aristolochia saccata* roots against *Ae. albopictus* larvae followed by ethanol extracts of *A. saccata*, *Annona squamosa* leaf and methanol extract of *A. squamosa* leaf, respectively. Polarity of the solvent used for extraction is another important factor that has a high influence on the potency of extracted active biochemical from plants. Polar molecules are extracted by polar solvents and non-polar molecules with non-polar solvents. Steroids and alkaloids are generally extracted by the moderately polar solvents. However, solvents with minimum

polarity such as hexane or petroleum ether or with maximum polarity such as aqueous/ steam distillation are mostly used.

Malik *et al*., 2014 [31] compared larvicidal activity of *Lantana camara* Linn. whole plant extracts and *Bauhinia racemosa* Lam. leaf extracts, extracted in petroleum ether, chloroform and ethyl acetate, against malaria vector mosquito *Anopheles stephensi* and found that the petroleum ether extract of *L. camara* showed highest larvicidal activity in comparison to petroleum ether extract of *B. racemosa*, and ethyl acetate extract of *B. racemosa* showed highest larvicidal activity in comparison to chloroform extract of *L. camara*.

The larvicidal potential of different solvent crude (hexane, chloroform, ethyl acetate, acetone and methanol) leaf extracts of four plants (*Blepharis maderaspatensis*, *Elaeagnus indica*, *Maesa indica*, *Phyllanthus wightianus* and *Memecylon edule*) was tested against the fourth-instar larvae of *Aedes aegypti*. All the tested extracts showed moderate to good larvicidal activities. However, the maximum larval mortality was detected in acetone extract of *E. indica* (LC_{50} 90.89, LC_{90} 217.21 and LC_{99} 441.88 ppm) followed by *M. indica* acetone extract (LC_{50} 173.21, LC_{90} 289.86 and LC_{99} 441.04 ppm) [32].

Tennyson *et al.*, 2012 [33] screened twenty five plant extracts for larvicidal activity against *Cx. quinquefasciatus* at 1000 ppm concentration and found that the hexane extracts of *Cleistanthus collinus* and *Murraya koenigii* plants showed 100 percent mortality at 24 h bioassay followed by diethyl ether, dichloromethane and ethyl acetate extracts of *C. collinus, Leucas aspera, Hydrocolite javanica, M. koenigii*, *Sphaeranthus indicus* and *Zanthoxylum limonella* after 48 h exposure.

Larvicidal activities of three medicinal plant extracts were studied in the range of 4.69 to 1000 mg/l in the laboratory bioassays against early 4th instar larvae of *An. subpictus* and *Cx. tritaeniorhynchus* [34]. All plant extracts showed moderate effects after 24 h of exposure; however, the highest toxic effect of bark methanol extract of *Annona squamosa*, leaf ethyl acetate extract of *Chrysanthemum indicum* and leaf acetone extract of *Tridax procumbens* against the larvae of *An. subpictus* (LC_{50} = 93.80, 39.98 and 51.57 mg/l) and bark methanol extract of *A. squamosa*, leaf methanol extract of *C. indicum* and leaf ethyl acetate extract of *T. procumbens* against the larvae of *Cx. tritaeniorhynchus* (LC_{50} =104.94, 42.29 and 69.16 mg/l) respectively.

The hexane, chloroform, ethyl acetate, acetone, and methanol leaf, flower and seed extracts of *Abrus precatorius*, *Croton bonplandianum*, *Cynodon dactylon*, *Musa paradisiaca* and *Syzygium aromaticum* were tested against fourth instar larvae of *Anopheles vagus*, *Armigeres subalbatus* and *Culex vishnui* by Bagavan and Rahuman [4]. The highest larval mortality was found in seed ethyl acetate extracts of *A. precatorius* and leaf extracts of *C. bonplandianum*, flower chloroform and methanol extracts of *M. paradisiaca*, and flower bud hexane extract of *S. aromaticum* against *An. vagus* with LC_{50} values of 19.31, 39.96, 35.18, 79.90 and 85.90 µg/mL; leaf ethyl acetate and methanol extracts of *C. dactylon*, flower methanol extract of *M. paradisiaca*, flower bud methanol extract of *S. aromaticum* against *Ar. subalbatus* with LC_{50} values of 21.67, 32.62, 48.90 and 78.28 µg /mL, and seed methanol of *A. precatorius*, flower methanol extract of *M. paradisiaca*, flower bud hexane extract of *S. aromaticum* against *Cx. vishnui* with LC_{50} values of 136.84, 103.36 and 149.56 µg /mL, respectively

Table 1: Some recently tested plant species with promising mosquitocidal potential

S. No.	Plant species and plant part used for extract preparation	Target mosquito species	Reference
1.	*Phragmites australis* (leaf and stem)	***Culex Pipiens***	**Bream *et al.* (2009)** [51]
2.	*Jatropha curcas* (leaf)	***Anopheles arabiensis***	**Zewdneh *et al.* (2011)** [52]
3.	*Lavandula officinalis* (flower), *Melissa officinalis* (leaf), *Rosmarinus officinalis* (leaf), *Citrus limonum* (peel), *Eucalyptus globulus* (leaf)	***Anopheles stephensi***	**Shooshtaari *et al.* (2012)** [53]
4.	*Ocimum americanum* (leaf), *Jatropha curcas* (leaf), *Citrus limon* (fruit peel)	***Aedes Aegypti***	**Kazembe and Chaibva (2012)** [54]
5.	*Azadirachta indica* (leaf)	***Aedes aegypti, Culex quinquefasciatus***	**Maragathavalli *et al.* (2012)** [55]
6.	*Abutilon indicum, Cleistanthus collinus, Leucas aspera, Murraya koenigii* (leaves) aerial parts of *Hyptis suaveolens* and whole plants of *Citrullus colocynthis* and *Sphaeranthus indicus*	***Anopheles stephensi***	**Arivoli *et al.* (2012)** [26]
7.	*Vernonia cinerea, Prosopis juliflora and Cassia tora* (leaf)	***Anopheles stephensi***	**Tyagi *et al.* (2013)** [56]
8.	*Tagetes erecta, Lantana camara* and *Tanacetum cineriifolium* (leaves and flowers)	***Culex quinquefasciatus***	**Amrutha *et al.* (2013)** [57]
9.	*Solanum nigrum* (seeds)	***Anopheles stephensi***	**Singh and Mittal (2013)** [58]
10.	*Anamirta cocculus* (fruit), *Pogostemon paniculatus* (leaf)	***Culex pipiens***	**Pushpalatha *et al.* (2014)** [59]

4.2 Mode of action of botanical pesticides

The mechanism of action of plant secondary metabolites on insect body was reviewed by Rattan, 2010 [35]. Several physiological disruptions, such as inhibition of acetylcholinesterase (by essential oils), GABA-gated Chloride channel (by thymol), sodium and potassium ion exchange

disruption (by pyrethrin) and inhibition of cellular respiration (by rotenone) are reported in insects subjected to botanical treatment. Other mechanisms include the blockage of calcium channels (by ryanodine), of nerve cell membrane action (by sabadilla), of octopamine receptors (by thymol), hormonal balance disruption, mitotic poisoning (by azadirachtin), disruption of the molecular events of morphogenesis and alteration in the behavior and memory of cholinergic system (by essential oil), etc. Of all these mechanisms, the inhibition of acetylcholinesterase activity (AChE) is the most important as it is the key enzyme responsible for terminating the nerve impulse transmission through synaptic pathway; AChE has now been observed to be organophosphorus and carbamate resistant, and it is well-known that the resistance in AChE is one of the main resistance mechanisms in insect pests [36].

4.3 Behavioral changes, mosquito knockdown and morphological growth disruption effects of plant extracts

In a study conducted under laboratory conditions to monitor behavioral changes, it was revealed that *Aedes aegypti* larvae exhibited a natural behaviour with the siphon pointed up through the water surface and head hung down immediately after exposure to ethanol-extract of celery, *Apium graveolens* seeds in test solution [37]. All the larvae were found restless and performed aggressive selfbiting to their anal papillae with their mouth parts and formed a ring shape (head to siphon) between 5 to 10 minutes after treatment with the extract at concentration ranged between 200-500 ppm. Fifteen to thirty minutes after treatment, most of the larvae were found to be irritated showing erratic movements, wriggling up and down erratically and violently. This restless behavioral pattern persisted till the larval movement slowed down and the larvae failed to reach the water surface. At four hours after treatment high level larval knockdown was clearly seen onto the bottom of the glass beaker as a result of chronic paralysis. Knockdown rate varied in a concentration dependent manner and was found to gradually increase upon increasing the extract concentration from 100 to 500 ppm. The theoretical KD_{50} value was obtained as 238.15 ppm. This alteration in larval behaviour suggests that ethanol extract derived from the seed of celery could act as cytolysin, affecting the neuro-muscular coordination in the chemical synapse conduction, thus leading to aggressive self biting, trembling movement, spinning and uncoordinated activity and paralysis. These symptoms were observed to be similar to those caused by synthetic nerve poisons, i.e. excitation, convulsions, paralysis and death. Discharge of electrolytes has also been observed from the anal region of treated mosquitoes as a result of the photo enhanced cytotoxic activity of the extract [37]. Similar behavioral observations have been reported from the same plant by Choochote *et al*., 2004 [38]. Apart from contributing to the elucidation of mode of action of insecticides, observation of the poisoning symptoms of insecticides is also of practical importance for insect control. Extensively damaged and shrunken cuticle of the anal papillae in *Aedes* larvae was reported by Chaithong *et al.*, 2006 [39] when treated with pepper extract. Alpha-terthienyl when introduced into the water medium containing mosquito larvae, entered into the body and subsequently caused halide leakage, releasing all the electrolytes into the medium and leading to death of the larvae [40]. An increase in the superoxide dismutase activity from 1st instar to 4th instar *Aedes* larval stage was observed by Nivsarkar *et al.*, 1991 [41]. This increase seems to be a protective mechanism against hazardous oxygen derivatives generated by the action of the phototoxin alpha-terthienyl superoxide dismutase found in the entire gill, except in the tracheal network. Further studies revealed severe morphological disruption of anal papillae in dead *Cx. quinquefasciatus* larvae [42]. Damaged anal papillae, with a shrunken cuticle border and destroyed surface with loss of ridge-like reticulum were observed under light and scanning electron microscopy after treatment with ethanolic extract of *Kaempferia galanga* [42]. Similar distinct features of alteration were reported by Green *et al.* [43] such as highly swollen anal papillae of *Ae. aegypti* larvae after treatment with oil of *Tagetes minuta*. The root extract of *Derris urucu* has been reported to affect the peritrophic matrix structure of *Ae. aegypti* larvae causing damage to the midgut epithelium [44]. Midgut is main site of digestion and absorption in insects [45]. The non-cellular membranous structure, "peritrophic membrane" lining the midgut lumen, protects the mid gut cells from toxic substances and pathogens entering the midgut through food [46]. Gut disruption by the activity of phototoxic Alpha-terthienyl was also observed earlier in other insects [28]. Botanical extracts are believed to contain growth regulatory compounds which possibly generate hormonal imbalance in the insects' body. Formation of pupal-adult intermediates and ecdysal failure seem to be important cause of mortality. Treatment of immature mosquitoes with juvenile hormone (JH) analogues and chitin synthesis inhibitors also reported similar abnormalities [47]. The natural plant products detrimentally affect insect growth and development. Ecdysis, shedding of old cuticle of insects, is under the influence of the hormone ecdysone. When the active plant compounds enter into the body of the insect, they may die due to abnormal regulation of hormone-mediated cell or organ development. Death may also occur either from a prolonged exposure at the developmental stage to other mortality factors or from an abnormal termination of a developmental stage itself. In particular, there often appears to be an incomplete extrication of the pupal stage from the larval cuticle, while several adults are stuck to the chitin inner lining of the puparium [48, 49]. Sakthivadivel and Thilagavathy, 2003 [50] reported that the acetone fraction of the petroleum ether extract of *A. mexicana* seeds exhibited larvicidal activity, formation of larval-pupal intermediates and formation of pupal-adult intermediates.

5. Conclusion and Future Implications

Mosquito borne diseases are major human and animal health problem in all tropical and subtropical countries. There has been a paradigm shift towards botanicals to overcome the problems associated with the use of synthetic compounds in mosquito management. Plant products can be used as mosquitocides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites. However, only a very few botanicals have moved from laboratory to the field use, which may be due to the light and heat instability of phytochemicals as compared to synthetic insecticides. Although the activity of botanicals is generally attributed to some particular compounds but if a synergistic phenomenon is established among these metabolites it may result in an increased bioactivity compared to isolated components, thus enhancing the effectiveness. At present, phytochemicals make one percent of world's pesticide market [2]. Identification, isolation and mass synthesis of bioactive compounds of plant origin against mosquito menace are imperative for the management of mosquito borne diseases. The successful results of preliminary studies on mosquitocidal potential of plant extracts encourage further effort to investigate the bioactive compounds in those extracts that might possess good

larvicidal properties when isolated in pure form. In addition, novel drug delivery systems of plant based active substances are need of time. Identifying plant based insecticides that are efficient as well as suitable and adaptive to local ecological conditions, biodegradable and have the wide spread mosquitocidal property will work as a new weapon in the arsenal of insecticides and in the future may act as a suitable alternative product to fight against mosquito-borne diseases.

APPENDIX

- Ppm: Parts per million
- Hrs: Hours
- LC_{50} : Lethal concentration that kills 50 percent of the exposed population
- LC_{90} : Lethal concentration that kills 90 percent of the exposed population
- KD_{50} : Dose that leads to knock down effect on 50 percent of the exposed population

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