

Original Article

Mosquito Larvicidal Potential of *Gossypium hirsutum* (Bt cotton) Leaves Extracts against *Aedes aegypti* and *Anopheles stephensi* larvae

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Abstract

Background: We aimed to extract the ingredients from leaves of *Gossypium hirsutum* (Bt cotton) using different solvents and evaluate for potential use to control different larval stages of mosquito species, *Aedes aegypti* and *Anopheles stephensi*.

Methods: Qualitative and quantitative estimation of ingredients from *Go. hirsutum* (Bt) plant extract was carried out and their inhibitory action against mosquito larvae was determined using mosquito larvicidal assay.

Results: LC₅₀ values of water, ethanol, ethyl acetate and hexane extracts for *Ae. aegypti* were 211.73±21.49, 241.64±19.92, 358.07±32.43, 401.03±36.19 and 232.56±26.00, 298.54±21.78, 366.50±30.59, 387.19±31.82 for 4th instar of *An. stephensi*, respectively. The water extract displayed lowest LC₅₀ value followed by ethanol, ethyl acetate and hexane. Owing to the comparatively better activity of water extract, its efficacy was further evaluated for mosquito larvicidal activity, which exhibited LC₅₀ values of 133.95±12.79, 167.65±11.34 against 2nd and 3rd instars of *Ae. aegypti* and 145.48±11.76, 188.10±12.92 against 2nd and 3rd instars of *An. stephensi*, respectively. Crude protein from the water extract was precipitated using acetone and tested against 2nd, 3rd and 4th instars of *Ae. aegypti* and *An. stephensi*. It revealed further decrease in LC₅₀ values as 105.72±25.84, 138.23±23.18, 126.19±25.65, 134.04±04 and 137.88±17.59, 154.25±16.98 for 2nd, 3rd and 4th instars of *Ae. aegypti* and *An. stephensi*, respectively.

Conclusion: Leaves extracts of *Go. hirsutum* (Bt) is potential mosquito larvicide and can be used as a potent alternative to chemical insecticides in integrated pest management.

Keywords: Cotton, leaves extract, mosquito, toxicity, *A. aegypti*

Introduction

Mosquitoes are top most insect vectors related to human health (Chakkaravarthy et al. 2011). Different mosquito species like *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* are widely distributed in tropical and subtropical zones and acting as vectors for pathogen of diseases like malaria, dengue, filaria, Japanese encephalitis, yellow fever, chikungunya (Redwane et al. 2002). According to WHO report 2012, an estimated 3.3 billion people were at risk of malaria in 2011 around the globe (WHO

2012). A total of ninety-nine countries had on-going malaria transmission. There were about 219 million cases of malaria in 2010 and an estimated 660,000 deaths, majority of them were from Africa continent. Analogous to that about 50 million people in the world are infected with dengue. Malaria is an entirely preventable and treatable disease. Report also stated that in endemic countries in the year 2011, 278 million courses of artemisinin-based combination therapies (ACTs) were procured by the public and

private which were 182 million in 2010, and just 11 million in 2005. This indicates that these diseases are major cause of morbidity and mortality (WHO 2012).

Drug resistance to antimalarial drugs is a major concern for the global effort to control malaria. *P. falciparum* resistance to artemisinins has also been detected in about four countries, therefore vector control through the use of insecticide is one of the most important measures for control of malaria. Solution to control population of these mosquitoes involve the use of different organophosphate and carbamate insecticides like temephos, fenthion, malathion, distillate of crude oil, malaria larvicide oil (MLO) but their persistent use leads to development of resistance in species (Raghvendra 2002). In addition, chemical insecticides few examples such as methoprene, pyriproxyfen, diflubenzuron, DDT have several disadvantages like interference in food chain, toxicity to nontarget organism, soil and water contamination, long environmental persistence. Besides this, mosquito resistance to at least one insecticide used for malaria control has been identified in 64 countries around the world (WHO 2012). Therefore, use of these insecticides needs to be restricted and search for alternatives to traditionally used pesticides is urgently needed.

Use of larvicidal agents from natural sources has shown to reduce the harmful effects of chemical pesticides on environment (Cheng et al. 2009), therefore, mosquito larvicidal agents from plants and microorganisms can be considered as alternatives to chemical insecticides (Sharma et al. 2009, Tchicaya et al. 2009, Patil et al. 2011, Salunkhe et al. 2011). Besides this, botanical insecticides are easily available, cheap and are ecofriendly.

Researchers have screened and reported natural products for their mosquito larvicidal potential. Crude plant extracts attracted most attention as mosquito larvicidal agents. Aina

et al. (2009) have evaluated larvicidal potential of seed extracts from *Piper guineense* which gave 83% inhibition of second instar larvae of *An. gambiae*. *Euphorbia hirta* leaves extract in carbon tetrachloride, methanol and petroleum ether were tested and found effective on *An. stephensi* by Sharma et al. (2009). The leaves and root extract of *Cissampelos mucronata* and *Tephrosia villosa* from Tanzania coast region (Nondo et al. 2011) leaves of *Morinda citrifolia* (Kovendan et al. 2012), were found effective against mosquito larvae.

Other researchers isolated active principles from crude plant extract and analysed their insecticidal potential. The piperonaline, a piperidine alkaloid from *Piper longum* (Lee SE, 2000), Goniotalamin from *Bryonopsis laciniosa* (Kabir et al. 2003), methanol extract of *Acorus calamus* rhizome (Hidayatulfathi et al. 2004), mosquito larvicidal protein from *Solanum villosum* leaves (Chowdhury et al. 2008), plumbagin from *Plumbago zeylanica* (Plumbaginaceae) and saponins from *Cestrum nocturnum* (Solanaaceae) (Patil et al. 2011), hexane and petroleum ether extracts of *Citrus limletta* peels Kumar et al. (2012), Limonin and Nomilin from *Citrus* cultivars (Bilal et al. 2012), essential oil from plant *Melaleuca cajuputi* (Abu Baker et al. 2012), pectolinarigenin from chloroform extract of *Clerodendrum phlomidis* L. (Lamiaceae) (Muthu et al. 2012).

Gossypium hirsutum (Malvaceae: Malvales) is commonly known as upland cotton or Mexican cotton (Fig. 1). It is native from Central America and Mexico and an important fibre crop and most widely cultivated species. Worldwide around 35 mha land is under cotton crop. India alone constitutes 9.5 mha about one quarter of global area under this crop. The country reported a little over 21% of the global cotton production in 2008–2009 and stands second in cotton production having 4.9 million tons behind China having 7.8 million tons (Karihaloo and Kumar 2009). However, this production is

less as compared to land under cultivation. Low production is majorly attributed to attack by sap sucking pest *Helicoverpa armigera* commonly known as bollworm. Advancement in plant biotechnology with introduction of genetically modified (GM) variety of cotton known as 'Bt- cotton' by Mahyco seeds in collaboration with Monsanto has improved situation of *He. armigera* attack and production of cotton in India. The Bt cotton variety involves incorporation of Cry1Ac gene from common soil bacterium *Bacillus thuringiensis*, which gives protection against bollworm. Apart from prime source of fibre used in textiles cotton crop also reported to have some medicinal uses. Leaves of cotton plant show antibacterial activity against clinically important bacteria like *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella dysenteriae* (Omojasola and Awe 2004). Leaves of cotton in combination with other plant extracts are effective against malaria (Igwe et al. 2012) and good remedy for uterine fibroid and cancer (Hartwel 1971) and as antifertility agent (Randel et al. 1992).

In the present study, we have tried to extract the ingredients from leaves of *Go. hirsutum* (Bt cotton) using different solvents and evaluate for potential use to control different larval stages of mosquito species, *Ae. aegypti* and *An. stephensi*.

Materials and Methods

Collection of plant material

The healthy and fresh leaves of *Go. hirsutum* (Bollgaard II), a Bt cotton variety and non-Bt variety (Y1) were collected from local farms around the area of Jalgaon (210 00 24.5 N, 750 29 45.5 E, elevation 218m) district. Leaves were thoroughly washed 2–3 times with distilled water and shade dried for three months at 27±2 °C.

Extraction method

Hundred grams of shade dried leaves of three month old *Go. hirsutum* (Bollgaard II) and local non-Bt variety (Y1) were ground to make powder and were dissolved in 500ml each of different solvents (water, ethanol, ethyl acetate and hexane) in separate 1000ml capacity conical flasks and were kept at shaking condition for 48h. The mixture was then filtered through Whatman filter paper so as to remove the undissolved plant materials. These extracts were then concentrated using vacuum evaporator (Roteva, Equitron, India) and air dried. The dried materials were weighed; stock concentrations were prepared by dissolving in respective solvents and used for their evaluation as mosquito larvicidal agents. Acetone precipitation was done to obtain crude protein from the water extract of leaves, which was also inspected for its larvicidal potential.

Test organisms

For the laboratory trial, locally collected early second, third and fourth instar larvae of *Ae. aegypti* and *An. stephensi* were used as experimental mosquitoes. The larvae were kept in plastic enamel trays containing dechlorinated tap water. They were maintained as per previous report (Patil et al. 2011).

Mosquito larvicidal bioassay

Stock solution (1000ppm) was prepared by dissolving 100mg of crude semisolid extract from respective solvents in 100mL of distilled water. Different concentrations of these stocks were prepared by dilution in dechlorinated tap water making the final volume 100mL and were used as working stocks. The larvicidal activity was assessed by the procedure of WHO (1996) with some modifications and as per the previous method (Patil et al. 2012 a,b).

For bioassay test, 25 larvae of IInd, IIIrd and IVth instars of *Ae. aegypti* and *An. stephensi* were added in 249mL of water in

four different batches and 1.0mL of the desired extract concentration. The control was set up with dechlorinated tap water. The numbers of dead larvae were counted after 48 hours of exposure, and the percentage mortality was recorded for the average of four replicates. The experimental media, in which 100% mortality of larvae occurred, was selected for the dose-response bioassay (data not shown).

Dose response bioassay

Based on the preliminary screening results, crude extract of experimental plants were subjected to dose-response bioassay for larvicidal activity against the IInd, IIIrd and IVth instars larvae of *Ae. aegypti* and *An. stephensi*. Different concentrations ranging from 100 to 500ppm were prepared and number of dead larvae was counted after 24 hours of exposure. The percentage of mortality was reported from the average of three replicates. To evaluate further partially purified protein i.e. the acetone precipitate of water extract were dried and used to evaluate toxicity against the IInd, IIIrd and IVth instars larvae of *Ae. aegypti* and *An. stephensi*.

Phytochemical analysis

Extracts from all four solvents were subjected to qualitative phytochemical analysis which was carried out to detect the presence of different metabolites as per method of Kokate et al. (1999).

Qualitative and quantitative estimation of protein

Water extracts of Bt and non Bt cotton (*Go. hirsutum*) leaves were precipitated with acetone, dried and dissolved in phosphate buffer (pH 7.5) and used for qualitative and quantitative analysis of proteins by method of Lowry et al. (1951).

Statistical analysis

Mortality was calculated and corrected

using Abbott's formula (Abott 1925). The dose-response data were subjected to probit regression analysis (Finney 1971). The lethal concentrations in parts per million (LC₅₀, LC₉₀) and the 95% confidence intervals of LC₅₀ (upper confidence limit) and (lower confidence limit) were calculated.

Results

Differential extraction of 100 gm of dried leaves from *Go. hirsutum* (Bollgaurd II) yielded crude extracts of 0.570gm, 0.498gm, 0.287gm, 0.345gm with the solvents water, ethanol, ethyl acetate and hexane, respectively. All extracts showed the larvicidal efficacy within 48h of exposure. Mortality rate (Y) was found to be directly proportional to the concentration of dose (X) indicating that mortality rate increases with the increasing dose. Efficacy of leaves extracts from water, ethanol, ethyl acetate and hexane were evaluated against both the targets which showed LC₅₀ values 211.73±21.49, 241.6±19.92, 358.07±32.43, 401.03±36.19 against IVth instar larvae of *Ae. aegypti* and 232.56±26.00, 298.54±21.78, 366.50±30.59, 387.19±31.82 for IVth instar larvae of *An. stephensi*, respectively (Table 1).

With these values we can infer that water extract showed comparatively high potential than the other solvent extracts. Water extract was further evaluated for mosquito larvicidal activity, which showed LC₅₀ values of 133.95±12.79, 167.65±11.34 against IInd and IIIrd instar larvae of *Ae. Aegypti* and LC₅₀ values of 145.48±11.76, 188.10±12.92 against IInd and IIIrd instar larvae of *An. stephensi*, respectively (Table 2).

We further purified the active component from crude water extract by using acetone precipitation technique. Qualitative test of the precipitated material revealed protein as a major component. Dried acetone precipitate of water extract showed protein content

of 350 µg/gm from dry leaves. Acetone extracted protein material was further solubilised in phosphate buffer (50–500ppm) and screened for mosquito larvicidal activity on IInd, IIIrd and IVth instar larvae of *Ae. aegypti* and *An. stephensi*. Crude protein showed the larvicidal efficacy within 48h of exposure. The crude protein when subjected for evaluation against different larval stages showed lower LC₅₀ values compared to water and other solvent extracts. LC₅₀ for crude protein was 105.72±25.84, 126.19±25.65, 137.88±17.59 and 134.04±24.04, 138.55±23.18, 154.25±16.98 against IInd, IIIrd and IVth instars of *Ae. aegypti* and *An. stephensi*, respectively (Table 3).

Qualitative analysis as per Kokate et al. (1999) of different solvent extracts of Bt leaves showed presence of alkaloids, phenolic compounds, terpenoids, tannins, saponins, flavonoids and proteins (Table 4). Water extract showed strong positive test for all metabolites under study. Ethanol extract showed Y strong positive test for terpenoids and moderate positive for alkaloids, phenolic compounds, tannins, saponins, flavonoids and devoid of proteins. Ethyl acetate extract showed absence of phenolic compounds and proteins while hexane extract remarkably showed presence of only saponins (Table 4).

The leaves extracts of non-Bt cotton variety (Y1) in different solvents did not show any substantial effect on the mortality of both mosquito larvae. Only the hexane extract showed 30 and 40% mortality for the both the mosquito

species at 450 and 650ppm, respectively (Data not shown). The different solvent extracts of this variety also showed presence of alkaloids, saponins, phenol, tannins and proteins. Acetone precipitation for leaves water extract revealed protein content of 300µg/gm but did not show any larvicidal effect. Therefore, results of extracts of non-Bt cotton variety are not presented in tables.



Fig. 1. A) Bt cotton (*Gossypium hirsutum*) plant with flower and fruit, (B) Bt cotton plantation in field.

Table 1. Larvicidal activity of differential extracts from leaves of *Gossypium hirsutum* against IVth instar larvae of *Aedes aegypti* and *Anopheles stephensi*

Mosquito species	Leaves Extract(Btcot)	LC ₅₀ ±SE (mg lit ⁻¹)	95% Fiducial limits (LCL-UCL)	LC ₉₀ ±SE (mg lit ⁻¹)	95% Fiducial limits (LCL-UCL)	Regression equation
<i>Aedes aegypti</i>	Water	211.73±21.49	165.01-253.86	528.59±52.02	448.88-673.31	Y=1.93+0.0143 X
	Ethanol	241.64±19.92	201.07-282.76	536.98±48.96	460.90-669.48	Y=1.24+0.0154 X
	Ethyl acetate	358.07±32.43	303.74-	750.27±96.81	609.91-	Y=0.811+0.0117X

Table 1. Countinued...

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	<i>Hexane</i>	401.03±36.19	342.34-501.14	780.38±99.93	635.45-1086.77	Y=0.399+0.0114X
	<i>Water</i>	232.56±26.00	176.31-286.13	625.49±77.94	514.72-866.47	Y=2.18+0.0121 X
<i>Anopheles stephensi</i>	<i>Ethanol</i>	298.54±21.78	257.97-347.87	603.70±57.19	515.61-760.30	Y=0.561+0.0150X
	<i>Ethyl acetate</i>	366.50±30.59	315.32-446.32	722.72±85.06	597.08-974.12	Y=0.418+0.0125X
	<i>Hexane</i>	387.19±31.82	334.72-471.24	735.99±85.30	609.77-987.03	Y= 0.212+0.0123

Y mortality rate (significant at P< 0.05 level), X concentration (significant at P< 0.05 level), LC₅₀ lethal concentration that kills 50% of the exposed larvae, LC₉₀ lethal concentration that kills 90% of the exposed larvae, SE standard error (all values are mean of four replicates), LCL lower confidence limit, UCL upper confidence limit

Table 2. Larvicidal activity of water extracts from leaves of *Gossypium hirsutum* against IInd and IIIrd instar larvae of *Aedes aegypti* and *Anopheles stephensi*

Mosquito species	Larval stage (Instars)	LC ₅₀ ±SE (mg lit ⁻¹)	95% Fiducial limits (LCL-UCL)	LC ₉₀ ±SE (mg lit ⁻¹)	95% Fiducial limits (LCL-UCL)	Regression equation
<i>Aedes aegypti</i>	II	133.95±12.79	105.21-157.26	283.64±18.12	253.49-328.26	Y= 2.00+0.0222X
	III	167.65±11.34	143.50-189.24	312.32±18.07	282.11-356.18	Y=0.631+0.0252X
<i>Anopheles stephensi</i>	II	145.48±11.76	119.71-167.34	287.18±17.43	258.07-329.72	Y=1.45+0.0237X
	III	188.10 ±12.92	160.59-212.88	364.92±23.01	327.15-422.15	Y=0.524+0.0234X

Y mortality rate (significant at P< 0.05 level), X concentration (significant at P< 0.05 level), LC₅₀ lethal concentration that kills 50% of the exposed larvae, LC₉₀ lethal concentration that kills 90% of the exposed larvae, SE standard error (all values are mean of four replicates), LCL lower confidence limit, UCL upper confidence limit

Table 3. Larvicidal activity of crude protein from water extract of *Gossypium hirsutum* (BollgaurdII) against IInd, IIIrd and IVth instar larvae of *Aedes aegypti* and *Anopheles stephensi*

Mosquito species	Larval stage (Instars)	LC ₅₀ ±SE (mg lit ⁻¹)	95% Fiducial limits (LCL-UCL)	LC ₉₀ ±SE (mg lit ⁻¹)	95% Fiducial limits (LCL-UCL)	Regression equation
<i>Aedes aegypti</i>	II	105.72±25.84	35.50-147.30	273.28± 25.56	232.00-345.43	Y=4.53+0.0127X
	III	126.19±25.65	56.87-168.45	314.17±27.89	269.46-391.62	Y=3.73+0.0140X
	IV	137.88±17.59	96.13-169.37	350.23± 30.23	302.8-432.062	Y=2.29+0.0195X
<i>Anopheles stephensi</i>	II	138.23±23.18	78.18-177.71	317.83±27.09	274.38-392.16	Y=3.30+0.0150X
	III	134.04±04	70.56-174.23	315.33±27.05	95.71-389.51	Y=3.30+0.0150X
	IV	154.25± 16.98	115.178-185.6	371.42±32.6	320.54-460.49	Y=1.92+0.0197X

Mortality rate (significant at P< 0.05 level), X concentration (significant at P< 0.05 level), LC₅₀ lethal concentration that kills 50% of the exposed larvae, LC₉₀ lethal concentration that kills 90% of the exposed larvae, SE standard error (all values are mean of four replicates), LCL lower confidence limit, UCL upper confidence limit

Table 4. Qualitative analysis of phytochemicals present in differential solvent extracts from leaves of *Bt*-cotton (*Gossypium hirsutum*)

Phytochemical constituent	Water extract	Ethanol extract	Ethyl acetate extract	Hexane extract
Alkaloids	+++	++	++	-
Phenolic compounds	+++	++	-	-
Terpenoids	+++	+++	++	-
Tannins	+++	++	+	-
Saponins	+++	++	+++	++
Flavonoid	++++	++	++	-
Protein	+++	-	-	-

+++ Strong positive, ++ Moderate positive, + Less, -Not detected

Discussion

The phytochemical constituents of plant extracts represent a large source of potentially bioactive molecules. *Go. hirsutum* (Bt) is a genetically modified variety, which has ability to express a protein toxin in its tissues. The differences in the levels of toxicity in each extract depends on the insecticidal ingredients of plant extracts, solubility in different solvents which can vary significantly depending on plant species, plant part, age of plant part, solvent used for extraction, seasonal variation and target insect species.

Among the evaluated different solvent extracts of leaves from Bt and non-Bt *Go. hirsutum*, the water extract of non-Bt cotton (Y1var) leaves did not show any mortality while water extract of Bt cotton was found to be most effective (LC₅₀ 211.73 and 232.56ppm) against IVth instar larvae of *Ae. Aegypt* and *A. Stephensi* respectively. Water extract and precipitated protein from water extract were further studied for their potential against various instars of *Ae. aegypti* (LC₅₀ 137.88 ppm) and *An. stephensi*. (LC₅₀ 154.25ppm). This manifestation might be due to the high water solubility of effective ingredients than in other solvents. In addition, it was found that protein obtained from water extract was more effective than water extract itself suggesting the major role of protein component in

larvicidal action. The water extract of non-Bt cotton leaves also precipitated by acetone but it did not show any significant larvicidal activity.

In the present study, LC₅₀ values of 211.73 ±21.49, 241.64±19.92, 358.07±32.43, 401.03 ±36.19 and 232.56±26.00, 298.54±21.78, 366.50±30.59, 387.19±31.82 for water, ethanol, ethyl acetate and hexane extracts of *Bt* cotton (*Go. Hirsutum*) against the IVth instar mosquito larvae of *Ae. aegypti* and *An. stephensi*, respectively. The lowest LC₅₀ value was found for water extract displayed compared to ethanol, ethyl acetate and hexane. Water extracts showed LC₅₀ values of 133.95 ±12.79, 167.65±11.34 against 2nd and 3rd instars of *Ae. aegypti* and 145.48±11.76, 188.10 ±12.92 against 2nd and 3rd instars of *An. stephensi*, respectively. Further lower LC₅₀ values of 105.72±25.84, 138.23±23.18, 126.19 ±25.65, 134.04±04 and 137.88±17.59, 154.25 ±16.98 were found for acetone precipitated crude protein against 2nd, 3rd and 4th instars of *Ae. aegypti* and *An. stephensi*, respectively.

Our results of mosquito larvicidal capacities of Bt cotton leaves extract are comparable to the investigations by other researchers on plant extracts. Efficiency of leaf chloroform extract of *Nyctanthes arbortristis* have been reported with LC₅₀ value of 526.3,

780.6ppm (24 hours) and 303.2, 518.2 (48h) for *Ae. aegypti* and *An. stephensi* (Mathew et al. 2009). Valencia late (*Citrus sinensis*) was the best in terms of LC₅₀ (297ppm), % mortality (97%) and LT₅₀ (18.49h) then freutrall early (*Citrus reticulata*) with LC₅₀ (377.4 ppm), % mortality (88%) and LT₅₀ (31 hours), while nomilin gave lowest LC₅₀ (121.04 ppm) than limonin (382.22ppm) after 72h of exposure against *Aedes albopictus* larvae (Bilal et al. 2012). Kovendan et al. (2012) reported *Morinda citrifolia* leaf extract in hexane, chloroform, acetone, methanol, and water had values of LC₅₀ 345.10, 324.26, 299.97, 261.96, and 284.59ppm and 361.75, 343.22, 315.40, 277.92, and 306.98ppm for third instar larvae of *An. stephensi* and *Ae. aegypti* respectively.

Isolated proteins have more potential larvicidal activity as compared to crude extracts. In the previous reports, the proteins isolated from mature leaves of *Solanum villosum* were found to have larvicidal activity against IIIrd instar larvae of *An. stephensi*, *Cu. quinquefasciatus* and *Stegomyia aegypti* mosquitoes and molecular weights of the protein bands ranged between 69–109 kDa (Chowdhury et al. 2008). The larvicidal activities of the essential oils separated from *H. persicum*, *F. vulgare* and *C. sativum* plants against *An. stephensi* revealed that the lethal dosage (LC₅₀) ranged between 20.10 and 120ppm (Sedaghat et al. 2011). In case of *M. cajuputi* 5 and 10% essential oil aerosol spray shows mortality of 20.00±2.85, 48.05±0.37 for *Ae. aegypti* and 22.90±4.22, 44.20±2.10 *Ae. Albopictus* (Abu Baker et al. 2012).

The phytochemical studies indicate presence of alkaloids, phenolic compounds, terpenoids, tannins, saponins, flavonoids and protein. Botanical pesticides have different mode of action like feeding and oviposition deterrence (Su and Mulla 1999). Phytochemicals have targeted action on midgut epithelial cells of mosquito (Cohen et al. 2012). Morya et al. (2007), stated that, the crude extract were

more effective than individual active compound because of natural synergism that discourages the emergence of resistance in mosquito vectors. The crude extract can be obtained by simple procedure than purification of active compounds and are more effective against insect and less toxic to aquatic organism (Prakash and Rao 1997).

Use of plant products may be considered as one of the best policy in integrated pest management (IPM) for control of mosquito larvae. Fibres of cotton are used in textile mills but other parts of cotton crop like leaves, roots are mostly used conventionally for cooking and burning purpose in India. Owing to such huge plantation of Bt cotton, there is need to utilize the agro-waste into useful products. Bt cotton leaves possess qualities of ideal bioinsecticide having ecofriendly, biodegradable nature, as it is less harmful to aquatic species than chemical insecticides and have low production cost, easy availability, easy utilization, and simple storage. This can even create employment opportunities to local population and can prove to be helpful to reduce the use of costly insecticides. Though agricultural waste is abundant but it is remained unutilized, so greater attention is needed to be paid towards converting it into useful products (Nand 1998).

Conclusion

Control of various mosquito species at various developmental larval stages is a useful measure to mitigate transmission of pathogens by mosquito vectors. In conclusion present study confirms that leaves extracts of *Go. hirsutum* (Bt) have potential mosquito larvicidal activity. Phytochemical studies revealed presence of proteins that play major part in killing larval stages. This may give an additional insight for the use of genetically modified agricultural waste to control the development of mosquitoes and eventually to

control spread of different life threatening diseases. Bt Cotton has added advantage of being largely cultivated worldwide for the production of fibre. The present study throws light on the additional advantage of cotton plant leaves for well-being of humans. As per our knowledge this is the first report on use of cotton (Bt) leaves as mosquito larvicidal agents. Further studies on extraction, characterization, size estimation of bioactive compounds in leaves and determination of mode of action of protein individually and synergistic performance of the ingredients from the extracts will be needed for recommendation as bioinsecticide in future.

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