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Plant proteins as natural, biodegradable, low cost larvicides against mosquitoes

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Vectors spread infectious and deadly diseases among human beings and animals. The best-known vector of many infectious diseases is mosquito. Some other vectors are flies, ticks, fleas, aquatic snails and triatomine bugs. Mosquitoes are the major group of arthropods which causes millions of deaths every year. The availability of vector control methods, drugs and transmission blocking vaccine reduced the number of deaths caused by mosquito but the emergence of insecticide-resistant mosquitoes and drug- resistant parasites leads to the requirement of a new and safe method to control mosquito borne diseases. Plant extracts have been used as an alternative mosquito control strategy from ancient times. Plant contains many phytochemicals which act as mosquito repellents, larvicidal agents, insect growth regulators and transmission blocking sources. There are many reports available showing larvicidal activities of plant crude extracts and some compounds isolated from crude extracts. But there is very little information available about plant proteins as larvicides. Plant proteins have been used as own protective system against insect pest. Plant proteins have several deleterious effects in target organism. Mosquitoes breed in the water and the larval stage is attractive target for botanical insecticides to deal with them in their habitat. The present study shade light on mosquito larvicidal role of crude plant proteins extracts, some toxic proteins (lectins, RIPs, proteinases inhibitors) derived from different plants and plant-based expression system for transmission blocking vaccine. This review may straighten out the complete investigations of the potency of anti-larvicidal properties of plant derived proteins and these proteins could be targeted for drug formation, natural bio-insecticide and transmission blocking agents for the control of vector borne diseases.

Keywords: Bio-insecticide, Larvicidal, Lectin, Mosquitoes, PPIs, RIP, Transmission blocking
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Vector- borne diseases are accountable for more than 0.7 million deaths every year and ratio of all transmittable diseases is 17% approximately[1]. In tropical areas, vector borne diseases remain a serious health problem[2]. In all over the world, mosquitoes are one of the deadliest insects. In India 40% of vector borne diseases are transmitted by the mosquitoes[3]. Mosquito transmit various diseases such as Dengue, Filariasis, Chikungunya, Zika, West nile, Malaria and Japanese encephalitis etc.[4,5]. Filariasis is transmitted by *Culex quinquefasciatus*. Malaria is a largest world health issue and caused by *Plasmodium* parasite and is passing by female *Anopheles*[6]. In 2017, estimated malaria cases were 219 million, affecting 87 countries. *Plasmodium falciparum* and *Plasmodium vivax* parasites are generally responsible for one million death annually and risk of infection in three billion human beings[7]. Dengue is mainly transmitted by the yellow fever mosquito (*Aedes aegypti*) and is prevalent in the tropical and sub-tropical regions[8]. Japanese encephalitis is caused by JE virus and is transmitted by *Culex vishnui* group. The vector of West nile virus is *Culex pipienspallens* and is distributed throughout Africa, the Middle East and Asia[9]. The availability of vector control methods, drugs and transmission blocking vaccine reduced the number of deaths caused by mosquito. The emergence of insecticide-resistant mosquitoes and drug- resistant parasites revealed that existing malaria control approaches are not demonstrable in last decade and required a new and safe method to control mosquito borne diseases.

Current control strategies are mainly targeting vector population against these diseases which are based on the integrated vector management (IVM)[10]. Vaccination against mosquito borne diseases is the most coherent method to eradicate these infectious diseases. There is no completely effective vaccine available against malaria. The Mosquirix (RTS,S) is a most effective vaccine against the malaria parasite pre-erythrocytic stage[11]. But this vaccine is not completely proficient, hence requires a vaccine of second generation. In this regard, plant compounds are more apparent that are environmentally safe and new tools in vector management[12]. Plants are used to produce high value

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pharmaceuticals such as plant-based vaccines to block malaria transmission from infected to non-infected person. At present, different expression techniques have been developed in plants. Plants are an alternative source of agent for drugs synthesis, vaccine formation and natural insecticides because plant derived products are environmentally safe, biodegradable, low cost and less toxic for humans[13]. Plant based products are cheap alternative treatment for mosquito borne disease. Further-more vaccine value of malaria antigens expressed in plants and making malaria antigens which is plant derived is an interesting platform to examine.

Plant cell wall is made up of cellulose which is bound by lipid-protein membrane. Plant contains various secondary metabolites (alkaloids, resins, saponins, phenolics, tannin etc.) which are beneficial for plants in protection and showed antimicrobial, antifungal, insecticidal property and useful against many disorders. Proteins are naturally occurring biomolecules made up of amino acids their content varies from plants to plants. The highest amount of protein is present in seeds, 10 to 50% of its dry weight[15]. Plant contains storage proteins which are present in different organs viz., kernels, seeds, grains, nuts and some tubers and roots. Some proteins have showed protective role against different pests that attack on plants[16]. Plant proteins demonstrated the highest rate of antibacterial and antifungal properties[17]. Plant proteins have also reported efficacy towards death of mosquito larvae. Conventional pesticides used in the water sources create risks to human beings and the environment. The pesticides derived from plant which is natural bio-pesticides are more promising in this exposure.

Although a lot of work has been reported about the larvicidal activities of different crude extracts of plants but very little information is available about larvicidal activity of plant proteins and their characterization. Hence, in the present review an attempt has been made to shade light on phytoproteins which act as mosquito repellents, larvicidal agents, insect growth regulators and could be used for drug formation, natural bio-insecticide and transmission blocking agents for the control of vector borne diseases.

Insecticidal activity of plant proteins against mosquito

Plant contains defensive proteins to protect themselves against insect pests. The function of plant proteins is displayed in defensive mechanism[18]. Proteins are digestive enzyme inhibitors, vicilins, lectins and chitinases act as insecticidal agents against insect and pest control[19]. The arcelins, chitinases, canatoxin and modified forms of storage proteins are involved in the complex mechanism of defense[20-25]. 3-D structure of plant proteins has been shown in Figure 1.

(a) Lectin- Lectins are naturally occurring plant proteins and have one non-catalytic domain that binds to monosaccharide or oligosaccharide[26,27]. Lectin is a multivalent protein and can adhere to the cells. Lectins protect against external pathogens that attack on plants. Hololectins, Merolectins, Chimerolectins and Superlectins are the main four classes of plant lectins, based on their characteristics and number of domains. Plant lectins played a biological function in antifungal, insecticide, antiproliferative, antiparasitic, biosensors and drug delivery processes[28]. Lectins have shown entomologic effects in insect pests Orders such as Diptera, Coleoptera, Homopetra and Lepidoptera. Powell *et al*.[29] first demonstrated the insecticidal property of lectin in plant *Galanthus nivalis* against aphids. Lectin extracted from *Glechoma hederacea* known as Gleheda lectin and *Phycella australis* have strong insecticidal activities against the green peach, pea aphid and larvae of *Leptinotarsa decemlineata*[28]. Water soluble lectin (WSMoL) and the coagulant lectin (cMoL) was isolated from the *Moringa oleifera* seeds and the larvicidal and enzymatic activity was checked against 4th instar stage of organophoshate susceptible larvae (Rockefeller L_4) and organophosphate resistance population (Rec-R) of *A. aegypti*. The LC_{50} value was 0.197 mg/mL against Rockefeller L_4. WSMoL killed Rockefeller larvae by stimulation of digestive enzyme activity and induced gut lumen proteolysis leading to

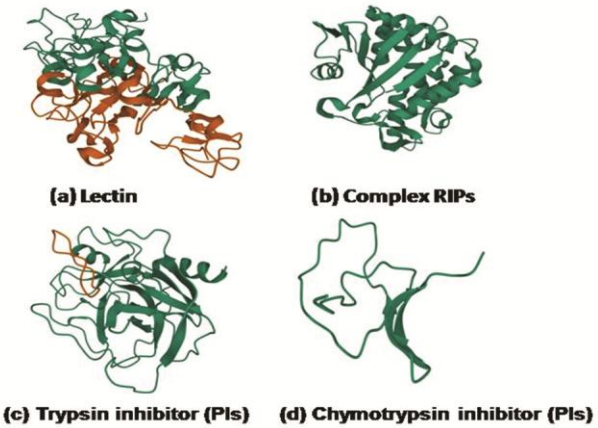

Fig. 1- 3 — D structure of plant proteins (www.rcsb.org).

degradation of important proteins and do not promote mortality in larvae of *A. aegypti Rec-R*[30]. The seed extracts of different plants found in Caatinga biome e.g. *Croton sonderianus, Caesalpinia ferrea, Cnidoscolus phyllanthus, Piptadenia viridiflora, Amburana cearenses, Genipa Americana, Erythrina velutina, Anadenanthera colubrine* etc. have showed the potential larvicidal and egg laying inhibitors in ovitraps in *A. aegypti*. The crude extracts of caatinga plant seeds contain variety of proteins, some are chitin-binding proteins, enzyme inhibitors and lectins which have insecticidal potential. The crude extracts of some plant killed 100% larvae after 48 h of exposure. *Genipa americana* did not show any mortality after 48 h[31].

Crude soluble protein and mull (lectin) were extracted from the *Myracrodruon urundeuva* leaf and both showed the larvicidal potential against *Aedes aegypti* larvae at different concentrations. The LC_{50} value of soluble protein was 10.9 mg/mL and 0.202 mg/mL for MuLL. Proteases, trypsin and α amylase were isolated from larva gut and checked for their activity against MuLL. MuLL had inhibitory effect on proteases, trypsin and α amylase with K_i value 2.8 µM. MuLL interfered in enzyme activities of gut and killed larvae due to resistance to proteolysis by gut enzymes[32]. Lectins isolated from different plants such as *Moringa oleifera, Myracrodruon urundeuva* and *Agelanthus brunneus* have shown ovicidal, larvicidal, oviposition stimulation and embryocidal activities against *A. aegypti* and *Culex quinquefasciatus*[33-37].

Lectins bind to the peritrophic membrane of the epithelial cells of insect's intestine[38,39]. Lectins act by binding to the glycoproteins present in insect gut epithelium, eventually causing death of insect by inhibiting absorption of nutrients. Plant lectins affect the insects by means of disturbing the glycosylation (N and O glycosylation) and many physiological changes in insects and thus considered to be a useful strategy for controlling insects[40]. The other toxic effect of lectin is to bind the glycosylated digestive enzymes of insect. Lectin bind to carbohydrate chain moieties on insect gut membrane and act as recognition molecules in cell-matrix or cell-cell interactions and these proteins are resistant to proteolytic degradation by insect digestive enzymes. It inhibits the digestion and absorption of food and as a result the mosquito larvae became dead[41,42].

(b) RIPs- Ribosome Inactive Proteins (RIPs) have various biological functions such as antifungal[43,44] antiviral[45] and insecticidal activities[46,47]. Plant RIPs are mainly of three types: RIP I, RIP II and RIP III. RIP-I and RIP- II isolated from apple (*Malus domestica* Borkh) and their over expression in tobacco showed strong insecticidal activity[48]. The *Jatropha curcas* contains many toxic components e.g. saponin, phorbol ester, protease inhibitors, curcalonic acid and curcin[49,50]. The protein Jc-SCRIP was extracted from the mature seed coat of *Jatropha curcas*, it is a type 1 ribosome inactivating protein (RIP). RIP is a toxin protein present in the net kernel known as curcin. This protein was compared for anti-larvicidal potency against *Aedes aegypti* and *Cx. quinquefasciatus* late third instar larva to the plant seed kernels (JSKCP) and seed coats (JSCCP) of crude protein extracts. The protein induced 100% mortality at 3.0 mg/mL concentration after exposure of Z72 h in *Aedes aegypti* and 100% mortality at 1.5 mg/mL concentration of protein after exposure of 12 h of in *Cx. quinquefasciatus.* Jc-SCRIP protein showed the rRNA N- glycosidase activities and hemagglutination assay[51]. Zhou *et al*.[52] isolated the type II[nd] RIP from the camphor seed (*Cinnamomum camphora*) and induced the LC_{50} value 168 ppm against the larvae of *Culex pipinespallens*.

Ribosome inactivating proteins (RIPs) cleaved the nucleotide *N-C* glycosidic bonds and these are group of cytotoxic N-glycosidases and render the ribosomes incapable of further translation. RIPs are localized extracellularly in different host cells. Many of these proteins have N-terminal signal sequences that target for co-translational entry into the endo-membrane system[53]. Recent studies have suggested that these proteins induce cell death by apoptosis[54].

(c) Proteinases inhibitor (PIs) – Protease inhibitors are complex of protein which regulate and inhibit their proteolytic activities. In plants near about 10 families of protease inhibitors are found[55]. Proteases are proteolytic enzymes which catalyze the peptide bonds cleavage. They are categorized according to the catalysis mechanism and amino acid present in their active centers-

(1) Cysteine proteinases
(2) Serine proteinases
(3) Metalloproteinases
(4) Aspartic proteinases[56]

Carica papaya contains laticifers cells which spread throughout most of the plant tissues and secrete latex. The cotyledon and the tegument aqueous crude extracts (1:10, w/v) of the *C. papaya*

seeds did not show mosquito larvicidal activity but both extracts mixture was lethal for the larvae[57]. The mixing of 17 µg/mL tegument protein content with 27 µg/mL cotyledon protein content have showed the most potent toxicity and 100% mortality of larvae. The enzyme tegupain was purified from the tegument extract by size exclusion chromatography. Tegupain has been identified as a cysteine proteinase enzyme due to the proteolytic effect on cotyledon protein which is endogenous[57]. Protease inhibitors isolated from *Lonchocarpus sericeus* seeds, *Alocasia macrorrhizos*, *Leucaena leucocephala* have insecticidal activity against *A. aegypti*[58-60].

Serine and cysteine proteinase inhibitors have deleterious effects which lead to decrease in weight and fecundity, increased mortality and severe deformations in coleopteran and Lepidopteran insects[61,62]. In the intestinal tract of insects, there is the presence of proteinaceous inhibitors that leads to the inhibition of digestive proteinases enzyme[63,64].

(d) α amylase inhibitors – α amylase are widely distributed digestive enzymes present in many insects which catalyze the hydrolysis of starch and glycogen into shorter oligosaccharides. α amylase inhibitors act as a source of natural defense mechanism in many plants. These inhibitors are important tools against pests in transgenic plants[65-67]. This protein induces insecticidal property by binding to peritrophic insect gut membrane that interferes in absorption of nutrient and cause degradation of gut membrane[68]. The toxic effect of that protein is due to the digestive amylase inhibition.

(e) Arcelins – It is an insecticidal protein and belongs to the lectin- like family[69] and contains two PHA-L and PHA-E subunit of phytohemagglutin. Arcelins affected the development of *Z. subfasciatus* larva by its inhibition[70,71].

(f) Canatoxin - Canatoxin is an entomologic protein and found lethal in insects which depend on digestive enzymes cathepsins B and D. The protein is most potent neurotoxic[72]. Mosquitocial activity of alpha amylase inhibitors, arcelins and canatoxin is still not identified.

(g) Crude proteins - The crude protein isolated from *Manilkara zapota* seed showed 100% mortality rate of all 4th instar *Aedes aegypti* larvae within exposure of 24 has been downloaded from PDB has been downloaded from PDB h. The LC_{50} value was 2.64 to 4.68 mg/mL and LC_{90} value was 6.24 to 8.33 mg/mL against all four instars of *A. aegypti* without affecting then on aquatic zooplankton[73]. The protein isolated from this plant exhibited moderate mosquito larvicidal activitiy against *Cx. quinquefasciatus*, *An. stephensi* and *St. aegypti*[74].

Larvicidal activity of plant proteins has been shown in Table 1.

Plant based expressed protein to produce transmission blocking antibodies

Vaccination is one of the productive and successful procedures for eradicating infectious diseases of humans throughout the world. Transmission blocking immunity is provided by specific antibodies which inhibit the parasite development in mosquito and block the transmission of infectious diseases from infected to non-infected person. Pfs25 is a surface protein of zygotes and ookinetes and expressed during the sexual stage of *Plasmodium.* Plants represent as a substitute for expression of different complex proteins. The Pfs25 protein was fused to the LicKM (modified Lichenase carrier). The resulting vector was introduced into *A. tumefaciens* and transformed bacteria were introduced into leaves of

Table 1 — Mosquito larvicidal activity of plant proteins

Plant name	Part used	Extract	Mosquito spp.	LC_{50} & LC_{90} / Mortality rate	Ref.
Manilkara zapota	seed coat	Crude protein	*A. aegypti*	4.68 mg/mL and 8.33 mg/mL	73
Caatinga biome	Seed	Chitin binding protein, lectin	*A. aegypti*	70 to 100%	31
Moringa oleifera	Seed	Crude extract and lectin, chitin binding protein, enzyme inhibitors	*A. aegypti*	51.65%	30
Carica papaya	Cotyledon and tegument	Crude protein and tegupain enzyme	*A. aegypti*	90%	57
Jatropha curcas Linn.	Seed coat	Jc- SCRIP protein	*Cx. Quinquefasciatus* and *A. aegypti*	100%	51
Myracrodruon Urundeuva	Leaves	MuLL protein Crude protein	*A. aegypti*	0.202 mg/mL 10.9 mg/mL	32
Solanum villosum	Leaves	Crude protein	*An. stephensi, Cx. quinquefasciatus* and *St. aegypti*	50 to 70%	74

N. benthamiana by agro infiltration. Plant expressed protein Pfs25-FhCMB were isolated from aerial tissues and characterize by SDS-PAGE and MS analysis, resulted that the disulfide pattern of arrangement is homogenous and complete. The mice is immunized by using 2- dose vaccination regimen in mice with Alhydrogel that showed the high level of transmission blocking antibodies (TBA) governed by ELISA and SMFA (Standard Membrane feeding Assay). SMFA data showed that the 95% reduction of oocyst formation at a dose of 1 µg of Pfs25-fhCMB[75].

Pfs230 protein existed on the surface of *Plasmodium falciparum* gametes. Pfs230 protein was transformed in *N. benthamiana* and the expressed protein Pfs230CMB was purified and characterized. The animal immunized with anti Pfs230CMB in presence of Freund's adjuvant generated antibodies resulted in 99% inhibition of formation of oocyst[76].

Mechanism of action

Plant proteins bind to insect glycoprotein which is found in gut membrane, effect glycosylation of insects and cause death of insect. They effect the insect's feeding behavior, survival and fecundity. The mechanism of insecticidal activity is still not completely clear[28]. The complete reasons for death of insects in various stages due to the action of plant proteins and their mechanism of action need to be investigated. Several studies have reported the proteolytic degradation in insect gut membrane[29], apoptosis[53], imbalance the function of digestive enzymes[62] Leading to inhibition in the food digestion, absorption, gut degradation, cell death and death of the organism. The mechanism of action is shown in Figure 2.

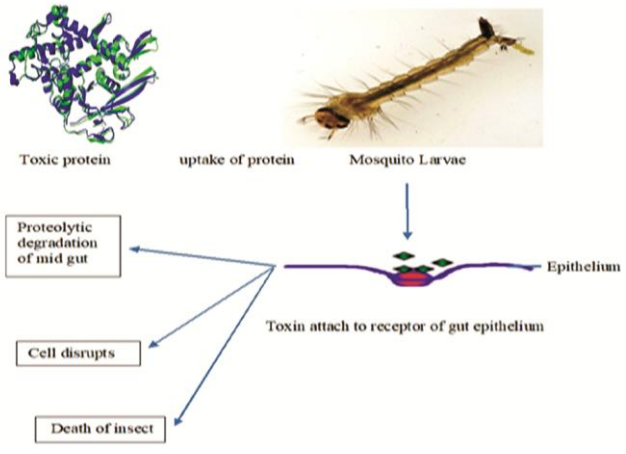

Fig. 2 — Mechanism of action towards anti-larvicidal activity of plant proteins

Discussion and Conclusion

Plant contains different toxic proteins which have larvicidal, ovicidal, oviposition stimulant activity against different order of insects. The main inhibitory mechanism is to bind the protein with gut of insect and block the various activities needed for their function. Overall, this review provided the recent information about the plant proteins which have been used as larvicides, ovicides specially mosquitoes. The crude extracts of seed coat proteins of *Manilkara zapota* and *Jatropha curcas Linn*. and seed proteins of Caatinga biome and *Moringa oleifera* showed significant larvicidal activity against *Aedes aegypti*. Larvicidal activity against Anopheline and Culicidae mosquitoes has also been observed in *Solanum villosum.* Although very less work has been done on plant proteins to check their potential as larvicide in spite of the fact that the plants are natural, safe, biodegradable and economically low-cost alternative for the existing dangerous insecticides or pesticides. Hence, the present review provides the basis and good scope for Pharmaceutics to develop new drugs and natural insecticide against mosquito because plants derived proteins are safe and advantageous as compared to synthetic products. These eco-friendly mosquitocidal tools should be promoted for vaccine development for complete eradication of mosquito borne diseases throughout the world. There is need of complete identification and characterization of the plant proteins along with their complete mode of action against larvae. Lastly, the botanical extracts could be employed as useful reservoir for development of bio-insecticides, drugs, mosquito repellent, transmission blocking vaccine and transgenic mosquito against different types of mosquito, which spread deadly diseases throughout the world.

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Conflict of Interest

There are no competing interests.

Authors' Contributions

MK collected the data and wrote original draft and NS review and edited the manuscript.

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