Pest potential of Pisonia alba extracts and fractions against mosquito-borne disease (Diptera: Culicidae)

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Pest potential of *Pisonia alba* extracts and fractions against mosquito-borne disease (Diptera: *Culicidae*)

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4 University of Nigeria, Enugu state, Nigeria

Abstract

Mosquitocidal activity of *Pisonia alba* leaf extracts was tested against *Anopheles stephensi*, *Culex quinquefasciatus*, *Aedes aegypti*. Totally Twenty five early fourth instars larvae of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* (*A. stephensi*, *C. quinquefasciatus* and *A. aegypti*) were exposed to various concentrations (50-250 ×10⁶) and the 24 hrs LC₅₀ values of the *Pisonia alba* extracts was determined by probit analysis and ovicidal activity, determined against *A. stephensi*, *C. quinquefasciatus* and *A. aegypti* to various concentrations ranging from 1.0, 2.0 and 4.0 mg/cm² ppm under laboratory conditions. The eggs hatchability was assessed 48 hrs post treatment. The LC₅₀ and LC₉₀ values of *Pisonia alba* petroleum ether extract against *A. stephensi* were 100.62 and 117.80 ×10⁶, respectively; *C. quinquefasciatus* was 98.52 and 112.75 ×10⁶, respectively; For, *A. aegypti* were 111.29 and 141.16 ×10⁶, respectively. The ovicidal activity of *Pisonia alba* exerted 100% mortality at 240, 300 and 360 ×10⁶ against *C. quinquefasciatus* and for repellency activity was definite against *A. stephensi*, *C. quinquefasciatus* and *A. aegypti* species at three concentration like 1.0, 2.0 and 4.0× 10⁻² kg.m⁻² under the laboratory conditions. The petroleum ether extract of *Pisonia alba* establish to more repellent than the additional extracts. A higher concentration of 4.0 ×10⁻² kg.m⁻² provided 100% protection up to 120, 160 and 200 minutes, respectively. The outcome clearly shows that larvicidal ovicidal and repellent activity was dose reliant. From the results it can be concluded the petroleum ether extract of *Pisonia alba* was an outstanding potential for controlling the vector mosquito *C. quinquefasciatus*.

Keywords: *Anopheles stephensi*, *Culex quinquefasciatus*, *Aedes aegypti*, larvicidal, ovicidal and repellent activity, *Pisonia alba*

1. Introduction

The plant realm is considered an asset for various kinds of potential drugs. In ancient days, many of the diseases were cured using plant products, and now again, there is an increasing awareness among people about the significance of plants and their medicinal ethics1 World Health Organization, more than 80% world population were cured using plant products, and now again, there is an increasing awareness among people about the significance of plants and their medicinal ethics. The plant realm is considered an asset for various kinds of potential drugs. In ancient days, many of the diseases went back to traditional medicines2. *Pisonia alba* leaf extract (Nyctaginaceae) is widely spread all over India33 and it is an evergreen commonly grown lettuce tree. Leaves, stem and root of this species are extensively used by the tribal’s in the preparation of several folk medicines. It has been extensively used in Indian traditional medicine as an ant diabetic, anti-inflammatory agent, and used in the treatment of ulcer, dysentery and snake bite4. *Pisonia alba* has good potential therapeutic plant5. Mosquito-borne diseases menace the living and livelihood of millions of people worldwide. Malaria continues to be a paramount disease, blighting 300 million Africans globally, despite the advancement and achieved using indoor residuary spraying and pesticide treated bed nets. It is increasingly difficult to control due to the spread of insecticide defiance in the mosquito vectors and also defiance of the parasite to the available drugs. In terms of dengue, 2.5 billion people live at danger fever of infection with one or more serotypes of the virus, which cause an estimated 390 million infections per year, and the affected area has been increased rapidly in the past 30 years. Chikungunya breakouts in Europe have worn the notice of that western world to this disease extend by the Asian tiger mosquito, *Ae. albopictus*6-14. Malaria is one in all the grave scourges inflicted upon human beings. It causes human mortality and morbidity alongside giant economic loss. Roughly all tropical regions of the planet area unit expertise the recovery and reoccurrence of 1 of the world’s an outsized quantity deadly diseases, ie. malaria and India is not any omission. Malaria afflicts one year of the planet folks i.e. 2020 million in 107 countries and territories placed within the tropical and semitropical regions15. In line with the newest estimates, there have been regarding 198 million cases of malaria in 2013 and a calculable 584,000 deaths. Most deaths occur among youngsters living in continent,
wherever a baby dies each minute from malaria. Malaria mortality rates among youngsters in continent are
reduced by Associate in nursing calculable fifty eight since 200016. The dipteran Cr. quinquefasciatus is a
crucial feature inflicting filariasis, West Nile virus, Avion malaria and St. Louis encephalitis. Cr. quinquefasciatus, besides known as the southern house dipterans, is extensively studied because it transmits
crucial diseases17. In 2014, estimate is impure with lymphatic filariasis parasites and more than 20 for every
penny of the planet populace is at danger of getting roundworm disease. In Asian nation it's calculable that
regarding 554.2 million folk’s area unit at hazard of humor disease unhealthiness in a pair of 43 districts 18.
Worldwide, twenty five million men clumsy person with sex organ sickness and over 15 million folks are
afflicted with lymphoedema19.

These diseases challenge the urbanized and rising countries of the world for irradiation. Pesticides have
numerous beneficial effects. These comprise crop protection, preservation of food and material and preclusion
of vector-borne diseases. Hence, there is emergency need to carry out research on the use natural plant extracts
in reducing the mosquito population.

2. Material and methods

Collection of Plant

Pisonia alba was collected from the natural population in Mariyappa Nagar, Chidhambaram, Cuddalore
district of Tamil Nadu, India, and identified in Department of Zoology, Annamalai University, Annamalai
Nagar, Tamil Nadu, and India. The complete plant dried under darkness at room temperature for about 15 days.

Extraction

The solid leaves were washed with sterile refined water, darkness dried, and finely ground. the finely ground
leaf powder (500 g/ml) was extracted with hexane, chloroform, diethyl ether, ethyl acetate and methanol
exploitation Soxhlet extraction equipment, and therefore the extraction was continued until visibly no more
extraction is feasible (by discerning the color of the extracted portion). The solvent from the extract area unit
removed employing a rotary vacuum evaporator to gather the crude extract and keep at 4ºC. Normal stock
solutions were ready at 1 % by dissolving the residues in plant product. From this stock solution, totally
different concentrations were ready and this solution is employed for larvicidal activity.

Mosquito Rearing

The mosquitoes, A. aegypti, A. stephensi and C. quinquefasciatus were procured from the Centre for research in
Medical entomology (ICMR), Viruddhachalam, reared within the laboratory condition, Department of zoology,
Annamalai University. The larvae be gobbled dog biscuits  and yeast powder within the 3:1 magnitude relation.
Adults were supplied from 100 percent sucrose solution and one week previous chick for feed. Mosquitoes were
control at (28±2) ºC, 70%-85% ratio (RH), with a photo amount of 14 h lightweight, 10 h dark.

Larvicidal activity

The larvicidal activity of Pisonia alba extract fractions were assessed according to the convention beforehand
portrayed10. In view of the wide range and thin range tests, all concentrates tried disappearing from 10-50 ppm
were ready and they were tried against the newly shed (0-6 hrs) third instar hatchlings of choosing mosquito
species. The plants concentrate were disintegrated in 1 ml DMSO (Dimethyl sulfoxide) and afterward weakened
in 249 ml of dechlorinated faucet water to acquire each of the fancied focuses. The control was readied utilizing
1ml of DMSO as a part of 249 ml of dechlorinated water. The total hatchlings test species (25) were obtainable
in 250 ml plastic glass containing 250 ml of fluid medium (249 ml of dechlorinated water + 1ml of Dimethyl
Sulfoxide) and the required to measure of the compound synthesis was incorporated. The total larval mortality
was recorded after 24 h of post treatment. For each examination, five recruits were kept up at once. Percent of
mortality was rectified for control mortality utilizing 11.

Ovicidal activity

The method of 27 was followed to check the ovicidal activity. The leaf extracts was diluted within the several
solvent to attain completely different concentrations (60, 120, 180, 240, 300 and 360 ppm). The freshly ordered
egg raft containing 100 eggs of A. aegypti, An. stephensi and Cx. quinquefasciatus were exposed to every dose
of leaf extract till they hatched or died. Every concentration was replicated six times. Eggs exposed to several
solvents in water served 48 h post treatment by the subsequent formula:

\[
\text{Egg hatchability} \times 100 = \frac{\text{Number of hatched larvae}}{\text{Total number of eggs}}
\]

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Toxicity on non-target aquatic organisms

The methodology developed by 16 was used to assess the fractions effect on non-target organisms. The E. variegata fractions were tested for toxicity against three non-target mosquito predators, namely Diplonychus indicus, Anisops bouvieri and Gambusia affinis. These organisms were collected in the field and kept separated in cement tanks (85-cm wide and 30-cm deep), containing water at 27±3 °C and external relative humidity of 85%. The fractions of E. variegata were examined at concentrations that were 50 times higher than the LC50 doses for mosquito larvae. Five replicates were carried out for each concentration, accompanied by four iterations of untreated controls. In additions, non-target organisms beneath test were observed successively for ten days to investigate the post-treatment influence of the extract on their continued existence and swimming ability.

Data analysis

Mortality data were subjected to probity analysis. LC90 (LD90) and LC50 (LD50) were estimated via Finney methodology23. ANOVA analysis, followed by Tukey’s HSD test (P>0.05) were in employment to investigate ovicidal data. The Suitability Index (SI) was used to assess biotoxicity on non-target organisms; the Index was calculated through the following formula20.

\[
SI = \frac{LC50 of \ non\ - \ target\ organisms}{LC50 of \ target\ vector\ species}
\]

Data analysis was carried out using the SPSS Statistical Software Package version 16.0. The significance of differences between values was assessed at the 0.05 probability level.

3. Results

Medicinal plants are produce on vector control. These plants can be used to develop environmentally it present terpenoids compound present in primary screening (Table 1) safe vector and pest administration agents. The regression equation (Based on Probit Analysis) between the concentrations of petroleum ether, acetone, benzene and hexane solvent extracts against Anopheles stephensi, Culex quinquefasciatus and Aedes aegypti after 24 h exposure are represented in (Table 2) and the petroleum ether extract of Pisonia alba reported in the present study showed the larvicidal exploit in the plant betokening their utilize in mosquito population control (Table 2-4). The result clearly stimulated that petroleum ether and benzene solvent plant extract at very low concentrations was toxic against the entire three mosquito species tested when compared to acetone and hexane extracts. The LC50 and LC90 value for petroleum ether extract of Pisonia alba against Cx. quinquefasciatus at 24 h post treatment was 98.95 ×10^6 and 112.75 ×10^6 respectively. Petroleum ether extracts were also effectual against An. stephensi larvae with LC50 and LC90 value of 100.62 ×10^6 and 117.80 ×10^6, respectively. The LC50 and LC90 value of petroleum ether against A. aegypti after 24 h post treatment was 111.29 ×10^6 and 141.16 ×10^6, respectively. Among the extracts tested for ovicidal activity against Cx. quinquefasciatus, the petroleum ether extract of Pisonia alba exerted 100% mortality (i.e., no hatchability was recorded this research) at 240, 300 and 360 ppm, respectively.

4. Discussion and conclusions

In our results outcomes demonstrated that, the chemical composition of Pisonia alba leaf have noteworthy larvicidal, ovicidal movement against human vector malarial mosquito, An. stephensi, Cx. quinquefasciatus and Ae. aegypti. The investigated that the larvicidal, pupicidal, repellent and adulticidal activity of Citrus sinensis against An. stephensi, A. aegypti and Cx. quinquefasciatus. The most noteworthy concentrations of 450 ×10^6 provided over 180 and 150 min. protection in ethanol extracts of Citrus sinensis against Cx. quinquefasciatus. Among three vectors tested, the highest adulticidal activity was observed in high mortality followed by An. stephensi, A. aegypti and Cx. Quinquefasciatus35. The larvicidal, oviposition deterrent and repellent activity of Annona squamosa against A. aegypti, An. stephensi and Cx. quinquefasciatus. The LC50 and LC90 values of 219.41 and 394.87×10^6 severally. In oviposition deterrent activity the best concentration of 0.1%, Annona squamosa manufacture 92.4% against A. aegypti. Skin repellent check at 0.02 ppm concentration of Annona squamosa offers the entire protection time ranges from 50.4 to 271 minutes. The Annona squamosa exerted the best protection time of 126.2 minutes 38-34 examination that the larvicidal potential of Murraya exotica essential oil against A. aegypti, An. stephensi and Cx. quinquefasciatus. After 12 h of introduction period, the larvicidal action are LC50=74.7 and LC90=152.7 ppm; after 24 h presentation period were LC50=35.8 and LC90=85.4 ppm, respectively against A. aegypti. The most noteworthy mortality was found in acetone extract against Ae. aegypti with LC50 and LC90 estimations of 4.1783 and 9.3884 g/l individually. Smoke poisonous quality was seen at 10 min interrim for 40 min, and the mortality information was recorded 22. The investigated that the larvicidal, ovicidal and repellent activity of Polygala arvensis benzene and methanol extracts tested against A. aegypti, An. stephensi and Cx. quinquefasciatus with maximum LC50 and LC90 values of methanol extract of Polygala arvensis were 58.21, 46.37 and 42.68 ×10^6 208.45, 189.82 and 130.44×10^6, respectively. The maximum ovicidal activity of methanol extracts against A. aegypti, An. stephensi and Cx. quinquefasciatus at
200 ppm concentration. The highest repellent activity of methanol extracts provided 100% protection against A. aegypti, An. stephensi and Cx. quinquefasciatus for 280 minutes. Among the different extracts of the plants screened the hexane extract of Pisonia albarecorded the highest ovicidal activity of 79.2% at 500 ppm concentration against the eggs of Cx. quinquefasciatus. Among the Aegle marmelos, Limonia acidissima, Sphaeranthus indicus, Sphaeranthus amaranthoides and Chromolaena odorata extract screened, the hexane extract of Pisonia alba noted the 100% oviposition deterrent activity at tested concentrations against Cx. quinquefasciatus and A. aegypti adult females. The highest lethal activity was recorded against Gnetum ula extract in the experimental larvae of An. stephensi (LC₅₀ = 82.86 ppm). Ovicidal activity revealed that Spermacoce hispida showed more than 50% activity against A. aegypti, An. stephensi and Cx. quinquefasciatus. Notably, at 200 ppm concentration of all the plants showed 100% ovicidal activity against An. stephensi, followed by A. aegypti and Cx. quinquefasciatus. The selected two plants, Gnetum ula, Spermacoce hispida extract offers 100% protection against An. stephensi, A. aegypti and Cx. quinquefasciatus adult female mosquitoes as far as repellency up to 120 minutes of presentation periods. Hence this study clearly reveals that the extracts of S. indicum seem to be made in phytochemicals, ovicidal and repellent activity, wide utilized in traditional drugs to combat and cure varied ailments. The antispasmodic, anti-inflammatory, antidiuretic drug and antianalgesic is attributed to their high steroids, terpenoids, tannins and saponins and extraction of traditional drugs to combat and cure varied ailments. The antispasmodic, anti-inflammatory, antidiuretic drug and antianalgesic is attributed to their high steroids, terpenoids, tannins and saponins and extraction of traditional drugs to combat and cure varied ailments.

In conclusion, the present study results that medicinal plant Pisonia alba have the potential for the development of new and safe control products and exhibits larvicidal and ovicidal activity against important mosquitoes. Furthermore, the results of the present study may donate to a diminution in the relevance of synthetic insecticides, which in turn increases the opening for nature control of various medically significant mosquitoes. Osong. Public. Health. Res. Perspect. 2015, 6(3), 232-235. The authors declare that they are no conflict of interest regarding this manuscript.

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Conflict of Interest:
The authors declare that they are no conflict of interest regarding this manuscript.

REFERENCES


**Table 1.** Phytochemical screening of plant extract of *Pisonia alba*

<table>
<thead>
<tr>
<th>S.no</th>
<th>Phyto constituents</th>
<th>Methanol</th>
<th>Ethyl acetate</th>
<th>Acetone</th>
<th>Benzene</th>
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<tr>
<td>2</td>
<td>Flavonoids</td>
<td>++</td>
<td>++</td>
<td>--</td>
<td>+</td>
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<tr>
<td>3</td>
<td>Saponins</td>
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<td>--</td>
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<tr>
<td>4</td>
<td>Steroids</td>
<td>--</td>
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<td>--</td>
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<td>Tannins</td>
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<td>+++</td>
<td>++</td>
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<td>++</td>
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<td>Anthraquinones</td>
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<td>++</td>
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<td>Amino acid</td>
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<td>12</td>
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<tr>
<td>13</td>
<td>Protein</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>Phytosteroids</td>
<td>+++</td>
<td>+</td>
<td>--</td>
<td>+</td>
</tr>
</tbody>
</table>

“+++” Strongly positive phytochemical group, “++” Positive phytochemical group, “+” Trace phytochemical group, “-” Absence of phytochemical group
Table 2
Larvicidal activity of the *Pisonia alba* extracts against third instar larvae of *An. stephensi* *Cx. quinquefasciatus* and *Ae. aegypti*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Extracts</th>
<th>LC₅₀ (ppm)</th>
<th>LCL-UCL</th>
<th>LC₉₅ (ppm)</th>
<th>Slope</th>
<th>Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>An. Stephensi</strong></td>
<td>Petroleum ether</td>
<td>100.62</td>
<td>97.62-103.71</td>
<td>117.80</td>
<td>2.029896</td>
<td>Y=2.029x+0.508</td>
</tr>
<tr>
<td></td>
<td>Acetone</td>
<td>107.41</td>
<td>103.90-111.03</td>
<td>129.23</td>
<td>2.16047</td>
<td>Y=2.160x-0.185</td>
</tr>
<tr>
<td></td>
<td>Benzene</td>
<td>104.81</td>
<td>101.47-108.25</td>
<td>124.96</td>
<td>1.969739</td>
<td>Y=1.969x+0.358</td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td>111.52</td>
<td>107.53-115.67</td>
<td>137.71</td>
<td>2.424203</td>
<td>Y=2.424x-0.934</td>
</tr>
<tr>
<td><strong>Cx. quinquefasciatus</strong></td>
<td>Petroleum ether</td>
<td>98.52</td>
<td>95.95-101.16</td>
<td>112.75</td>
<td>2.274699</td>
<td>Y=2.274x+0.179</td>
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<td>Acetone</td>
<td>103.92</td>
<td>100.58-107.37</td>
<td>123.92</td>
<td>2.00234</td>
<td>Y=2.002x+0.358</td>
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<tr>
<td></td>
<td>Benzene</td>
<td>101.03</td>
<td>98.21-103.93</td>
<td>117.15</td>
<td>2.159682</td>
<td>Y=2.159x+0.213</td>
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<tr>
<td></td>
<td>Hexane</td>
<td>108.44</td>
<td>104.65-112.36</td>
<td>132.45</td>
<td>2.002549</td>
<td>Y=2.002x+0.106</td>
</tr>
<tr>
<td><strong>Ae. aegypti</strong></td>
<td>Petroleum ether</td>
<td>111.29</td>
<td>106.80-115.98</td>
<td>141.16</td>
<td>1.724065</td>
<td>Y=1.724x+0.609</td>
</tr>
<tr>
<td></td>
<td>Acetone</td>
<td>122.48</td>
<td>116.44-128.84</td>
<td>168.06</td>
<td>1.852355</td>
<td>Y=1.852x+0.017</td>
</tr>
<tr>
<td></td>
<td>Benzene</td>
<td>115.60</td>
<td>110.77-120.64</td>
<td>149.13</td>
<td>2.071039</td>
<td>Y=2.071x+0.300</td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td>127.76</td>
<td>120.74-135.19</td>
<td>183.71</td>
<td>1.626349</td>
<td>Y=1.626x+0.409</td>
</tr>
</tbody>
</table>

Value represents mean of five replications. Mortality of the after 24 h of exposure period LC₅₀=Lethal concentration brings out 50% mortality and LC₉₅=Lethal concentration brings out 95% mortality. LCL= lower confidence limit; UCL= upper confidence limit; values in a column with a different superscript alphabet are significantly different at \(P<0.05\) level DMRT Test.
### Table 3

Ovicidal activity of the *Pisonia alba* extracts against third instar larvae of *An. stephensi* *Cx. quinquefasciatus* and *Ae. aegypti*.

<table>
<thead>
<tr>
<th>Species</th>
<th>Extract</th>
<th>Concentration used (ppm)</th>
<th>Percentage of egg hatch ability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>120</td>
</tr>
<tr>
<td><em>An. stephensi</em></td>
<td>Petroleum ether</td>
<td>71.3±2.5a</td>
<td>53.8±2.5a</td>
</tr>
<tr>
<td></td>
<td>Acetone</td>
<td>92.1±2.2c</td>
<td>71.5±2.3c</td>
</tr>
<tr>
<td></td>
<td>Benzene</td>
<td>88.5±3.8b</td>
<td>67.8±3.0b</td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td>97.5±1.7d</td>
<td>78.1±2.5d</td>
</tr>
<tr>
<td><em>Cx. quinquefasciatus</em></td>
<td>Petroleum ether</td>
<td>66.1±2.7a</td>
<td>39.5±2.5a</td>
</tr>
<tr>
<td></td>
<td>Acetone</td>
<td>89.3±1.6c</td>
<td>67.3±2.3c</td>
</tr>
<tr>
<td></td>
<td>Benzene</td>
<td>80.1±2.0b</td>
<td>59.8±1.7b</td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td>94.5±1.7d</td>
<td>79.8±1.3d</td>
</tr>
<tr>
<td><em>Ae. aegypti</em></td>
<td>Petroleum ether</td>
<td>85.3±1.8a</td>
<td>60.8±2.4a</td>
</tr>
<tr>
<td></td>
<td>Acetone</td>
<td>95.3±1.7c</td>
<td>77.8±2.5c</td>
</tr>
<tr>
<td></td>
<td>Benzene</td>
<td>92.5±1.7b</td>
<td>76.1±2.1b</td>
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<tr>
<td></td>
<td>Hexane</td>
<td>98.8±0.9d</td>
<td>82.3±1.6d</td>
</tr>
</tbody>
</table>

NH- No hatchability; values are mean of six replicates ±SD. of five replications. Different alphabets in the column are statistically significant at $P \leq 0.05$ level DMRT Test.