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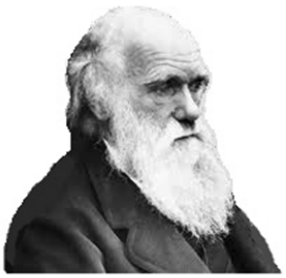

World News of Natural Sciences

An International Scientific Journal

WNOFNS 43 (2022) 1-10 EISSN 2543-5426

# Mosquito larvicidal and ovicidal properties of *Pelargonium graveolens* L. Herit. (Family: Geraniaceae*)* essential oil against three mosquito species

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**ABSTRACT**

The use of botanicals as an alternative to the chemical compounds is gaining tremendous momentum because of its multifarious advantages. In view of its increasing interest, an attempt was made in the present study to assess the larvicidal and ovicidal potential of important plant like *P. graveolens* against three mosquito species. The third instar larvae were exposed to different concentrations (i.e. 10, 20, 30, 40, 50 ppm) of methanol, diethyl ether, chloroform and ethyl acetate oil extracts of seed of *P. graveolens* plant. The mortality was recorded after 24 hrs exposure and $LC_{50}$ and $LC_{90}$ were determined. The ovicidal activity was determined against three mosquito species to different concentrations ranging from 70–350 ppm lower than the laboratory conditions. The present investigation revealed that the $LC_{50}$ values methanol, diethyl ether, chloroform and ethyl acetate extracts of *P. graveolens* against *An. stephensi* larvae were 21.54, 24.21, 26.34 and 22.83 mg/L respectively. Ovicidal activity of methanol extract was assessed by assessing the egg hatchability. Highest concentrations 210, 280 and 350 ppm of extract exhibited 100% ovicidal activity. The results clearly show that larvicidal and ovicidal activity was dose reliant. The highest larvicidal and ovicidal activity against *An. stephensi* was obtained with methanol extract of *P. graveolens.*

***Keywords*:** *Pelargonium graveolens, Aedes aegypti, Anopheles stephensi, Culex quinquefasciatus,* larvicidal and ovicidal

( Received 29 March 2022; Accepted 18 April 2022; Date of Publication 20 April 2022 )

## 1. INTRODUCTION

Mosquitoes are one of the mostly significant insect pests that affect the health and welfare of humans and domestic animals worldwide. The most important disease transmitting and nuisance causing mosquitoes belong to the genera *Aedes, Anopheles, Culex.* In India, the various species of *Aedes, Anopheles,* and *Culex* are important as carriers of diseases, malaria, dengue, chikungunya, filariasis, Japanese encephalitis and leishmaniasis causing millions of deaths every year in India (William, 2000; Ramesh *et al.,* 2010). The dengue fever incidence has increased fourfold since 1970 and nearly half the world's population is now at risk. In 1990, almost 30% of the world population, 1.5 billion people, lived in regions where the estimated risk of dengue transmission was greater than 50% (Hales *et al*., 2002). *An. stephensi* are major malaria vectors in India. With an annual incidence of 300-500 million, malaria is still one of the most important communicable diseases. Currently, about 40% of the world's population lives in areas where malaria is endemic (Subash *et al.,* 2019; Baranitharan *et al.,* 2019a; 2020; Kavitha *et al.,* 2020; Irrusappan *et al.,* 2022). *Cx. quinquefasciatus*, a vector of lymphatic filariasis, is widely distributed in tropical zones with around 120 million people infected worldwide and 44 million people having common chronic manifestation (Bernhard *et al.,* 2003; Jabanesan *et al.*, 2020; Muni Raj *et al.,* 2017).

Many studies on plant extracts against mosquito larvae have been conducted around the world. Ethyl acetate, hexane, chloroform and acetone extracts of *C. aromaticus* against third instar larvae of *Ae. aegypti, An. stephensi, Cx. quinquefasciatus* (Baranitharan *et al.,* 2014; 2015; 2016; 2017; 2019b; Dhanasekaran *et al.,* 2018). The phytochemicals derived from plant sources possess a complex of chemicals with unique biological activity. *Pelargonium graveolens* L. Herit. (Geraniaceae) is commercially cultivated in India. It is one of the important aromatic plants, yielding an essential oil which is highly prized for its very profound and strong rose-like odour. The oil also contains α-pinene, β-pinene, α-terpinene, myrcene, α-phellandrene, limonene, cis-ocimene, trans-ocimene, p-cymene, terpinolene, cis-roseoxide, trans-rose oxide, methone, trans-linalool, iso-menthone, caryophyllene, geranyl acetate, nerol, geranylformate, geranayl butyrate and geraniol. It's otherwise called Geranium. The pure geranium oil is almost a perfume by itself and blends well with all other perfumes. Therefore the here study was carried out to decide the larvicidal and ovicidal activity of *P. graveolens* seed extracts against vector mosquitoes.

## 2. MATERIAL AND METHODS

### 2. 1. Sample collection

Fresh, mature seeds of are collected from the Yercaudu of Nilgiris hills in India. Plant and seeds are properly authenticated in the Department of Botany, Annamalai University. Fresh, mature seeds are rinsed with distilled water and dried in a shed before powered.

### 2. 2. Extraction method

The dried powder of seeds was subjected for oil extraction with methanol, diethyl ether, chloroform and ethyl acetate, using Soxhlet apparatus. After collecting the crude oil, it was then allowed to condense in Rotary Vacuum Evaporator. The condensed crude oil extract was stored in refrigerator until required for investigation for larvicidal and ovicidal activity.

### 2. 3. Test mosquitoes

All tests were carried out against laboratory reared vector mosquitoes *viz.*, *Aedes aegypti*, (*Ae. aegypti*), *Anopheles stephensi* (*An. stephensi*) and *Culex quinquefasciatus* (*Cx. quinquefasciatus*) free of exposure to insecticides and pathogens. Cyclic generations of vector mosquitoes were maintained at 25-29 °C and 80-90 % relative humidity in the insectariums. Larvae were fed on larval food (powdered dog biscuit and yeast in the ratio of 3:1) and adult mosquitoes on 10 % glucose solution. Adult female mosquitoes were periodically blood-fed on restrained albino mice for egg production.

### 2. 4. Larvicidal activity

Standard WHO protocol with slight modifications was adopted for the study (WHO 1996). From the stock solution, the concentration of 10, 20, 30, 40 and 50 ppm was prepared. Early third instar larvae were introduced in 250 ml plastic cups containing 200 ml of water with each concentration. A control was prepared by the addition of acetone to water. Mortality was recorded after 24 hours. For each experiment, four replicates were maintained at a time. The observed percentage mortality was corrected by Abbott's Formula (Abbott, 1925).

### 2. 5. Ovicidal activity

Evaluation of the *P. graveolens* extracts for ovicidal activity was carried out by following the method of Su and Mulla (1998). Eggs were exposed to different concentrations ranging from 70 to 350 ppm. The desired concentrations of the test solutions were achieved by adding 1.0 ml of an appropriate stock solution to 99 ml of tap water. Each eggs raft containing 100 eggs of *Ae. aegypti, Cx. quinquefasciatus* and hundred eggs of *An. stephensi* were exposed to each dose of extract for 48hr. counting of eggs was done under a microscope. DSMO served as control. Four replicates for each concentration were maintained. After 24 hrs of incubation, the egg rafts or eggs exposed to each concentration were transferred to distilled water cups. The hatch rates were calculated by the following formula.

$$\text{Mortality} = \frac{\text{Mortality at treatment} - \text{Mortality at control}}{100 - \text{Mortality at control}} \times 100\%$$

### 2. 6. Statistical analysis

The analysis program probit (Finney, 1971) was used for the determination of $LC_{50}$, $LC_{90}$ and other statistics at regression, chi-square, slope, mean and standard deviation values were calculated using the SPSS 12.0 software.

## 3. RESULTS

The result of the larvicidal activity of crude methanol, diethyl ether, chloroform and ethyl acetate solvent extracts of *P. graveolens* against *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus.* The methanol extract of *P. graveolens* reported in the present study showed the mosquitocidal properties in the plant signifying their use in mosquito population control (Table 1). The data are presented in Table 1.

**Table 1.** Larvicidal activity of the *P. graveolens* extracts against *Ae. aegypti*, *An. stephensi* and *Cx. Quinquefasciatus.*

| Mosquitoes | Solvents | Mortality (%) ± SD | | | | |
|---|---|---|---|---|---|---|
| | | 50 ppm | 100 ppm | 150 ppm | 200 ppm | 250 ppm |
| ***Ae. aegypti*** | Methanol | 18.4±1.81 | 35.8±1.30 | 59.2±2.48 | 74.4±1.51 | 98.2±0.44 |
| | Diethyl ether | 12.8±2.28 | 29.6±2.19 | 49.8±2.16 | 67.4±2.19 | 92.8±1.30 |
| | Chloroform | 12.2±1.48 | 28.4±2.70 | 48.2±1.78 | 65.6±1.81 | 89.2±2.16 |
| | Ethyl acetate | 15.8±1.92 | 32.4±2.19 | 56.4±2.30 | 70.2±2.28 | 95.2±1.30 |
| | Hexane | 4.8±1.30 | 16.4±1.81 | 34.2±1.48 | 59.4±1.67 | 79.6±2.07 |
| ***An. Stephensi*** | Methanol | 22.2±1.09 | 41.8±2.28 | 65.6±1.94 | 89.8±2.77 | 100.0±0.00 |
| | Diethyl ether | 15.2±1.48 | 32.8±1.92 | 52.6±2.50 | 78.6±2.60 | 100.0±0.00 |
| | Chloroform | 14.4±2.30 | 30.2±2.48 | 50.6±1.67 | 76.4±1.51 | 100.0±0.00 |
| | Ethyl acetate | 18.8±1.64 | 38.4±1.81 | 57.8±1.64 | 82.2±1.48 | 100.0±0.00 |
| | Hexane | 6.8±1.64 | 19.4±1.51 | 36.2±1.78 | 64.2±2.68 | 92.6±1.67 |
| ***Cx. quinquefasciatus*** | Methanol | 24.4±181 | 44.2±1.78 | 68.4±1.81 | 93.6±1.94 | 100.0±0.00 |
| | Diethyl ether | 16.8±1.92 | 33.4±1.81 | 57.2±1.48 | 81.6±1.94 | 100.0±0.00 |
| | Chloroform | 15.8±1.48 | 32.6±1.94 | 56.2±1.78 | 79.8±2.16 | 100.0±0.00 |
| | Ethyl acetate | 20.4±2.19 | 40.2±1.78 | 60.2±2.48 | 89.6±2.30 | 100.0±0.00 |
| | Hexane | 12.8±1.48 | 28.6±1.51 | 53.4±1.34 | 72.2±1.09 | 100.0±0.00 |

Significant at P<0.05 level

**Table 2.** Probit analysis of Larvicidal activity of *P. graveolens* extracts against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefaciatus*

| Species | Extract | $LC_{50}$ | 95% Confidence limits | | $LC_{90}$ | $x^*$ | Regression |
|---|---|---|---|---|---|---|---|
| | | (mg/L) | LCL | UCL | (mg/L) | | |
| *Ae. aegypti* | Methanol | 129.04 | 118.40 | 139.14 | 231.76 | 7.136 | y=3.761x-2.611 |
| | Diethyl ether | 141.75 | 132.12 | 151.26 | 236.37 | 4.523 | y=3.384x-2.110 |
| | Chloroform | 145.84 | 136.04 | 155.60 | 243.44 | 3.620 | y=3.191x-1.777 |
| | Ethyl acetate | 137.59 | 126.93 | 147.91 | 253.56 | 4.325 | y=3.443x-2.103 |
| | Hexane | 164.56 | 154.12 | 175.44 | 272.09 | 1.431 | y=3.472x-2.740 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *An. stephensi* | Methanol | 111.66 | 101.67 | 120.90 | 200.01 | 4.732 | y=-2.666x+9.778 |
| | Diethyl ether | 133.23 | 102.58 | 161.41 | 223.73 | 9356 | y=-2.577x+9.328 |
| | Chloroform | 137.25 | 104.89 | 167.82 | 227.47 | 10.559 | y=-2.558x+9.240 |
| | Ethyl acetate | 123.32 | 113.33 | 132.77 | 216.79 | 7.658 | y=-2.723x+9.746 |
| | Hexane | 157.99 | 148.59 | 167.63 | 251.56 | 5.432 | y=3.855x-3.332 |
| *Cx. quinquefasciatus* | Methanol | 105.74 | 95.75 | 114.88 | 191.32 | 5.244 | y=-2.618x+9.769 |
| | Diethyl ether | 128.08 | 118.60 | 137.21 | 217.77 | 7.358 | y=-2.584x+9.404 |
| | Chloroform | 130.57 | 103.00 | 155.71 | 220.14 | 7.857 | y=-2.566x+9.334 |
| | Ethyl acetate | 118.91 | 108.76 | 128.42 | 212.31 | 6.709 | y=-2.601x+9.590 |
| | Hexane | 150.28 | 140.76 | 159.86 | 244.79 | 4.361 | y=-2.506x+9.094 |

Values represent mean of five replications. Mortality of the after 24 h of exposure period $LC_{50}$ = Lethal concentration brings out 50% mortality and $LC_{95}$ = Lethal concentration brings out 95% mortality. LCL = lower confidence limit; UCL = upper confidence limit

The methanol extracts of *P. graveolens* showed larval mortality. *An. stephensi* was more vulnerable followed by *Cx. quinquefasciatus* and *Ae. aegypti*. The methanol extract of *P. graveolens* exhibited the maximum larvicidal activity with $LC_{50}$ and $LC_{90}$ values of 21.54 and 27.73 mg/L against the larvae of *An. stephensi* (Table 2). Among the extracts tested for ovicidal activity against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*, the methanol extract of *P. graveolens* exerted 100% mortality (*i.e.*, no hatchability was recorded; Table 3) at 210, 280 and 350 ppm, respectively.

**Table 3.** Ovicidal activity of the *P. graveolens* extracts against *Ae. aegypti*, *An. stephensi* and *Cx. Quinquefasciatus*

| Species | Extract | Percentage of egg hatch ability | | | | | |
|---|---|---|---|---|---|---|---|
| | | Concentration (ppm) | | | | | |
| | | Control | 50 | 100 | 150 | 200 | 250 |
| *Ae. aegypti* | Methanol | 100±0.0 | 80.8±1.78 | 63.6±1.94 | 29.2±2.16 | 7.4±1.81 | NH |
| | Diethyl ether | 100±0.0 | 87.8±1.64 | 72.4±2.30 | 44.2±2.16 | 19.2±1.48 | 6.4±1.51 |
| | Chloroform | 100±0.0 | 90.6±1.81 | 79.2±2.16 | 52.6±2.30 | 24.2±2.16 | 16.2±1.48 |
| | Ethyl acetate | 100±0.0 | 85.4±2.30 | 66.8±1.48 | 31.6±1.81 | 14.2±2.04 | 3.6±1.67 |
| | Hexane | 100±0.0 | 96.8±1.64 | 80.6±1.81 | 64.2±2.16 | 42.6±2.30 | 24.2±1.48 |
| *An. stephensi* | Methanol | 89.2±2.5 | 75.4±1.51 | 45.8±2.28 | 21.6±2.30 | NH | NH |
| | Diethyl ether | 100±0.0 | 82.6±2.30 | 66.4±1.67 | 36.2±1.48 | 10.4±1.94 | NH |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Chloroform | 100±0.0 | 86.6±1.51 | 71.2±1.48 | 53.4±1.81 | 31.6±1.94 | 9.4±1.34 |
| | Ethyl acetate | 100±0.0 | 79.4±2.50 | 50.8±2.16 | 28.4±1.18 | NH | NH |
| | Hexane | 100±0.0 | 89.4±2.30 | 75.6±1.51 | 59.4±1.81 | 36.8±2.16 | 12.4±1.81 |
| *Cx. quinquefasciatus* | Methanol | 99.2±0.9 | 60.8±1.78 | 20.6±2.19 | NH | NH | NH |
| | Diethyl ether | 100±0.0 | 79.8±1.64 | 59.6±2.19 | 29.4±2.30 | NH | NH |
| | Chloroform | 100±0.0 | 84.2±1.78 | 63.6±2.30 | 45.4±1.94 | 20.8±2.16 | NH |
| | Ethyl acetate | 100±0.0 | 70.8±2.16 | 31.8±2.58 | 7.4±1.51 | NH | NH |
| | Hexane | 100±0.0 | 87.6±1.81 | 71.2±2.16 | 56.4±1.18 | 33.2±1.30 | 10.8±1.48 |

Values represent mean ± SD of the five replications

**Table 4.** Repellent activity of the *P. graveolens* extracts against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* at 5.0 mg/cm$^2$

| Species | Extract | % of repellency | | | | | |
|---|---|---|---|---|---|---|---|
| | | Time post application of repellent (min) | | | | | |
| | | 40 | 80 | 120 | 160 | 200 | 240 |
| | Methanol | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 |
| *Ae. aegypti* | Diethyl ether | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 | 97.4±1.14 |
| | Chloroform | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 | 96.2±1.92 | 88.4±1.81 |
| | Ethyl acetate | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 |
| | Hexane | 100±0.00 | 100±0.00 | 100±0.00 | 97.8±1.64 | 90.2±2.16 | 78.4±2.60 |
| | Methanol | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 |
| *An. stephensi* | Diethyl ether | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 | 97.8±1.64 | 89.6±2.19 |
| | Chloroform | 100±0.00 | 100±0.00 | 100±0.00 | 96.8±1.30 | 86.2±3.27 | 75.2±1.64 |
| | Ethyl acetate | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 | 96.8±2.48 |
| | Hexane | 100±0.00 | 100±0.00 | 96.6±1.94 | 87.6±2.30 | 77.2±2.16 | 66.2±1.48 |
| | Methanol | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 |
| *Cx. quinquefasciatus* | Diethyl ether | 100±0.00 | 100±0.00 | 100±0.00 | 97.6±1.67 | 88.4±2.50 | 78.6±2.88 |
| | Chloroform | 100±0.00 | 100±0.00 | 94.4±1.81 | 84.2±2.38 | 72.6±2.60 | 61.8±2.48 |
| | Ethyl acetate | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 | 100±0.00 | 92.8±2.38 |
| | Hexane | 100±0.00 | 92.8±2.16 | 83.2±2.68 | 72.2±2.16 | 61.6±2.30 | 49.2±2.28 |

Values represent mean ± SD of the five replications

## 4. DISCUSSION

The results of present study are comparable with earlier reports Dhanasekaran *et al.*, (2013) have that the $LC_{50}$ of ethanol crude extracts of selected indigenous medicinal plants are *G.ula* extract in the experimental larvae of *An. stephensi* ($LC_{50}$ = 82.86 ppm), followed by *S. hispida* ($LC_{50}$ = 89.45ppm). Baluselvakumar *et al.,* (2012) reported that the$LC_{50}$ and $LC_{90}$ values of methanol *O. esculentum* leaf extract against *An. stephensi* were 63.84 and 122.48 ppm, respectively. Krishnappa *et al.,* (2012) reported that the $LC_{50}$ and $LC_{90}$ values of methanol extract against *An. stephensi* were 78.18 and 155.42 mg/l, and *A. digitata* exerted 100% up to 150 min at 4 and 6 mg/cm$^2$. Cent percent repellency of *An. stephensi* was noticed up to 180 min with chloroform extract at concentrations. But, hexane extract showed 100% repellency up to 120min, whereas methanol extract showed strong repellency up to 210 min except the minimal concentration (2 mg/cm$^2$).

Baranitharan and Dhanasekaran (2014a) reported that the evident larvicidal activity of ethyl acetate extract followed by hexane, chloroform and acetone extracts of *C. caudata* showed $LC_{50}$ values of *Ae. aegypti* are 97.19, 112.85, 99.17 and 109.67 mg/L; *An. stephensi* are 96.04, 104.16, 97.13 and 106.53 mg/L; *Cx. quinquefasciatus* are 94.76, 102.95, 95.98 and 105.09 mg/L, respectively. Krishnappa *et al.,* (2013) reported that the $LC_{50}$ and $LC_{90}$ values of *C. quadrangularis* and *C. ovalifolium* methanol extract against *An. stephensi* were 37.48 and 74.53 mg/L, respectively. Among two plant solvents tested, *C. quadrangularis* extracts were found to be most significant ovicidal activity 100% eggs mortality observed at 50 ppm and 350 ppm for *C. quadrangularis*. Gokulakrishnan *et al.,* (2012) reported that the larvicidal and ovicidal efficacy of different solvent leaf extract of *A. indica* against *An. stephensi*.

The hatch rates were assessed 48 h after treatment. The $LC_{50}$ and $LC_{90}$ values of acetone, benzene, chloroform, hexane and methanol extracts of *A. indica* against *An. stephensi* larvae in 24 h were 76.29, 58.82, 53.59, 65.84, 51.78 and 205.85, 193.23, 185.16, 196.72 and 181.00 ppm, respectively. The larvicidal activity of different solvents extracts of *C. sparciflrorus* were tested against the three vector mosquitoes. Among the different solvents the maximum larvicidal activity was observed in ethyl acetate. The $LC_{50}$ and $LC_{90}$ values of *C. sparciflorus* against three vector mosquitoes of *A. aegypti, An. stephensi* and *Cx. quinquefasciatus* were 34.02, 28.88 and 36.22 ppm, 74.57, 65.35 and 79.89 ppm, respectively (Baranitharan *et al.,* 2014b).

Baluselvakumar *et al.,* (2012) reported that the methanol plant extract of *M. maderaspatana* had ovicidal and repellency against *Ae. aegypti* with the methanol extract of *M. maderaspatana* exerted 100% egg mortality at 120, 160, 200 and 240 ppm for *Ae. aegypti*, and a higher concentration of 3.0 mg/cm$^2$ methanol extract of *M. maderaspatana* provided 100% protection up to 80, 100,120 and 140 min. Baranitharan and Dhanasekaran (2014b) reported that the larvicidal activity of diethyl ether followed by hexane, benzene and acetone extracts of *C. aromaticus* showed 73.49, 85.93. 76.03 and 80.56 mg/L, respectivey. Elumalai *et al.,* (2012) reported that the *E. roseum* acetone and methanol extracts of $LC_{50}$ values of 121.65 and 139.86 ppm, it was that 100% mortality was noted from the acetone and methanol extracts of 100 ppm.

The leaf extract of *C. vulgaris* with different solvents were tested for repellent activities against *A. stephensi* and showed that Skin repellent test at 1.0, 2.5 and 5.0 mg per cm$^2$ concentration gave the mean complete protection time ranged from 119.17 to 387.83 minutes with the four different extracts tested (Mullai 2008). These results could encourage the search for new active natural compounds offering an alternative to synthetic repellents and insecticides from other medicinal plants.

## 5. CONCLUSION

In conclusion, an effort has made to evaluate the role of *P. graveolens* different extracts for their larvicidal and ovicidal activities against three mosquito species. As these plant species is distributed throughout the Tamilnadu, it can help to generate local employment, minimize the dependence on expensive synthetic pesticides and also stimulate local efforts to enhance public health. Further studies on the larvicidal and ovicidal mode of action, their efforts on non-target organisms and formulations for improving the insecticidal potency of methanol extract of *P. graveolens* are in progress.

**ACKNOWLEDGEMENTS**

Authors are thankful to the Professor & Head, Department of Zoology and Botany, Annamalai University, Chidambaram, and grateful to the University Grant Commission, New Delhi, for providing financial assistance under UGC-Major Research Project (Letter No. F 42-597/2013 (SR), dt. 25.03.2013).

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