Assessment of *Achyranthes aspera* induced toxicity and molecular analysis of RAPD-PCR profiles of larval genomic DNA of *Aedes aegypti* L. (Diptera: Culicidae)

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Abstract Current studies investigated the anti-mosquito potential of *Achyranthes aspera* against the dengue vector, *Aedes aegypti*. The stems and leaves of *A. aspera* were extracted in hexane and evaluated for their toxicity against early fourth instars of *A. aegypti*. The larvicidal efficacy of the extract was validated as per WHO protocol. The mortality counts were made after 24 h and LC values were calculated at different levels. The adverse impact of extracts was also explored on the larval genomic DNA. The larvae were exposed to extracts at LC₅₀ levels and the alterations in g-DNA was evaluated through RAPD-PCR technique using three random primers; MA-09, MA-12 and MA-26. Our investigations ascertained the larvicidal efficacy of both the leaf and stem extracts of *A. aspera* resulting in respective LC₅₀ values of 0.068 and 0.082 mg/mL. The extracts also caused variable genotoxic effects with significant changes in the RAPD profiles. The results showed appreciable modifications in larval g-DNA with loss of certain bands and gain of unique bands with 82.35% DNA polymorphism. These alterations suggest the probable DNA damage and mutations in the larval g-DNA caused by certain phytochemicals which could be the possible reason of larval mortality. Our studies evidenced

the anti-mosquito potential of *A. aspera* extracts against *A. aegypti* causing appreciable larval mortality and significant changes in g-DNA. The *A. aspera* extracts are suggested as efficient and eco-friendly control agent against *A. aegypti*, yet further investigations are needed to identify the bioactive constituent and ascertain its effectiveness in the field conditions.

Keywords *Achyranthes aspera* · *Aedes aegypti* · Larvicidal · RAPD · PCR · g-DNA

Introduction

Aedes aegypti, the chief vector of Dengue and Chikungunya Virus in India and Zika virus in South America, Central America and the Caribbean, has engrossed extensive research interest at global level because of the massive rise in these diseases. Since last decade, dengue has emerged as a prime health concern in tropical and sub-tropical expanse of the world resulting in increased mortality every year. Since past five decades, an alarming rise of 30-fold has been observed in the occurrence of dengue at the global level with roughly 50% of the world's population estimated to be at the threat of dengue fever (WHO 2016). It is significant to note that despite of global spread, almost three-fourth of the world population experiencing dengue resides in the Asia-Pacific region. In India, Ministry of Family and Health Welfare has reported 1, 11, 880 cases of dengue and 227 fatalities in 2016; with highest figures of 17,702 cases and 34 deaths reported in West Bengal. Apart from this, a total of 58,136 cases of Chikungunya have also been reported in 2016 which is shockingly highest in figure since last 6 years and almost double than those reported in previous year (NVBDCP 2016).

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Several measures have been attempted to control dengue vector. The prime methodology adopted for their control and prevention of diseases is based on the disruption of cycle of disease-transmission utilizing chemical insecticides. Nonetheless, recurring and unmethodical utilization and application of toxic mosquitocides in the environment has been reported to cause variable resistance to insecticides in mosquitoes and toxicity to the non-target organisms. Along with, non-degradability of insecticides has led to a great harm to the environment and caused their entry in the food chain (Kumar et al. 2002, 2004; Borase et al. 2013).

Taking all these undesirable impacts of insecticides in consideration, researchers have transformed their interest towards botanicals and started exploring phytochemical constituents for mosquito management. The possible use of plant products against mosquito control has been explored by many researchers (Mehlhorn et al. 2005; Amer and Mehlhorn 2006a, b, c; Elango et al. 2009; Ghosh et al. 2012; Warikoo and Kumar 2014; Sharma et al. 2016). Shaalan et al. (2005) had comprehensively reviewed various larvicidal plant species, processes of their extraction, bioactive constituents inhibiting larval growth and reproduction, ovicidal effects of extracts, and synergistic and antagonistic impacts of mixtures. The possible effects of phytochemicals on non-target organisms, the resistance and screening methodologies and promising advances made in their research have also been discussed. Several reports available in the literature reveal the efficacy of different plant parts as larvicides, oviposition deterrents and ovicides against mosquitoes without posing toxicity hazards to humans (Kumar et al. 2012a, b; Warikoo and Kumar 2013; Warikoo and Kumar 2014). In fact, plant-derived constituents and their metabolites are often reported to be effective at very low concentrations against young instars of *Aedes*, *Ochlerotatus*, *Anopheles* and *Culex*. Consequently, researchers have advocated many plant extracts as innocuous substitutes to the synthetic pesticides resulting in the formulation of an ecologically sound and environmentally safe mosquito vector control program (Warikoo and Kumar 2013). Botanicals are also known to be utilised for the rapid synthesis of nanoformulations, mosquitocidal formulations and repellents. Currently, mosquito control studies are based on behavioural aspects, and different kill approaches such as sound traps and manipulation of swarming behaviour (i.e. lure and kill approach) are being discussed and formulated (Benelli and Mehlhorn 2016).

Achyranthes aspera L. (Fam., Amaranthaceae), commonly called devil's horsewhip, is a widespread local weed in India. The seeds and leaves of *A. aspera* are consumed by human beings and are also a part of Indian religious ceremonies (Ragupathy and Newmaster 2009). A few reports uncover the prothyroidic and anti-peroxidative

effects of *Achyranthes* leaf extracts in rats (Tahiliani and Kar 2000). In contrast, the leaf and stem extracts of *A. aspera* have caused larvicidal toxicity in *A. aegypti* besides behavioural and morphological modifications (Sharma et al. 2015). Bagavan et al. (2008) observed larvicidal efficacy of the extracts prepared from its leaves, flowers and seeds against the IV instars of *A. subpictus* Grassi and *C. tritaeniorhynchus* Giles. They also isolated saponins from the ethyl acetate extract of *A. aspera* leaves and found them as potential larvicides against *A. aegypti* and *C. quinquefasciatus*.

The toxins present in environment probably cause cidal effects and other changes in the organisms due to the adverse effects on the genome of the organisms causing DNA damage. The genotoxic effects of toxins may be in the form of genetic rearrangements, mutation and several other genomic alterations. Researchers have explored the random amplified polymorphic DNA (RAPD) assay as the genetic marker for uncovering the DNA alterations in organisms after the exposure to genotoxins (Savva 2000; Lalrotluanga et al. 2011). The RAPD-PCR technique is considered one of the most consistent, reliable, sensitive, cost-effective and uncomplicated technique (Atienzar et al. 2001). It can be utilized against any organism for detecting DNA damage and does not require prerequisite knowledge of DNA sequence for the targeted gene as the amplification stops at the site of the damage (Lalrotluanga et al. 2011). Possibly, the prime reason for the success of RAPD-PCR method is the gain of a large number of genetic markers that require small amounts of DNA without the need of cloning, sequencing or any other form of the molecular characterisation of the genome of the species (Kumar and Subramanian 2011). The RAPD method has been also used to detect genetic instability in tumors and genomic DNA alterations induced by DNA damaging agents other than plant extracts, such as copper (Atienzar et al. 2001), benzopyrene, heavy metals, UV radiation (Lalrotluanga et al. 2011) and X-ray (Kuroda et al. 1999).

Till date, however, the effect of phytochemicals on the genomic DNA of mosquito larvae has not been studied much, though RAPD-PCR technique has been utilized to assess DNA damage in insects induced by various plant extracts. Lalrotluanga et al. (2011) have reported modifications in RAPD profiles of *C. quinquefasciatus* larvae exposed to extracts prepared from *Curcuma longa* and *Melia azedarach*. They showed variations in the band intensity and gain or loss of bands suggesting the probable cause of variation as DNA damage and mutations or structural rearrangements caused by genotoxins, which may have affected the primer sites and/or interpriming distances. Likewise, essential oil of *Prangos ferulacea* has been reported to induce DNA changes in *Ephestia kuehniella* Zeller larvae (Ercan 2015).

Keeping all the above reports and investigations in view, the present work was carried out to explore the larvicidal potential of hexane extracts of leaf and stem of *A. aspera* and the impact of these extracts on the genomic DNA of early fourth instars of *A. aegypti* by RAPD-PCR technique. The investigations were based on SDS-based DNA extraction protocol which has been though reported time-consuming, but is considered cost-effective providing purity level yielding good quality of DNA as compared to use of DNA extraction kits (Gupta and Preet 2012). The results of the present investigations might be beneficial in mosquito management program aiming at the formulation of new eco-friendly substitute for chemical insecticides utilizing beneficial properties of this undesirable weed.

Materials and methods

Preparation of plant extracts

Fresh, young and mature plants of *A. aspera* were randomly harvested from surrounding areas of New Delhi, India. Healthy and disease-free stems and leaves were separated and air-dried under shade. Plant extracts were prepared by following maceration method. The dried parts were grounded into fine powder using electric blender, sieved and kept in sealed container. The powdered leaves and stems were extracted by soaking 50 g each separately in 200 mL of hexane. After a week, the extracts, thus obtained, were concentrated under low pressure using a vacuum evaporator (Buchi Type) at a temperature below its boiling point 60 °C. The final concentrated extracts were stored at low temperature of 4 °C for assays.

Rearing of *A. aegypti*

The colony of dengue and Chikungunya vector *A. aegypti* was maintained in an insectary under controlled conditions of $80 \pm 5\%$ RH, 28 ± 1 °C temperature and 14/10: L/D photoperiod as per the protocol described by Sharma et al. (2015).

Larvicidal bioassays

The larvicidal bioassay was conducted on the early fourth instar larvae of *A. aegypti* at 28 ± 1 °C (Sharma et al. 2015) in accordance with the procedure described by WHO with slight modifications (WHO 2005). The graded series of the extracts was prepared using ethanol as the solvent. Sets of 20 early fourth instar larvae of *A. aegypti* were taken in enamel bowls containing 99 mL of distilled water. These were transferred to glass jar containing 100 mL of distilled water and 1 mL of a particular concentration of *A.*

aspera extract. Three replicates were conducted concurrently for each test concentration. Controls were exposed to the ethanol alone.

The data was subjected to regression analysis using computerized SPSS 19.0 Program. The LC₃₀, LC₅₀ and LC₉₀ with 95% fiducial limits, Chi square and regression coefficient were computed.

Genomic DNA assays

The early fourth instar larvae of *A. aegypti* were exposed to LC₅₀ dosage of hexane leaf and stem extract of *A. aspera*, separately for a period of 24 h. The exposed larvae were collected and preserved in ethanol to explore the probable alterations in genomic DNA caused by phyto-components.

Extraction of larval genomic DNA

The genomic DNA (g-DNA) of early fourth instars of *A. aegypti* was extracted with slight alterations in the procedure proposed by Ballinger-Crabtree et al. (1992). Three replicates were conducted simultaneously. Each assay had ethanol-preserved larvae, in batches of five, which were completely homogenized in 200 µL of lysis buffer (1 M Tris HCl, pH: 8.0, 10% Sodium dodecyl sulphate, 5 M NaCl and 0.5 M EDTA). The homogenized mixture was incubated with 5 µL of proteinase K (20 mg/mL) at 55 °C for 16 h followed by centrifugation at 8000 rpm for 10 min. The supernatant was collected and added with an equal volume of phenol–chloroform (24:25). This mixture was again centrifuged at 8000 rpm for 10 min. The procedure was repeated twice after which DNA was extracted by adding 0.2 volume of 5 M NaCl and 2.0 volumes of ethanol at room temperature. The final mixture was incubated overnight at (–)20 °C and spun at 12,000 rpm for 10 min to obtain pellet which was re-suspended in 50 µL of sterilized distilled water and stored at 4 °C for RAPD-PCR assay.

DNA purification

The g-DNA was purified using Zymoclean DNA Extraction kit. Three volumes of Agarose Dissolving Buffer (ADB) were added to 3 volumes of each g-DNA sample which were then transferred in their respective columns followed by centrifugation at 12,000 rpm for 1 min. A volume of 500 µL wash buffer was added to these columns and centrifuged again at 12,000 rpm for 1 min. The left over flow-through was discarded and remaining ethanol was eluted from the sample by centrifugation of the columns at the same speed for 2 min. An amount of 20 µL of elution buffer was added and kept for 2–5 min followed by centrifugation at 12,000 rpm for 1 min.

Table 1 Larvicidal activity of the leaf and stem hexane extracts of *Achyranthes aspera* against early fourth instars of *Aedes aegypti*

Part of the plant	Larvicidal activity			SE	χ^2 (df)	Regression coefficient
	LC ₃₀ (mg/mL)	LC ₅₀ (mg/mL)	LC ₉₀ (mg/mL)			
Leaves	0.066 (0.056–0.074)	0.082 (0.073–0.091)	0.139 (0.120–0.177)	0.851	6.738 (6)	5.600
Stems	0.054 (0.021–0.070)	0.068 (0.042–0.092)	0.115 (0.086–0.440)	0.901	15.910 (5)	5.630

No mortality was observed in the control

LC₃₀, 50, 90– lethal concentration that kills 30, 50 and 90% of the exposed larvae

Values in parentheses indicate the lower and upper 95% fiducial limits

SE standard error, χ^2 Chi square, df degree of freedom, RC regression coefficient

Test samples were transformed into log covariant ($\log_{10}$)

$p < 0.05$, level of significance

Rapid amplified polymorphic DNA (RAPD)-PCR

RAPD amplification was performed with 1 μ L of extracted DNA added to 15 μ L PCR mix (1X PCR buffer –1.5 μ L, 1 mM MgCl₂ –0.6 μ L, 0.2 mM dNTP –0.3 μ L, 0.533 μ L/mL BSA –0.8 μ L, Primers –0.3 μ L, 1.5 unit of Taq polymerase –0.3 μ L and sterile deionized water to fill up till the final volume). Three primers; MA-09, MA-12 and MA-26 were randomly selected for RAPD analysis in accordance with the protocol of Lalrothuanga et al. (2011). The cocktail components were thoroughly mixed by a short spin followed by loading onto a thermal cycler. The PCR assay involved an initial denaturation step at 94 °C for 4 min followed by 45 cycles at 94 °C for 1 min for DNA denaturation. The annealing of DNA was carried out at 32 °C/s for primer MA-09 and MA-26 and 34 °C/s for primer MA-12 (Table 1) followed by extension at 72 °C for 2 min and final extension at 72 °C for 10 min.

Agarose gel electrophoresis

The amplified g-DNA and 1 kb DNA ladder as standard marker were subjected to electrophoresis in 1.5% agarose gel in (1X) TAE buffer. Molecular sizes of the marker used were 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp. The gel was run for 30 min at 150 V and stained with ethidium bromide (final concentration-0.5 μ g/mL). The bands were visualized on an ultraviolet trans-illuminator (Cell Biosciences AlphaImager Gel Documentation System), analysed using software Alpha View (Version: 3.2.2.0) Cell Biosciences, Inc. and photographed.

Results

The results of larvicidal bioassay performed with the hexane leaf and stem extracts of *A. aspera* against early fourth instars of *A. aegypti* are presented in Table 2. Our studies showed the larvicidal efficacy of both the extracts

Table 2 Random primers used for RAPD analysis in *Aedes aegypti* early fourth instars exposed to *Achyranthes aspera* leaf and stem hexane extracts at LC₅₀ values

Primer	Sequence (5'–3')	Annealing Tm °C/s
Primer MA-09	GACGGATCAG	32
Primer MA-12	ACCGCGAAGG	34
Primer MA-26	GACGTGGTGA	32

exhibiting LC₅₀ values below 0.1 mg/mL. The 24 h larvicidal bioassay with hexane stem extract of *A. aspera* resulted in LC₅₀ value of 0.068 mg/mL against early fourth instars of *A. aegypti* (Table 2), while larval exposure to hexane leaf extracts revealed 1.21-fold higher value with LC₅₀ value of 0.082 mg/mL, the hexane stem extract thus found to be 0.82-fold more toxic than the leaf extract.

The investigations on the impact of *A. aspera* extracts on the g-DNA of early fourth instars of *A. aegypti* exposed to LC₅₀ values for 24 h revealed varying DNA modifications when compared to the control set. The alterations in RAPD profiles of exposed larvae were observed in the form of variations in intensity of bands and gain or loss of bands.

The g-DNA samples isolated from the control larvae of *A. aegypti* and amplified with MA-09, MA-12 and MA-26 showed the presence of a total of 06, 06 and 05 bands, respectively (Table 3; Fig. 1) with corresponding molecular size ranging from 357 bp to 1.64 kb, 184 bp to 1.20 kb and 259 bp to 1.07 kb (Table 4). In contrast, the g-DNA isolated from the larvae exposed to hexane leaf extract of *A. aspera* and PCR-amplified resulted in only 4 bands in amplification with MA-09 and caused in disappearance of 2 bands of 500 bp and 1.30 kb, while that with MA-12 caused disappearance of 1 band of 304 bp and reappearance of a unique band of 1.611 kb. On the other hand, amplification with MA-26 resulted in reappearance of a unique band of 491 bp molecular weight and a smeared region ranging from 700 to 902 bp.

When g-DNA extracted from the larvae exposed to the hexane stem extract of *A. aspera* was analyzed with

Table 3 Total number of bands obtained in RAPD-PCR analysis of *g*-DNA samples of *Aedes aegypti* larvae exposed to the leaf and stem extracts of *Achyranthes aspera* at LC₅₀ values for 24 h

Primer	<i>Achyranthes aspera</i> Hexane Extract	LC ₅₀ (mg/mL)	No. of bands
MA-09	Control	–	06
	Leaf extract	0.082	04
	Stem extract	0.068	05
MA-12	Control	–	06
	Leaf extract	0.082	06
	Stem extract	0.068	05
MA-26	Control	–	05
	Leaf extract	0.082	04
	Stem extract	0.068	02

RAPD-PCR, the disappearance of only one band of 1.64 kb molecular weight was observed on amplification with MA-09. Amplification with MA-12 resulted in disappearance of one band of approximately 650 bp molecular weight. On the other hand, amplification with MA-26 strikingly showed disappearance of 04 bands ranging from 517 bp to 1.07 kb and reappearance of one unique band of 473 bp in the exposed larvae (Fig. 1; Table 4).

The results revealed that amplification with MA-26 showed maximum reduction in the number of *g*-DNA

bands of dengue vector larvae exposed to *A. aspera* stem extracts (Table 5). The RAPD-PCR assay of larval *g*-DNA of *A. aegypti* amplified with three primers resulted in a total of 17 bands in control larvae. Nevertheless, larval exposure to *A. aspera* extracts caused appearance of a total of 03 and disappearance of 11 bands. Thus, the polymorphism value due to loss and/or gain of bands in the *g*-DNA profile of control larvae as compared to that of larvae exposed to extracts was found to be 82.35% under the influence of treatment with three random primers (MA-09, MA-12 and MA-26) (Table 5).

Discussion

Extensive use of synthetic chemical insecticides has resulted in environmental degradation, hazards and development of resistance to these insecticides in mosquito vectors. Since past few years, scientists have stressed upon the formulation of a more potent and environment-friendly insecticide and diverted their interest towards the use of plant extracts for mosquito management. A large number of plant species have been identified which possess chemical factors and metabolites of importance in pest control programmes. Several plant extracts have been reported to possess varied activities against different species of mosquitoes (Kumar et al. 2012a).

Fig. 1 RAPD-PCR profiles of DNA isolated from control/untreated early fourth instars of *Aedes aegypti* and from larvae treated with hexane leaf and stem extracts of *Achyranthes aspera* using three random primers MA-09, MA-12 and MA-26, M-Maker DNA of 1 kb. 1. DNA isolated from untreated larvae and amplified with MA-09. 2. DNA isolated from larvae exposed to *A. aspera* leaf extract and amplified with MA-09. 3. DNA isolated from larvae exposed to *A. aspera* stem extract and amplified with MA-09. 4. DNA isolated from

untreated larvae and amplified with MA-12. 5. DNA isolated from larvae exposed to *A. aspera* leaf extract and amplified with MA-12. 6. DNA isolated from larvae exposed to *A. aspera* stem extract and amplified with MA-12. 7. DNA isolated from untreated larvae and amplified with MA-26. 8. DNA isolated from larvae exposed to *A. aspera* leaf extract and amplified with MA-26. 9. DNA isolated from larvae exposed to *A. aspera* stem extract and amplified with MA-26

Table 4 Alterations in RAPD-PCR profiles of *Aedes aegypti* early fourth instars exposed to leaf and stem extract of *Achyranthes aspera* at LC₅₀ values for 24 h

S. no.	MA-09			MA-12			MA-26		
	Control	Leaf	Stem	Control	Leaf	Stem	Control	Leaf	Stem
1	1648	1833	–	–	–	–	–	–	–
2	1303	–	1394	–	1611	–	–	–	–
3	1045	1015	1091	1201	1227	1000	1076	1045	–
4	–	–	–	826	815	761	902	SMEAR	–
5	717	700	837	650	642	–	700	–	–
6	500	–	550	482	473	482	517	692	–
7	357	375	375	304	–	312	–	491	473
8	–	–	–	–	–	–	259	241	227
9	–	–	–	184	194	194	–	–	–

Figures indicate the molecular weight of the DNA bands (bp) in each lane

Current investigations were carried out on the larvae exposed to stem and leaves of *A. aspera* extracted with hexane. Our studies have ascertained the efficacy of both the stem and leaf extracts against early fourth instar larvae of *A. aegypti* resulting in respective LC₅₀ values of 0.068 and 0.082 mg/mL. Bhattacharya and Chandra (2013) had reported comparable LC₅₀ values, ranging from 35.46 to 63.39 ppm against different instars of *C. vishnui* when exposed to acetone leaf extracts of *A. aspera*. Larvicidal studies conducted by Warikoo and Kumar (2013) with hexane root extracts of *Argemone mexicana* against early fourth instars of *A. aegypti* resulted in respective LC₅₀ and LC₉₀ values of 91.331 and 156.684 ppm. They also

reported that hexane and petroleum ether extracts of *A. mexicana* were much more efficient as compared to the benzene, acetone and ethanol extracts. Leaves of another common weed, *Parthenium hysterophorus*, when extracted in hexane and petroleum ether extracts, have also been found effective against *A. aegypti* larvae leading to 100% mortality (Kumar et al. 2012b). Assays with crude leaf extract of *Leucas aspera* against *A. aegypti* and *C. quinquefasciatus* revealed highest larvicidal potency of the hexane extracts when compared to the chloroform and ethanol extracts (Maheswaran et al. 2008).

It is well known that various environmental pollutants and chemical contaminants can cause genotoxic effects in the organisms leading to DNA alterations. These alterations can be detected by simple but much sensitive, effectual, economical and reasonably effortless RAPD-PCR assays (Ercan 2015). It has been reported that SDS-based DNA extraction yields 1.4-times higher DNA as compared to other methods (Ballinger-Crabtree et al. 1992). Gupta and Preet (2012) recommended RAPD analysis an appropriate method for identification, genetic diversity evaluation and detection of DNA damage in different mosquito species; *A. aegypti*, *A. stephensi* and *C. quinquefasciatus*. Earlier, Atienzar and Jha (2004) had measured benzo(a)pyrene [B(a)P]-induced DNA changes in the water fleas, *Daphnia magna* to assess the potential of RAPD assay. They concluded that the RAPD method is a useful technique to qualitatively assess the kinetics of DNA changes, repair and trans-generational effects in the insects. Reports reveal that changes in genomic DNA of mosquito larvae may inhibit the chitin synthesis, resulting in malformations leading to the larval mortality (Abd-Alla et al. 2003).

Our investigations showed that 24 h exposure of the early fourth instars of *A. aegypti* to leaf and stem extracts of *A. aspera* at their LC₅₀ levels caused changes in RAPD

Table 5 RAPD profile variance in the number of bands obtained with three primers in *Aedes aegypti* early fourth instars exposed to leaf and stem extract of *Achyranthes aspera* at LC₅₀ values for 24 h

Primer	Concentration (mg/mL)	No. of bands	a*	b#
MA-09				
Control	–	06	–	–
Leaf extract	0.082	–	00	02
Stem extract	0.068	–	00	01
MA-12				
Control	–	06	–	–
Leaf extract	0.082	–	01	01
Stem extract	0.068	–	00	01
MA-26				
Control	–	05	–	–
Leaf extract	0.082	–	01	02
Stem extract	0.068	–	01	04
Total		17	a + b = 14**	

a* Appearance of extra bands

b# Disappearance of normal bands

a + b** Polymorphic bands

profiles with alterations in band intensity and in gain or loss of bands. Modified RAPD profile of extract-treated larvae with altered intensity of DNA bands, appearance of unique bands and loss of certain bands suggest probable DNA damage, mutations or structural rearrangements induced by genotoxins (Atienzar et al. 2002). Similar RAPD assay studies with random primers (MA-09, MA-12 and MA-26) conducted by Lalrotluanga et al. (2011) analyzed randomly amplified RAPD profile of genomic DNA of larvae of *C. quinquefasciatus* and reported the number of bands were increased when exposed to acetone extract of *M. azedarach* whereas the number of bands were decreased on exposure to *C. longa* extract. Changes in the RAPD profile of *E. kuehniella* DNA has been observed after treatment with *P. ferulacea* essential oil (Ercan 2015). Variances in *g*-DNA have been defined by losses and/or gains of bands and alterations in band intensity.

It is suggested that the *A. aegypti* larval exposure to the extracts of *A. aspera* could have caused DNA damage because of formation of adducts, breaks, point mutations, genomic rearrangements, insertions, deletions etc. which could have influenced primer sites. Appearance of unique bands in the RAPD profile of the *g*-DNA isolated from the extract-treated larvae of *A. aegypti* in contrast to the bands of control larvae implies the possible direct co-correlation of *g*-DNA to the greater accessibility of the marker primers to certain specific sites. This signifies that treatment with *A. aspera* extracts might have caused certain structural changes in the genomic DNA of *A. aegypti* larvae. Similar inference can be deduced from the disappearance of certain amplified PCR products from the genomic DNA of the extract-treated larvae. However, fading or loss of bands may be attributed to the probable formation of DNA lesions that could have induced structural changes and reduced polymerization in DNA, and/or blocked the *Taq* DNA polymerase.

Researchers have observed covalently bound adducts between chemical/its metabolites and the DNA of an organism when exposed to a plant extract. Inaccurate repair of these adducts may cause mutations and cytogenetic changes in DNA (Lalrotluanga et al. 2011). Further, exposure to a genotoxin may affect only certain cells or different sites on the different chromosomes. Since in the complete *g*-DNA sample, DNA would have originated from a number of cells, it seems unlikely that the entire *g*-DNA would have been damaged at the same site. The present data, hence, suggests that DNA damage and the possible occurrence of mutations appear to be the prime factors influencing RAPD patterns of larvae of *A. aegypti* treated with *A. aspera* extracts. The polymorphism in the RAPD is evidently indicative of the larval mortality on treatment with the *A. aspera* extracts.

Conclusion

Our investigations have ascertained the larvicidal potential of *A. aspera* extracts, the cidal response may be linked with the mutations induced at genomic level due to the presence of some active phyto-components in the hexane plant extracts that probably interferes with the molecular mechanisms involved in normal functioning of *A. aegypti* larvae and consequently leading to larval mortality. It suggests the possible use of these extracts as the potential larvicides against dengue vector. However, the range of types and levels of bioactive constituents in these extracts may be responsible for the variability in their potential against *A. aegypti*. Further investigations are needed to identify the bioactive constituent and ascertain its effectiveness in the field conditions.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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