Insecticidal and genotoxic activity of *Psoralea corylifolia* Linn. (Fabaceae) against *Culex quinquefasciatus* Say, 1823

Virendra K Dua1*, Arvind Kumar2, Akhilesh C Pandey1 and Sandeep Kumar1

**Abstract**

**Background:** Indiscriminate use of synthetic insecticides to eradicate mosquitoes has caused physiological resistance. Plants provide a reservoir of biochemical compounds; among these compounds some have inhibitory effect on mosquitoes. In the present study the larvicidal, adulticidal and genotoxic activity of essential oil of *Psoralea corylifolia* Linn. against *Culex quinquefasciatus* Say was explored.

**Methods:** Essential oil was isolated from the seeds of *P. corylifolia* Linn. Larvicidal and adulticidal bioassay of *Cx. quinquefasciatus* was carried out by WHO method. Genotoxic activity of samples was determined by comet assay. Identification of different compounds was carried out by gas chromatography- mass spectrometry analysis.

**Results:** LC50 and LC90 values of essential oil were 63.38±6.30 and 99.02±16.63 ppm, respectively against *Cx. quinquefasciatus* larvae. The LD50 and LD90 values were 0.057±0.007 and 0.109±0.014 mg/cm2 respectively against adult *Cx. quinquefasciatus*. Genotoxicity of adults was determined at 0.034 mg/cm2. The mean comet tail length was 6.2548±0.754 μm and 8.47±0.931 μm and the respective DNA damage was significant i.e. 6.713% and 8.864% in comparison to controls. GCMS analysis of essential oil revealed 20 compounds. The major eight compounds were caryophyllene oxide (40.79%), phenol,4-(3,7-dimethyl-3-ethenylocta-1,6-dienyl) (20.78%), caryophyllene (17.84%), α-humulene (2.15%), (+)- aromadendrene (1.57%), naphthalene, 1,2,3,4-tetra hydro-1,6-dimethyle-4-(1-methyl)-, (1S-cis) (1.53%), trans- caryophyllene (0.75%), and methyl hexadecanoate (0.67%).

**Conclusion:** Essential oil obtained from the seeds of *P. corylifolia* showed potent toxicity against larvae and adult *Cx. quinquefasciatus*. The present work revealed that the essential oil of *P. corylifolia* could be used as environmentally sound larvicidal and adulticidal agent for mosquito control.

**Keywords:** Larvicidal activity, Adulticidal activity, Genotoxicity, DNA damage, Essential oil, *Psoralea corylifolia*, *Culex quinquefasciatus*, GCMS

**Background**

Mosquitoes are an important public health concern around the world. They not only cause nuisance to humans but also transmit several diseases like malaria, filaria, Japanese encephalitis, dengue fever, chikungunya [1] and yellow fever [2]. These diseases affect the health and quality of life of millions of people in subtropical and tropical countries [3]. Mosquitoes also cause allergic responses in humans that include local skin and systemic reactions such as angioedema [4]. In 2010, The WHO reported 216 million cases of malaria in the world with an estimated 6,55,000 malaria deaths [5]. An estimated 120 million people in tropical and subtropical areas of the world are infected with lymphatic filariasis [6]. Three billion people in the endemic areas are at risk of infection with Japanese encephalitis and incidence of the disease is 30,000–50,000 cases annually [7]. Over 40% of the world’s population (approximately 2.5 billion) is at risk from dengue, WHO estimated 50–100 million dengue infections worldwide, annually [8]. Moreover, there are an estimated 200,000 cases of yellow fever (causing 30,000 deaths) worldwide annually [9]. *Culex*
quinquefasciatus Say, 1823 (widely distributed mosquito in India) is a vector of important diseases, such as West Nile virus, filariasis, Japanese encephalitis, St. Louis encephalitis, avian malaria and bancroftian filariasis (Wuchereria bancrofti) [10]. Cx. quinquefasciatus is responsible for major public health problems in India with around 31 million microfilaraemics, 23 million cases of symptomatic filariasis, and about 473 million individuals potentially at risk of infection [11].

Synthetic insecticides were used extensively during the 1950s to control malaria in various countries by indoor residual spraying (IRS) as a larvicide [12]. Synthetic insecticides were also used to control adult mosquitoes by fogging [13]. The continuous use of synthetic insecticide such as malathion, DDT, HCH and deltamethrin for controlling mosquitoes has created diverse environmental problems such as toxicity to non target organisms [14], development of genetic resistance in mosquitoes [15], environment pollution [16] and their non degradable nature results in biomagnifications. Herein, the worldwide continuous efforts to eradicate and control this vector were found ineffective. Therefore, there is a need to search for environmentally safe, degradable and target specific insecticides. Plant derived essential oils are emerging as a potential source for mosquito control agents, since they constitute a rich source of bioactive compounds that are biodegradable and potentially suitable for controlling mosquitoes. Earlier researchers have reported the efficacy of several plant essential oils against mosquito larvae [17-21] and adults [22-26].

Psoralea corylifolia Linn. is an erect, herbaceous, and annual weed growing up to a height of 60–120 cm in the plains of central and eastern India, China, and in some parts of Arabia under semi arid conditions [27]. Seeds are usually brownish-black in color, smooth, and adhere to the pericarp [28,29]. The plant is widely used in several skin diseases such as psoriasis [30], leucoderma and leprosy [31]. The therapeutic action of P. corylifolia against various diseases such as asthma, diarrhoea, alopecia areata [32], impotency, menstruation disorder and uterine hemorrhage [33]. Moreover it has antitumor [34], antiallergic [35], antioxidant [36], insecticidal [37] and antimicrobial activity [38]. The plant has also been used for the treatment of enuresis, various kidney problems [39], depression [40], osteoporosis and bone fractures [27]. In the present study the larvicidal, adulticidal and genotoxic activity of volatile oil extracted from seeds of P. corylifolia against Cx. quinquefasciatus and the phytochemical analysis of volatile oil by GC-MS was determined.

The single cell gel electrophoresis (SCGE or Comet assay) is one of the most promising and imminent genotoxicity tests. It is less resource intensive than the usual genotoxic techniques and permits both qualitative and quantitative assessment of DNA damage in individual eukaryotic cells. The sensitivity of the SCGE technique has been applied in many areas, e.g. environmental monitoring [41], in vivo and in vitro genotoxicity testing [42] and epidemiological and biomonitoring studies in human populations exposed occupationally, environmentally or clinically [43,44]. This test procedure has been recommended in the Committee on Mutagenicity Guidelines of the UK Department of Health (COM) for determining in vitro mutagenicity of chemicals [45].

Methods
Plant material
Plants with seeds of P. corylifolia (Fabaceae) collected from Garhwal region of Himalaya were purchased from the Arya Vastu Bhandar Dehradun, Uttarakhand, India, which were further confirmed by Botanical Survey of India (BSI), Dehradun, India. The Voucher specimen of the whole plant and its seeds were kept in the Institute herbarium for future reference (Voucher Specimen No: NIMRHR-101PC-1).

Isolation of the essential oils
The collected seeds of P. corylifolia were washed with distilled water and dried under shade. The dried seeds (100g) were powderized with the help of a grinder and mixed in water (1:6); further steam distillation (in a Cleveger apparatus for 7 h), extracted the essential oil. The oil layer was separated from the aqueous phase using n-hexane with the help of a separating funnel. The volatile essential oil was dried using anhydrous sodium sulfate, and stored at 4°C until used.

GC–MS analysis
The GC-MS analysis was carried out on a Shimadzu (QP 2010) series GC-MS (Tokyo, Japan) system equipped with AOC-20i auto-sampler and coupled with DB-5 MS capillary column (Agilent technologies, made in USA), (30 m × 0.25 mm i.d., 0.25 μm). Helium was used as carrier gas at a flow rate of 1.28 ml/min; split ratio of 1: 50; mass scan 50–800; ionization energy, 70 eV; ion source temperature, 200°C; injector temperature, 250°C. Oven temperature was programmed as follows: initially at 40°C for 5 min, rising at 4°C/min to 220°C and then held isothermally (5 min) at 220°C. The oil sample (10 μl) was diluted (up to 2 ml) with dichloromethane (HPLC grade), sample injection volume was 1 μl. Individual components were identified by comparison of their mass spectra (MS) with NIST database and Adams libraries from the derived fragmentation pattern [46,47].
Test organisms
The test organism Cx. quinquefasciatus Say, 1823, was reared continuously from several generations in the Entomology Laboratory of the National Institute of Malaria Research, Field Unit, Hardwar, India. They were free of exposure to pathogens and insecticides and maintained at 26 ± 2°C and 60-80% relative humidity. The larvae were fed on dog biscuits and yeast powder in a ratio 3:2 until moulting to become pupae. Pupae were transferred into a mosquito cage. The emergent adults were fed with 10% glucose solution dipped in a piece of cotton in humidified cages.

Larvicidal bioassay
Larvicidal activities of the essential oil of P. corylifolia were determined in terms of LC50 and LC90 by using the standard procedure of WHO [48] with slight modification. Twenty early fourth instar larvae of Cx. quinquefasciatus, were transferred to 500 ml bowls containing 249 ml of dechlorinated tap water. The essential oil was dissolved in 1 ml aceton to prepare a serial dilution of test dosage and mixed in 249 ml tap water containing 20 early fourth instar larvae. Three replicates were run simultaneously with at least six dosages 25–100 μg/ml (ppm) along with control (1 ml of acetone alone to 249 ml of tap water). Bioassay was conducted at room temperature 26 ± 2°C with 60-80% relative humidity, during which time no food was offered to the larvae. Mortality of larvae was recorded 24 h post treatment and evaluated LC50 and LC90 by using probit analysis and StatusPlus2009 software.

Adulticidal bioassay
Adulticidal bioassay was performed according to WHO guidelines [49] against Cx. quinquefasciatus and lethal dose (LD50 and LD90) and knockdown time (KDT50 and KDT90) were evaluated. Different concentrations ranges of the essential oil of P. corylifolia were prepared in 2.5 ml of acetone and homogenously applied on Whatman no. 1 filter papers (size 12 × 15 cm²), control papers were treated with 2.5 ml of acetone under similar conditions and placed in WHO exposure tubes. 20 adult mosquitoes (2–5 days old glucose fed mosquitoes) were exposed on treated paper for one hour and knocked down and live mosquitoes were counted at 5 minute intervals. After one-hour exposure mosquitoes was transferred into WHO holding test tubes for a 24 hour recovery period. During this period the mosquitoes were kept at room temperature at 26 ± 2°C and 70-80% relative humidity. The mortality was observed after 24 hours [50]. Three replicates were run simultaneously with at least six doses (0.034, 0.055, 0.069, 0.104, 0.138 and 0.173 mg/cm²) to produce a range of mortality from 15 to 100% along with controls. Lethal dose (LD50 and LD90) and knocked down time (KDT50 and KDT90) were calculated by probit analysis using StatusPlus2009 software.

Positive control
Advance studies on adulticidal activity was carried out by using 0.05% deltamethrin (DM) impregnated paper as a positive control against female Cx. quinquefasciatus for determination of KDT50 and KDT90 value.

Genotoxicity testing by comet assay
The DNA damage studies were carried out using Single Cell Gel Electrophoresis (SCGE), commonly known as comet assay. The protocol (Alkali method) was followed as described by Singh et al. [51] with minor modifications as described below.

Slide preparation
Twenty mosquitoes were exposed to 0.034 mg/cm² and 0.069 mg/cm² concentrations of the essential oil of P. corylifolia as well as controls, for 1h in WHO exposure tubes and were homogenized in 10% (w/v) homogenizing buffer (0.075 M NaCl and 0.024 M EDTA). Homogenate was centrifuged at 1000 rpm for 10 minutes and the pellet was gently resuspended in 1ml of chilled homogenizing buffer for nuclei preparation. Frozen microscopic slides were then placed horizontally and a homogenous thin layer of 1% normal melting agarose was cast onto the slide, isolated nuclei and 1% low melting agarose [(1:4), 100 μl] were mixed and cast onto the precoated slides and kept at 4°C for 20 minute. The slides were immersed into the freshly prepared chilled lysis buffer (2.5 M NaCl, 100 mM EDTA pH 10, 5% DMSO, 1% and Triton-X 100) for 1 hour in the dark at 4°C. After complete lysis the slides were placed for 20 min in an ice cold electrophoresis chamber containing alkaline electrophoresis buffer (1mM EDTA and 300 mM NaOH, pH>13) to facilitate unwinding of DNA strands, the process was subsequently conducted for 20 minutes at 25 volts/300 mA. The slides were washed thrice with neutralizing buffer (0.4 M Tris pH 7.5) for 5 minutes and just before visualization. Slides were then stained with ethidium bromide (20 μg/ml, 40 μl/slide) for 10 min in the dark. Slides were then dipped once in chilled distilled water to remove excess ethidium bromide and subsequently cover slips were placed over them. The slides were stored in a dark, humidified chamber and analyzed within 3±4 h.

Comet capture and analysis
A total of 100 cells from each slide were analyzed by image analysis using a fluorescence microscope (Leica DM4000B) with an excitation filter of 515–560 nm and a barrier filter of 590 nm using X10 objectives. The
photographs of the individual cells were taken using a Leica Digital DFC 320R-II camera. Comet tail length and percentage of DNA damage in tail were measured with an Image Analysis System (Leica Qwin) and Comet Score software version 1.5 (TriTek Corporation, Sumeduck, VA).

Statistical analysis
Statistical analysis of the experimental data was performed using the computer software StatPlus 2009 (AnalystSoft, Canada) to find the lethal concentration/dose against larvae (LC50 and LC90) and adult (LD50 and LD90) in 24 h and also determines the knockdown time (KDT50 and KDT90) by probit analysis [52] with a reliability interval of 95%. To determine whether there was a statistically significant difference among different doses of *P. corylifolia* essential oils against mosquito larvae and adults, Student’s t-test was used to analyze the difference of the percentage of mortality. Results with P<0.05 were considered to be statistically significant.

The corrected percent mortality was evaluated by using Abbott’s formula:

\[
\text{Corrected Mortality} \% = \frac{MT - MC}{100 - MC} \times 100
\]

where MT and MC are percent mortality in treated and control experiment, respectively [53].

Results and discussion
The average yield of essential oil was 2% w/w according to their dry weight. Oil is a complex mixture of several compounds; chemical constituents of analyzed oils are displayed in Table 1.

### Phytochemical screening
A total of 20 compounds were identified in seeds of *P. corylifolia* essential oil. Compounds occurring in trace amounts are not reported in this article. Caryophyllene derivatives (caryophyllene oxide and caryophyllene) are major fractions of the essential oil (59.30%), among the caryophyllene derivatives, caryophyllene oxide and caryophyllene contributed 2/3 and 1/3 fraction, respectively. But 8 major compounds constituting 86.08% of the *P. corylifolia* seed essential oil were caryophyllene oxide, phenol, 4-(3,7-dimethyl-3-ethenyl)octa-1,6-dienyl, caryophyllene, α-Humulene, (+)- aromadendrene, naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methyl), (1S-cis), trans- caryophyllene, and methyl hexadecanoate (Table 1). The results differ from Kapoor [54] and Sharma *et al.* [55] who reported that the main constituent of essential oil of *P. corylifolia* seeds have limonene, α-elemene, γ-elemene, β-caryophylleneoxide, 4-terpineol, linalool, geranylacetate. We assume that the discrepancy might have been caused by the differences in the chemotypes of the species. Several studies have shown that caryophyllene oxide and caryophyllene present in the essential oils of different plants possess significant insecticidal activities against different species of mosquitoes [56-59]. A range of essential oils exhibited bioactive properties against the larvae of *Cx. quinquefasciatus* [21,60-62].

### Larvicidal activity
In the present study larvicidal activity of essential oil from *P. corylifolia* was evaluated at different concentrations (range: 25–100 ppm) on early fourth instar larvae of *Cx. quinquefasciatus* and 8.0, 18.5, 45.0, 85.0, 91.63 and 100.0% mortality was recorded at 25, 50, 65, 75, 90 and 100 ppm, respectively (Figure 1). The data were analyzed using Student’s t-test in which p values <0.05

### Table 1 Major identified compounds in the essential oil of *P. corylifolia* seeds by GCMS

| S. No. | Retention time (min) | Concentration (%) | Constituents | Mass fragmentation pattern | Match with library |
|-------|---------------------|-------------------|--------------|---------------------------|--------------------|
| 1     | 30.500              | 17.84             | Caryophyllene | [M+] 204, 189, 175, 161, 147, 133, 120, 105, 93, 79, 69, 55, 51 | NIST147.LIB |
| 2     | 31.688              | 2.15              | α-Humulene   | [M+] 204, 147, 121, 107, 93, 80, 67, 53 | WILEY7.LIB |
| 3     | 33.782              | 1.57              | (+)- Aromadendrene | [M+] 204, 187, 161, 147, 134, 119, 106, 96, 81, 69, 55, 51 | WILEY7.LIB |
| 4     | 33.842              | 1.53              | Naphthalene, 1,2,3,4-tetrahydro-1,6-dimethyl-4-(1-methyl), (15-cis) | [M+] 202, 159, 144, 131, 105, 69 | WILEY7.LIB |
| 5     | 35898               | 40.79             | (–)-Caryophyllene oxide | [M+] 173, 164, 149, 135, 123, 107, 96, 79, 69, 55, 51 | WILEY7.LIB |
| 6     | 40.466              | 0.75              | Trans- Caryophyllene | [M+] 236, 203, 189, 175, 161, 147, 133, 119, 105, 91, 79, 69, 55, 51 | WILEY7.LIB |
| 7     | 45.419              | 0.67              | Methyl Hexadecanoate | [M+] 270, 239, 227, 199, 185, 171, 161, 143, 129, 119, 101, 87, 74, 69, 55 | WILEY7.LIB |
| 8     | 52.073              | 20.78             | Phenol, 4-(3,7-dimethyl-3-ethenyl)octa-1,6-dienyl | [M+] 256, 213, 185, 173, 158, 145, 127, 121, 107, 93, 83, 69, 55, 53 | NIST147.LIB |
were taken to represent significant differences between mean values. Mean LC50 and LC90 (± standard error) values were 63.38±6.3 ppm and 99.02±16.63 ppm against Cx. quinquefasciatus larvae respectively (Table 2). In 2005, Dharmagadda and coworkers reported the larvicidal activity of Tagetes patula essential oil against the fourth instar larvae of Aedes aegypti, Anopheles stephensi, and Cx. quinquefasciatus, the LC50 and LC90 values were 13.57, 12.08, 22.33 and 37.91, 57.62, 71.89 ppm respectively [62]. In 2009, Pavela screened 22 essential oils for their larvicidal activity, the essential oil obtained from Thymus vulgaris, Satureja hortensis and Thymus satureioides plants found potent larvicidal activity with LC50 of 33, 36 and 44 g/ml, respectively against Cx. quinquefasciatus [60]. A series of plant essential oils were also reported as larvicide against different mosquito species [57,63].

Adulcicidal activity

A large number of plants are reported to have larvicidal activity, but very few of the plants are reported to have adulcicidal activity [22,24,50].

The present study demonstrates the adulcicidal activity of essential oil from P. corylifolia at different concentrations (0.034, 0.055, 0.069, 0.104, 0.138 and 0.173 mg/cm²) on Whatman no.1 impregnated filter paper, against female adult Cx. quinquefasciatus mosquitoes and showed 16.66, 45.00, 70.00, 85.00, 95.00 and 100% mortality, respectively (Figure 2). The data were analyzed using Student’s t-test in which p values <0.05 were taken to represent significant differences between mean values. The LD50 and LD90 values (± standard error) were 0.057±0.007 mg/cm² and 0.109±0.104 mg/cm² against adult Cx. quinquefasciatus respectively (Table 2); the results were compared with 0.05% deltamethrin (DM) impregnated paper. KDT50 and KDT90 values of the essential oil from P. corylifolia were 20.29±0.88, 18.06±1.32, 13.45±0.60, 11.16±0.49, 9.87±0.48 min and 47.87±3.18, 36.00±1.32, 25.75±1.25, 19.40±0.96, 17.85±0.95 min respectively at 0.055, 0.069, 0.104, 0.138 and 0.173 mg/cm² concentrations, respectively against Cx. quinquefasciatus (Figure 3). KDT50 and KDT90 values of 0.05% deltamethrin impregnated papers were 14.91±0.67 and 30.89±1.58 min, respectively against Cx. quinquefasciatus, with 96.7% mortality (Table 3). Dua and collaborators (2008) [50] reported the adulcicidal activity of essential oil of Valeriana jatamansi root against An. stephensi, An. culicifacies, Ae. aegypti, Ae. albopictus, and Cx. quinquefasciatus, with LD50 and LD90 values were 0.14, 0.16, 0.09, 0.08, 0.17 mg/cm² and 0.24, 0.34, 0.25, 0.21, 0.28 mg/cm², respectively; Whereas KDT50 and KDT90 values were 13, 13, 12, 13, 18 min and 24, 25, 21, 20, 42 min against An. stephensi, An. culicifacies, Ae. aegypti, An. albopictus and Cx. quinquefasciatus, respectively, using 0.28 mg/cm² impregnated papers. In 2010 Dua et al. [22], reported the adulcicidal activity of essential oil of leaves of Lantana camara against Ae. aegypti, Cx. quinquefasciatus, An. culicifacies, An. fluviatilis and An. stephensi, LD50 values were 0.06, 0.05, 0.05, 0.05 and 0.06 mg/cm² while LD90 values were 0.10, 0.10, 0.09, 0.09 and 0.10 mg/ cm² respectively. Whereas KDT50 values were 20, 18, 15, 12, 14 min and KDT90 values were 35, 28, 25, 18, and 23 min against Ae. aegypti,

Table 2 Insecticidal activity of essential oil from seeds of P. corylifolia against Cx. quinquefasciatus larvae and adults

| Concentration (mg/cm²) | % Mortality |
|------------------------|-------------|
| 0                      | 0           |
| 0.034                  | 16.66       |
| 0.055                  | 45          |
| 0.069                  | 70          |
| 0.104                  | 85          |
| 0.138                  | 95          |
| 0.173                  | 100         |

Six concentrations were tested with control (significantly different at P < 0.05) three replicates were taken. *Standard error, LC and LD values were determined by probit analysis.
Cx. quinquefasciatus, An. culicifacies, An. fluviatilis and An. stephensi, respectively on 0.208 mg/cm² impregnated paper. Adulticidal activity of five essential oils (Citrus sinensis, Mentha piperita, Carvocryl oil, Citronela oil and citral oil) at different concentrations and time intervals was determined by Yang et al. (2005), the Rutaceae oil (C. sinensis) was found as the most toxic against Cx. quinquefasciatus with LC₅₀ of 0.0513 [24].

Genotoxicity
The effect of the oil from P. corylifolia on DNA damage in individual cells of adult Cx. quinquefasciatus was assessed by two distinct types of DNA damage measurements: the length of DNA comet tail and the percentage of fragmented DNA present in the tail after electrophoresis. The DNA damage was deduced in adult Cx. quinquefasciatus when exposed to 0.034 mg/cm² and 0.069 mg/cm² concentrations of essential oil of P. corylifolia using the comet assay method. The observation showed that DNA damage was significant i.e. 6.713% and 8.864% at 0.034 mg/cm² and 0.069 mg/cm² concentrations of essential oil in comparison to controls (Figure 4). The comet tail length increases with increase in concentration of essential oil of P. corylifolia, the tail length was 6.2548±0.754 µm and 8.47±0.931 µm at 0.034 mg/cm² and 0.069 mg/cm² concentration respectively with reference to control, which confirms the genotoxicity (Figure 4). A literature study revealed that there was DNA damage in mid gut cells of third instar larvae of D. melanogaster on exposure to chlorpyrifos for 24 and 48 h, as assessed by comet assay. The tail length at 1.5 mg/l and 15.0 mg/l is 9.63 µm and 19.26 µm at 24 h, 9.87 µm and 28.21 µm at 48 h, respectively. The DNA damage was 9.65% and 18.94% at 24 h, 1.09 and 27.14% at 48 h, respectively [64].

The possible mechanism of essential oil toxicity is either to react with DNA or by the generation of ROS (reactive oxygen species) therefore causing DNA damage including in the adult Cx. quinquefasciatus. ROS are generated by inhibition of mitochondrial ATP synthesis through the uncoupling of oxidative phosphorylation that could lead to the generation of ROS [64,65]. During normal metabolism of the cell, ROS are generated in very low amounts and regulate various biological processes.

| Table 3 Mean knockdown time and corrected percent mortality of essential oil of P. corylifolia against adult Cx. quinquefasciatus at different concentrations |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|
| Concentration (mg/cm²)        | % kd in 1 hr.   | Kd₅₀            | Kd₉₀            | % Corrected mortality in 24 hr. |
| 0.034                         | 38.3            | ND              | ND              | 20               |
| 0.055                         | 91.65           | 20.29±0.88      | 47.8±3.18       | 45               |
| 0.069                         | 100             | 18.06±1.33      | 36.00±1.32      | 70               |
| 0.104                         | 100             | 13.45±0.60      | 25.75±1.25      | 85               |
| 0.138                         | 100             | 11.16±0.49      | 19.40±0.96      | 95               |
| 0.173                         | 100             | 9.87±0.48       | 17.85±0.95      | 100              |
| Control                       | 0               | -               | -               | 0                |
| 0.05% Deltamethrin            | 100             | 14.91±0.67      | 30.89±1.58      | 96.7             |

* Kd₅₀ Knockdown time (minutes)±standard error, ND not determined. Six concentrations were tested with control (significantly different at P < 0.05) three replicates were taken. Kd₅₀ values were determined by probit analysis and mortality was corrected using Abbott’s formula.
processes such as signal transduction pathways. At high and/or sustained levels, they can cause severe damage to DNA, protein and lipids [66]. Various stressors present in the environment including pesticides are capable of reacting with DNA and causing DNA damage, stressors also have the capability to generate ROS, one of the possible mechanisms for the induction of DNA damage may be through the generation of ROS [67].

Conclusion
In the present study essential oil obtained from the seeds of *P. corylifolia* has shown potent toxicity against larvae and adults of *Cx. quinquefasciatus*. GC–MS analysis revealed the major constituents of essential oils were carophyllene oxide, phenol,4-(3,7-dimethyl-3-ethenylcota,1,6-dienyl) and carophyllene. The findings may be utilized for the development of eco-friendly insecticide, which could be used as an alternative for mosquito control.

Competing interests
The authors declare that they have no competing interest.

Authors’ contributions
VKD designed the work and supervised the manuscript. AK performed experiments, interpretation of data and drafted the manuscript ACP. VKD designed the work and supervised the manuscript. AK performed experiments, interpretation of data and drafted the manuscript ACP.

Acknowledgements
We gratefully acknowledge the Integrated Diseases Vector Control (IDVC) for financial support to perform the study. We also acknowledge the NIMR for technical support.

Received: 31 August 2012 Accepted: 27 December 2012 Published: 4 February 2013

References
1. Korgaonkar NS, Kumar A, Dash A, Yadav RS, Kabadi D, Dash AP: Mosquito biting activity on humans & detection of *Plasmodium falciparum* infection in *Anopheles stephensi* in Goa, India. *Indian J Med Res* 2012, 135:120–126.
2. Auguste AJ, Lemerie P, Pybus OG, Suchard MA, Salas RA, Adesiyun AA, Barrett AD, Tesh RB, Weaver SC, Carrington CVF: Yellow fever virus maintenance in trinidad and its dispersal throughout the Americas. *J Virol* 2010, 84:9967–9977.
3. Giriheko AK, Lindsay SW, Cordofanjei UE, Patz JA: Climate change and vector-borne diseases: a regional analysis. *Bull W H O* 2000, 78:1136–1147.
4. Peng Z, Yang J, Wang H, Simons FER: Production and characterization of monoclonal antibodies to two new mosquito *Aedes aegypti* salivary proteins. *Insect Biochem Mol Biol* 1999, 29:909–914.
5. World malaria report 2011, Switzerland: WHO, 2011.
6. Fox LH: Infectious Diseases Related To Travel. In CDC Health Information for International Travel 2012. Edited by Brunette GW. New York: Oxford University Press; 2011.
7. Solomon T: Control of Japanese encephalitis — within Our grasp? *N Engl J Med* 2006, 355:869–871.
8. Global burden of dengue, Dengue and severe dengue WHO, 2012.
9. Populations at risk. Yellow fever: WHO, 2011.
10. Pauly KP, Hoti SL, Balaraman K: Development of lymphatic filarial parasite *wuchereria bancrofti* (spirurida: onchocercidae) in mosquito species (diptera: culicidae) Fed artificially on microfilaricmic blood. *J Med Entomol* 2006, 43:1222–1226.
11. Agrawal VK, Sashindran VR: Lymphatic filariasis in India problems, challenges and new initiatives. *Med J Armed Forces India* 2006, 62:359–362.
12. Webb IA: The first large-scale Use of synthetic insecticide for malaria control in tropical Africa: lessons from Liberia, 1945–1962. *J Hist Med All Sci* 2011, 66:347–376.
13. Fradin MS: Mosquitoes and mosquito repellents: a Clinician’s guide. *Ann Intern Med* 1998, 128:931–940.
14. Atkar M, Paramasivam M, Sengupta D, Purkait S, Ganguly M, Baraneeja S: Impact assessment of pesticide residues in fish of Ganga river around Kolkata in West Bengal. *Environ Monit Assess* 2009, 157:97–104.
15. Brausch JM, Smith PN: Pesticide resistance from historical agricultural chemical exposure in *Thamnocephalus platyurus* (Crustacea: Anostraca). *Environ Pollut* 2009, 157:481–487.
16. Thakur JS, Prinja S, Singh D, Rajwanshi A, Prasad R, Panwana HK, Kumar R: Adverse reproductive and child health outcomes among people living near highly toxic waste water drains in Punjab, India. *J Epidemiol Community Health* 2010, 64:148–154.
17. Cheng S-S, Chang H-T, Chang S-T, Tsai K-H, Chen W-J: Bioactivity of selected plant essential oils against the yellow fever mosquito *Aedes aegypti* larvae. *Bioresource Technol* 2003, 89:99–102.
18. Zhu J, Zeng X, Liu T, Qian K, Han Y, Xue S, Tucker B, Schultz G, Coats J, Rowley W, Zhang A: Adult repellency and larvicidal activity of five plant essential oils against mosquitoes. *J Am Mos Control Assoc* 2006, 22:515–522.
19. Cheng S-S, Liu J-Y, Tsai K-H, Chen W-J, Chang S-T: Chemical composition and mosquito larvicidal activity of essential oils from leaves of different *cinnamomum osmophloeum* provenances. *J Agric Food Chem* 2004, 52:4395–4400.
20. Cheng S-S, Liu J-Y, Huang C-C, Hsu Y-R, Chen W-J, Chang S-T: Insecticidal activities of leaf essential oils from *Cinnamomum osmophloeum* against three mosquito species. *Bioresource Technol* 2009, 100:457–464.
21. Cheng S-S, Chua M-T, Chang E-H, Huang C-C, Chen W-J, Chang S-T: Variations in insecticidal activity and chemical compositions of leaf essential oils from *Cryptocarya japonica* at different ages. *Bioresource Technol* 2009, 100:845–870.
22. Dua VK, Pandey AC, Dash AP: Adulicidal activity of essential oil of *Lantana camara* leaves against mosquitoes. *Indian J Med Res* 2010, 131:434–439.
23. Jantani I, Yalverma MF, Ahmad NW, Jamal JA: Insecticidal activities of the leaf oils of eight *cinnamomum* species. *Species against aedes aegypti and aedes albopictus*. *Pharm Bio* 2005, 435:266–532.
24. Yang P, Ma Y, Zheng S: Adulicidal activity of five essential oils against *Culex pipiens quinquefasciatus*. *Pestic Sci Soc Japan* 2005, 59:307–314.
25. Cleik JE, Hallman CF, Johnson R: Effect of several commercially formulated essential oils against caged female *aedes albopictus* and *culex quinquefasciatus* when operationally applied via an automatic timed insecticide application system. *J Am Mos Control Assoc* 2011, 27:252–255.
26. Kalavani V, Senthil-Nathan S, Murugesan A: Biological activity of selected Lamiaceae and Zingiberaceae plant essential oils against the dengue vector *Aedes aegypti* L. *Diptera: Culicidae*. *Parasitol Res* 2012, 110:1261–1268.
27. Joshi SG: Medicinal Plants. New Delhi India: Oxford and IBH Publishing Co. Pvt. Ltd, 2000:206–207.
28. Panda H: In Herbs Cultivation and Medicinal Uses. New Delhi: National Institute Of Industrial Research; 2000:479–481.
29. Ukey SK, Yadav AS, Sharma AK, Rai AK, Raghuvanshi DK, Badhane Y: The botany, chemistry, pharmacological and therapeutic application of *Psoralea corylifolia* L. – A review. *Int J Phytomedicine* 2011, 2:100–107.
30. Khan MS, Siddiqui M, Aleem S: Effect of *Psoralea corylifolia* Linn. and *Marham-e-Gulabi* in Da-al-sadaf (psoriasis). *Indian J Traditional Knowledge* 2009, 8:425–430.
31. Sah P, Agarwala D, Garg S: Isolation and identification of furocoumarins from the seeds of *Psoralea corylifolia* Linn. *Indian J Pharm Sci* 2006, 68:769–771.
32. Qiao C-F, Han Q-B, Song J-Z, Mo S-F, Kong L-D, Kung H-F, Xu H-X: Chemical fingerprint and quantitative analysis of *Fructus Psoraleae* by high performance liquid chromatography. *J Sep Sci* 2007, 30:813–818.
33. Ruan B, Kong LT, Takaya Y, Niwa M: Studies on the chemical constituents of *Psoralea corylifolia* L. *J Asian Nat Prod Res* 2007, 9:41–44.
34. Latha PG, Evans DA, Panikkar KR, Jayavardhanan KK: Immunomodulatory and antitumour properties of Psoralea corylifolia seeds. Fitoterapia 2000, 71:223–231.
35. Matsuda H, Sugimoto S, Morikawa T, Matsuura K, Mizuguchi E, Nakamura S, Yoshikawa M: Bioactive Constituents from Chinese Natural Medicines: XX. Inhibitors of Antigen-Induced Degranulation in RBL-2H3 Cells from the Seeds of Psoralea corylifolia. Chem Pharm Bull 2007, 55:195–200.
36. Shinde AN, Malpathak N, Fulzele DP: Determination of isoflavone content and antioxidant activity in Psoralea corylifolia L. callus cultures. Food Chem 2010, 118:128–132.
37. Khatune NA, Islam ME, Rahman MAA, Baki MA, Sadik G, Haque ME: Pesticidal Activity of a Novel Coumarin Derivative Isolated from Psoralea corylifolia Linn. Against Tribolium castaneum Herbst. Adults and Larvae (Coleoptera:Tenebrionidae). J Agric J 2002, 1:1–12.
38. Zaidi SFH, Yamada K, Kadowaki M, Usmanghani K, Sugiyama T: Antidepressant-like effects of psoralidin isolated from the seeds of Psoralea corylifolia L. In vitro and in vivo assessment of genotoxic effects of etoposide and chlorothalonil by the comet assay. Mutat Res Genet Toxicol Environ Mutagen 2002, 516:148–152.
39. Godard T, Fessard V, Huet S, Mourot A, Deslandes E, Pottier D, Hyrien O, Yi L-T, Li Y-C, Pan Y, Li J-M, Xu Q, Mo S-F, Qiao C-F, Jiang F-X, Xu H-X, Lu Q, et al: Antidepressant-like effects of psoralin isolated from the seeds of Psoralea corylifolia in the forced swimming test in mice. Prog Neuropsychopharmacol Biol Psychiatry 2008, 32:510–519.
40. Dusinska M, Collins AR: Handbook of Ayurvedic Medicinal Plants. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988, 175:184–191.
41. Finney DJ: Probit Analysis By D J Finney, Volume 60. 3rd edition. 32 E 57th St. New York: Cambridge University Press; 1971.
42. Kapoor LD: A method of computing the effectiveness of an insecticide. J Am Mos Control Assoc 1925, 3:303–304.
43. Sharma PC, Yelne MB, Dennis T J: Database on Medicinal Plants Used in Ayurveda. 2nd edition. New Delhi: Central Council for Research in Ayurveda and Siddha; 2001:89–93.
44. Liu ZL, Liu QR, Chu SS, Jiang GH: Insecticidal Activity and Chemical Composition of the Essential Oils of Artemisia lavandulaefolia and Artemisia sieversiana from China. Chem Biodivers 2010, 7:2040–2045.
45. Silva WJ, Dósa GA, Maia RT, Nunes RS, Carvalho GA, Blank AF, Alves PB, Marçal RM, Cavalcanti SC H: Effects of essential oils on Aedes aegypti larvae: Alternatives to environmentally safe insecticides. Bioresource Technol 2008, 99:3251–3255.
46. de Morais SM, Facundo VA, Bertini LM, Cavalcanti ESB, Anjos Júnior JF, Ferreira SA, de Brito ES, de Souza Neto MA: Chemical composition and larvicidal activity of essential oils from Piper species. Biochem Syst Ecol 2007, 35:670–675.
47. Adams RP: Identification of essential oil components by gas chromatography/mass spectroscopy. 3rd edition. 32 E 57th St. New York: Cambridge University Press; 2004.
48. Committee on Mutagenicity of Chemicals in Food, Consumer Products and the Environment: [http://www.iacom.org.uk/index.htm].
49. World Health Organization: Guidelines For Laboratory and field Testing Of the susceptibility or resistance of adult mosquitoes to organochlorine, organophosphate and carbamate insecticides: diagnostic test. Geneva: WHO/VBC/81.807; 1981.
50. Dua VK, Alam MF, Pandey AC, Rai S, Chopra AK, Kaul VK, Dash AP: Larvicidal activity of Tagetes patula essential oil against three mosquito species. Bioresource Technol 2005, 96:1235–1240.
51. Singh NP, McCoy MT, Tice RR, Schneider EL: A method of computing the effectiveness of an insecticide. J Am Mos Control Assoc 1971.
52. Finney DJ: Probit Analysis By D J Finney, Volume 60. 3rd edition. 32 E 57th St. New York: Cambridge University Press; 1971.
53. Kapoor LD: A method of computing the effectiveness of an insecticide. J Am Mos Control Assoc 1925, 3:303–304.
54. Sharma PC, Yelne MB, Dennis T J: Database on Medicinal Plants Used in Ayurveda. 2nd edition. New Delhi: Central Council for Research in Ayurveda and Siddha; 2001:89–93.
55. Liu ZL, Liu QR, Chu SS, Jiang GH: Insecticidal Activity and Chemical Composition of the Essential Oils of Artemisia lavandulaefolia and Artemisia sieversiana from China. Chem Biodivers 2010, 7:2040–2045.
56. Silva WJ, Dósa GA, Maia RT, Nunes RS, Carvalho GA, Blank AF, Alves PB, Marçal RM, Cavalcanti SC H: Effects of essential oils on Aedes aegypti larvae: Alternatives to environmentally safe insecticides. Bioresource Technol 2008, 99:3251–3255.
57. de Morais SM, Facundo VA, Bertini LM, Cavalcanti ESB, Anjos Júnior JF, Ferreira SA, de Brito ES, de Souza Neto MA: Chemical composition and larvicidal activity of essential oils from Piper species. Biochem Syst Ecol 2007, 35:670–675.
58. Koliopoulos G, Pitarokili D, Koulou S, Michaelakis A, Tzakou O: Chemical composition and larvicidal evaluation of Mentha, Salvia and Melissa essential oils against the West Nile virus mosquito Culex pipiens. Parasitol Res 2010, 107:327–335.
59. Pawlak R: Larvicidal property of essential oils against Culex quinquefasciatus Say (Diptera: Culicidae). Ind Crop Prod 2009, 30:311–315.
60. Ansari MA, Vasudevan P, Tandon M, Razdan RK: Larvicidal and mosquito repellent action of peppermint (Mentha piperita) oil. Bioresource Technol 2000, 71:267–271.
61. Dharmagadda VS, Naik SN, Mittal PK, Vasudevan P: Larvicidal activity of Tagetes patula essential oil against three mosquito species. Bioresource Technol 2005, 96:1235–1240.
62. Liu Z, He Q, Chu S, Wang C, Du S, Deng Z: Essential oil composition and larvicidal activity of Saussurea lappa roots against the mosquito Aedes albopictus (Diptera: Culicidae). Parasitol Res 2010, 1–6.
63. Gupta SC, Mishra S, Sharma A, Deepak Balaji TG, Kumar R, Mishra RK, Chowdhuri DK: Chloropyruvate induces apoptosis and DNA damage in Drosophila through generation of reactive oxygen species. Ecotox Environ Safe 2010, 73:1415–1423.
64. Ishii N, Seno-Matsuda N, Miyake K, Yasuda K, Ishi T, Hartman PS, Furukawa S: Coenzyme Q10 can prolong C. elegans lifespan by lowering oxidative stress. Mech Aging Dev 2004, 125:41–46.
65. Lau ATY, Wang Y, Chiu J-F: Reactive oxygen species: Current knowledge and applications in cancer research and therapeutic. J Cell Biochem 2008, 104:657–667.
66. Wilson DM, Sofonowski TM, McNeill DR: Repair mechanisms for oxidative DNA damage. Front Biosci: a journal and virtual library 2003, 8:963–981.

Cite this article as: Dua et al.: Insecticidal and genotoxic activity of Psoralea corylifolia Linn. (Fabaceae) against Culex quinquefasciatus Say, 1823, Parasites & Vectors 2013 6:30.

Submit your next manuscript to BioMed Central and take full advantage of:

• Convenient online submission
• Thorough peer review
• No space constraints or color figure charges
• Immediate publication on acceptance
• Inclusion in PubMed, CAS, Scopus and Google Scholar
• Research which is freely available for redistribution

Submit your manuscript at www.biomedcentral.com/submit