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Impact of *Catharanthus roseus* leaf ethanol extract on survival, growth and development of *Dysdercus koenigii* (Heteroptera: Pyrrhocoridae)

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The effects of *Catharanthus roseus* leaf ethanol extract (*Cr*-LEE) on the survival, growth, and development of *Dysdercus koenigii* Fabricius were studied. The experiments were conducted on 0-12 hour-old fifth instar nymphs reared and maintained in the laboratory under optimum conditions. *Catharanthus roseus* leaf ethanol extract (*Cr*-LEE) was applied topically at doses 40, 100, 200, and 400 µg/Insect to the fifth instar nymphs. The results indicate that treating fifth-instar nymphs with *Cr*-LEE affected their survival and development. The effects on survival, growth, and development were dose-dependent. Treatment at doses 200 and 400 µg/Insect shows high mortality, especially during the later part of their life. Different developmental anomalies, such as supernumerary nymphal stage, adultoid, adult with deformed wings, adult with exuviae attached to different parts of the body, and adult with shrunken body, were observed. Often, the nymphs died due to abortive moulting. The gas chromatography and mass spectroscopy (GC-MS) analysis of the *Cr*-LEE revealed the presence of chemical compounds having insecticidal and insect-repellent activity and compounds that are intermediate in the juvenile hormone (JH) biosynthetic pathways. These compounds may act as JH mimics or Juvenoids for insect pests. Therefore, it is apprehended that the phytoconstituents of *Cr*-LEE singly or synergistically affect the survival, growth and development of *D. koenigii*.

Keywords: *Catharanthus roseus*, *Cr*-LEE, *Dysdercus koenigii*, GC-MS, Growth and development

IPC code; Int. cl. (2021.01)− A01N, A01N 25/00, A01N 65/00, A01P

Introduction

The cotton stainer *Dysdercus koenigii* F. (Heteroptera: Pyrrhocoriedae) is a destructive pest of cotton and many other crops of economic importance across many countries of Asia[1], including India. Both the adults and the nymphs suck the sap mainly from the seeds of the cotton and other plants of family Malvaceae. In this way, they reduce the germinating potential of the seed. Deposition of faecal matter and body fluids of the adults and the nymphs on the cotton balls causes staining of the lint; the lint quality is further deteriorated by the premature fall of the cotton balls[2,3]. The use of chemical pesticides is one of the most prevalent methods of controlling insect pests. However, chemical pesticides have serious concerns related to human health, environmental contamination, and harm to beneficial insects and non-target organisms. The insects often develop resistance against chemical insecticides, which makes the synthetic insecticides ineffective and leads to pest resurgence[4–6]. Therefore, a search for eco-friendly, sustainable, and environmentally safe agents to control insect pests is important. Green pesticides are plant-derived products or chemicals that can be used to control the insect pests population under the Integrated Pest Management (IPM) program[4].

Catharanthus roseus is an evergreen herb belongs to the family Apocynaceae[7]. It has several medicinal properties such as anti-microbial, antioxidant, anti-diabetic, hypolipidemic, wound healing, hypotensive, anti-ulcer, antidiarrhoeal, and memory enhancement[8]. Previous studies suggested that *C. roseus* has insecticidal, larvicidal, insect repellent,

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and antifeedant activities against many insects such as *Earias vittella*, *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles stephensi*[9,10]. Acetone extract of *C. roseus* showed IGR activities against the *Spodoptera litura* Fab. And *Helicoverpa armigera* Hub.[11]. Present research studied the growth regulatory activities of *C. roseus* leaf ethanol extract against *D. koenigii*.

Materials and Methods

Preparation of plant extract

Leaves of *C. roseus* were collected in the month of April 2023 from the Deshbandhu College Botanical garden, New Delhi, India (latitude: 28.540243, longitude: 77.255602). Leaves were washed three times with running tap water and then shade-dried at room temperature. The dried leaves were ground to make a fine powder with the help of a grinder. For phytochemical extraction, a Soxhlet apparatus was used[12,13]. Absolute ethanol was used as a solvent to extract the phytochemicals from the leaf powder. About 30 g of finely ground *C. roseus* leaf powder was extracted using 300 mL of absolute ethanol. The extraction process was run for 24 h to ensure the complete extraction of the phytochemicals. The crude extract was desiccated at 40°C, 120 rpm, by using the rotatory evaporator (Buchi)[14]. A stock solution of 20% *C. roseus* leaf ethanol extract (*Cr*-LEE) was prepared and stored at 4°C. Different test concentrations viz., 20, 10, 5, and 2% were prepared using absolute ethanol as a solvent by the serial dilution of the stock solution.

Rearing and Maintenance of *D. koenigii* Culture

A culture of *D. koenigii* was maintained in a BOD incubator at a temperature of 27±1°C, humidity of 70±5%, and 12 hours of light & 12 hours of dark photoperiod. Insects were reared in a 500 mL sterilized glass jar. Cotton seeds were given as food to the insects, and a cotton swab soaked in distilled water was kept in each jar as a water source. Newly emerged fifth instar nymphs of age group 0-12 hours were separated and used for the experimental purpose[14].

Treatment of Fifth Instar Nymphs of *D. koenigii* with *Cr*-LEE

The experimental fifth instar nymphs were treated with the *Cr*-LEE by topical application[15]. A volume of 2 µL of test concentrations viz. 20, 10, 5, and 2% was applied at the dorsum of the experimental insect. This constituted effective doses of treatments: 400, 200, 100, and 40 µg/Insect, respectively. In control, 2 µL ethanol was applied topically to the insect. Cotton seeds and distilled water were given to the insect. Ten insects were treated in each of the experimental setups[15]. All the experimental groups, along with the control, were replicated five times.

Assessment of Growth and Development

The impact of *Cr*-LEE on the survival of *D. koenigii* was analysed by recording mortality of fifth instar nymphs on a daily basis till moulting. Developmental anomalies were also recorded for different doses. The results were analysed to determine various parameters of survival, growth, and development. The growth index was calculated using the formula[14].

$$\text{Growth Index} = \frac{Percent\ fifth\ instars\ moulted\ into\ adults}{Average\ nymphal\ period\ of\ fifth\ instar\ nymphs}$$

Phytoconstituent Analysis of *Cr*-LEE by Gas Chromatography and Mass Spectroscopy (GC-MS)

Phytoconstituent analysis of *Cr*-LEE was performed using GC-MS. For this, the concentrated ethanol extract was dissolved in the ethanol. The extract was then injected into the Gas Chromatography unit. The Shimadzu GC-MS QP2010 served as the instrument for the GC-MS analysis. The injector temperature was set at 250ºC, and a flame ionisation detector, maintained at 280ºC, was used. The carrier gas (nitrogen) pressure was held at 10 psi. The oven temperature ranged from 60 to 280ºC, increasing gradually by 10ºC per minute. The extract was passed through a DB-5 MS column, 30 m in length and 0.25 mm in diameter, and the eluted components were detected by the flame ionisation detector, with the GC chromatogram being recorded. The extract constituents were separated based on retention time and identified by a mass spectrophotometer. The chromatogram, which plots intensity against retention time, was captured by the inbuilt software. Compounds were identified by comparing the graph data with existing software libraries such as WILEY08, NIST08, and NIST08s.

Statistical Analysis

The data were presented as Mean±SE. IBM SPSS 19 software was used for statistical analyses of the data. The difference between the groups was analysed using one-way ANOVA followed by a post-hoc Tukey Test[16].

Results

The data presented in Table 1 show that the fifth instar nymphs treated with *Cr*-LEE did not exhibit a significant decrease in the per cent survival after 24 h of the treatment. However, in the treatment with a dose of 400 µg/Insect, the per cent survival was reported to be 82%, which was statistically significant (p<0.05). The per cent survival of fifth instar nymphs on the seventh day of treatment at doses 200 and 400 µg/Insect was 86 and 68%, respectively; the results were statistically significant. No significant decrease in the survival of fifth-instar nymphs was observed in the treatments with doses of 40 and 100 µg/Insect. The results also indicate that the nymphs treated with a dose of 400 µg/Insect showed a high incidence of abortive moulting. Consequently, at this dose, only 48% out of 68% of nymphs could moult successfully (Table 1). Further, there was a significant decrease in the percentage of normal adult emergence in all the treatments (results significant at p<0.05).

The results of the day-wise survival pattern of the fifth instar nymph treated with *Cr*-LEE are presented in Fig. 1. In the control group, no mortality was observed till the nymphs moulted into the adults. The survival of the fifth instar nymphs moulted into the adults was statistically significant in the treatment with *Cr*-LEE at doses of 100, 200, and 400 µg/Insect. The total mortality observed in the treatment with *Cr*-LEE at doses 40, 100, 200, and 400 µg/Insect were 4, 6, 14, and 52% (p<0.05). In control, no mortality was observed in the fifth instar nymphs.

The data presented in Fig. 2 show the impact of *Cr*-LEE on duration of the nymphal stage of fifth-instar nymphs of *D. koenigii*. In the case of the control group, it was 6.90 days. There was a sharp increase in the nymphal period in the treatments with a dose of 400 µg/Insect; it was reported to 10.85 days. In the treatments with the doses of 100 and 200 µg/Insect, the nymphal period was 7.73 and 7.80 days, respectively. Treatment with the dose of 40 µg/Insect increased the nymphal period marginally. The nymphal period of the fifth instar nymph treated with the 400 µg/insect was significantly different (p<0.05) from the insects treated with the 40 µg/Insect, 100 µg/Insect, 200 µg/Insect, and control groups (p>0.05).

Cr-LEE also affected the day-wise pattern of adult emergence from the fifth instar nymph. The results

Table 1 — Survival of fifth instar nymphs of *D. koenigii* in response to *C. roseus* leaf ethanol extract

Dose of ethanol extract (µg/insect)	Percent of Fifth instar nymphs survived after 24 h of treatment	Percent of Fifth instar nymphs survived after 7 days of treatment	Percent of Fifth instar nymphs moulted successfully	Percent of Fifth instar nymphs moulted into normal adults
Control	100^{a}±0	100^{a}±0	100^{a}±0.00	100^{a}±0.00
40 µg	100^{a}±0	96^{a}±2.45	96^{a}±2.45	64^{b}±2.00
100 µg	96^{ab}±2.45	94^{a}±2.45	94^{a}±2.45	10.66^{cd}±3.17
200 µg	94^{ab}±2.45	86^{ab}±2.45	86^{a}±2.45	4^{d}±2.45
400 µg	82^{c}±8.0	68^{b}±12	48^{b}±12	27.61^{c}±9.69
p-value	0.02	0.00	0.00	0.00
Fvalue	3.61	6.61	13.86	71.41

Means followed by the same letter in a column are not significantly different at p<0.05; (One-way ANOVA followed by Tukey test

∗Average of five replicates, 10 insects per replicate

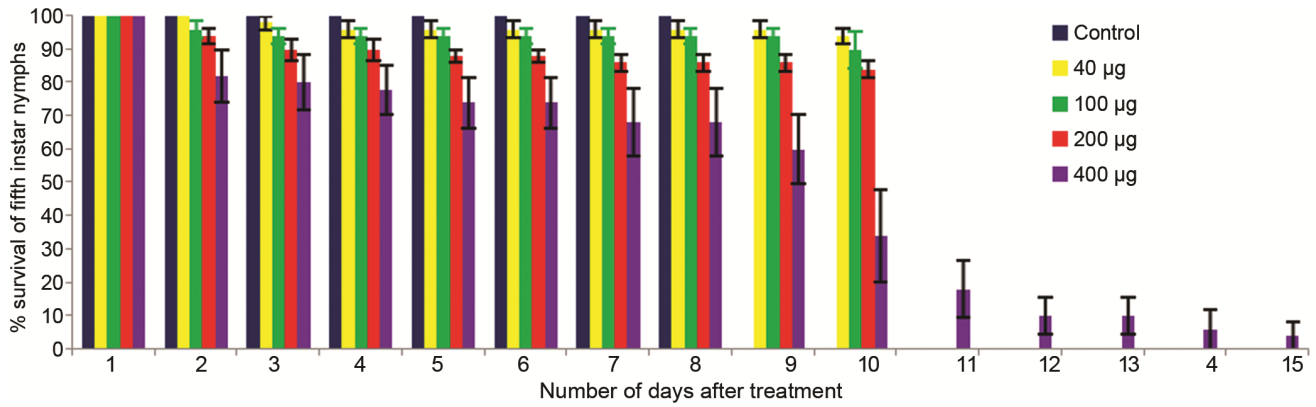

Fig. 1 — Day-wise survival of fifth-instar nymphs of *D. koenigii* after treatment with *Cr*-LEE.

presented in Fig. 3 indicate that treatment of *Cr*-LEE caused the delay in the moulting in a dose-dependent manner. In the control group, the adult emergence started on day 6 when 16% of the nymphs moulted into adults. On day seven, the percentage of fifth instar nymphs moulting into adults was 78%. All the nymphs mouted into adults by eight days (Fig. 3). At a dose of 400 µg/Insect moulting started on day 9 and continued up to 16 days. Although the moulting in the nymph treated with the *Cr*-LEE at doses 100 and 200 µg/Insect started on day 7, the percentage of adult emergence remained less. At a dose of 100 µg/Insect, the percentage of adult emergence on days 7, 8 and 9 was 34, 56, and 4, respectively. Treatment at a dose of 200 µg/Insect, the percentage of adult emergence on these days was 22, 58, and 6%, respectively. The trends of day-wise adult emergence in the treatments at 40 µg/Insect were similar to control (Fig. 3).

In the case of control groups, all of the fifth instar

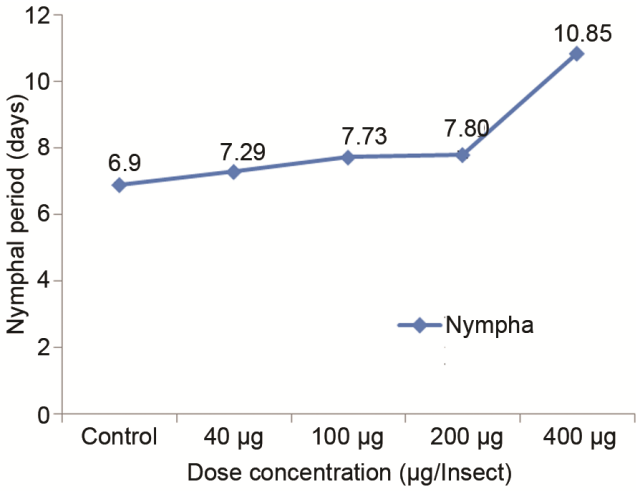

Fig. 2 — Nymphal period of *D. koenigii* treated with fifth instar *Cr*-LEE.

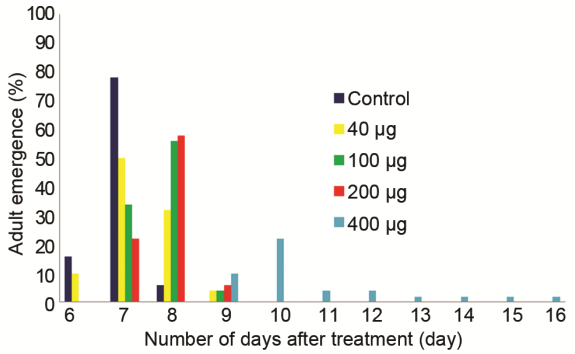

Fig. 3 — Day-wise adult emergence of *D. koenigii* fifth-instar nymphs treated with *Cr*-LEE.

nymphs moulted into normal adults. Fifth instar nymphs treated with *Cr*-LEE moulted into the stages with different types of developmental anomalies (Fig. 4). Some of the common deformities seen were supernumerary nymphal stage, adultoid, adult with wing deformed, adult having exuviae attached to different parts of body, adults with shrunken body (Fig. 4). In many cases the moulting was aborted leading to death of moulting nymphs. In the newly emerged fifth instar nymph, three pairs of transverse white bands were present on the abdominal sternum (Fig. 4a). The fifth instar nymphs were moulted into adults. The normal males were small in size compared to the females. The males have an external aedeagus at the posterior side of the body. The female has a broad abdomen (Fig. 4b-c). Some of the treated fifth instar nymphs started their moulting but did not complete it and died off. This showed abortive moulting (Fig. 4d). Supernumerary adults had transverse white bands on the dorsal side of the abdomen, similar to fifth-instar nymphs (Fig. 4e). Treatment of fifth instar nymphs with *Cr*-LEE also resulted in the formation of adultoid. They retained both adult and nymphal characteristics. Adultoids were characterized by the presence of short, stumpy wings and three round black abdominal spots on the dorsal surface of the abdomen, similar to the fifth instar nymphs (Fig. 4 g). Wing-deformed adults had

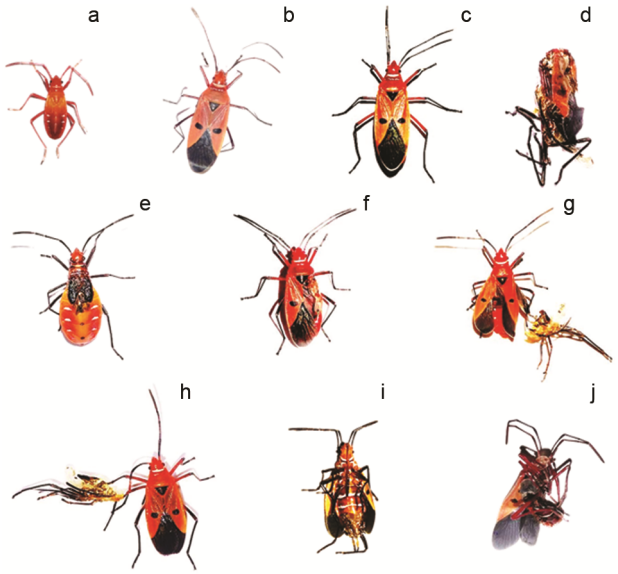

Fig. 4 — Developmental anomalies of *D. koenigii* after treatment with *Cr*-LEE; a) Normal fifth-instar nymph; b) Normal male; c) Normal female; d) Aborted moulting; e) Supernumerary nymphal instar; f) Wing-deformed adult; g) Adultoid; h) Adult with exuviae attached; i) Adult with the genital plug of exuviae; j) adult with a shrunken abdomen.

abnormal wings compared to normal adults (Fig. 4 f). In some of the treated fifth instar nymphs, the exuviae, remained attached to their body (Fig. 4 h). The treated fifth instar nymphs sometimes moulted into the adults with exuviae attached to their genital region. This prevented them from mating with their sexual partner (Fig 4 i). In some of the adults, the abdomen was shrunken just after the moulting (Fig. 4j); these adults failed to survive.

Fig. 5 shows that in the control group, 100% of normal adults emerged. In the case of treatment at a dose of 40 µg /Insect of *D. koenigii* with *Cr*-LEE, the most common developmental anomaly was an adult with deformed wings (27.11%). The treatment at dose 100 µg/Insect, common developmental abnormalities were adults with deformed wings (36.44%) and adultoid (35.78%); only 10.67% nymphs were moulted into normal adults. The percentage of adultoids remained high in the treatment with a dose of 200 µg/Insect, i.e. 44.72%. Treatment with a dose of 400 µg/Insect resulted in 27.62, 4.17, 45.24, 9.05, and 14.76% of normal adults, supernumerary, wing deformed, adultoid, and adults with exuviae attached, respectively.

Data presented in Table 2 show the impact of *Cr*-LEE on the growth index of the fifth-instar nymphs. The results show a decrease in the growth index of the fifth instar as a result of treatment in a dose-dependent manner. In control, the growth index was 14.50, whereas, at doses 400 µg per insect, it decreased to 4.5; more than three times a decrease was observed. The reduction in growth index was also significant in the treatments with doses 40, 100, and 200 µg, i.e.13.17, 12.17, and 11.12, respectively (Table 2)

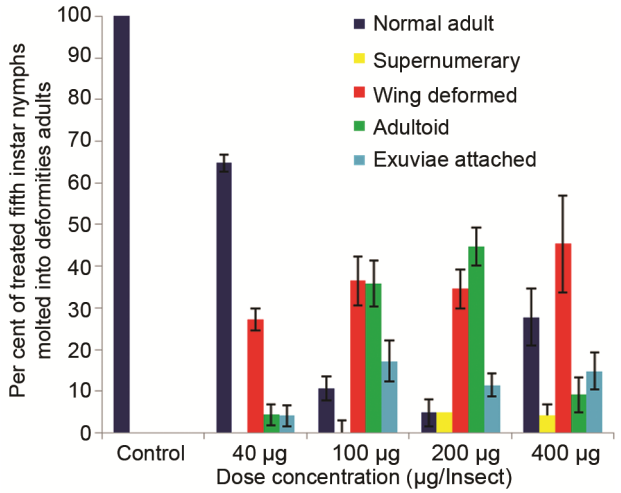

Fig. 5 — Fifth-instar nymph of *D. koenigii* treated with *Cr*-LEE showing developmental deformities.

Ethanol extraction of *Catharanthus roseus* presented 53 peaks, with retention times ranging from 7.486 to 46.697 and a peak area of 100% (Fig. 6). It contained chemical compounds such as cis-Z-Alpha-bisabolene epoxide, 1,2-Benzenedicarboxylic acid diethyl ester, Hexadecanoic acid trimethylsilyl ester, Phytol, Ethyl oleate, Alpha-linolenic acid trimethylsilyl ester, 2,20-Cycloaspidospermidine-3-carboxylic acid 6,7-didehydro methyl ester 2.alpha.,3.alpha.,5.alpha.,12.beta.,19.alpha.,20R, Geranylgeraniol, Hexacosane, Desmethoxyvindoline, Tetracontane, Vitamin E, 1H-indolizino[8,1-cd]carbazole aspidospermidine-3-carboxylic acid derivative, Stigmasta-5,22-dien-3-ol, Hexatriacontane, Stigmast-5-en-3-ol (3.beta.), Bicyclo[4.1.0]heptane 4,4-dimethyl-3-(3-methyl-3-butenylidene)-2-methylene, Methyl commate b, and 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Table 3). The biological properties of some of the phytoconstituents present in *Cr*-LEE are presented in Table 3.

Discussion

Insect's life cycle is divided into multiple stages: egg, nymph and adult in hemimetabolous insects, and egg, larva, pupa and adult in holometabolous insects[35]. Different stages and life processes can be targeted to control their population. *D. koenigii* is a hemimetabolous insect that causes economic damage to cotton and many other crops of economic importance[36]. In the present study, *Cr*-LEE was studied for its growth-suppressive activities against fifth-instar nymphs. Our study showed that the survival of the fifth instar nymph was decreased in response to treatment with the *Cr*-LEE. Although, the impact on survival was not significant after 24 h of treatment a significant reduction in survival was

Table 2 — Growth index of fifth instar nymphs treated with *C. roseus* leaf ethanol extract

S. No.	Dose/Insect	Growth Index
1	Control	14.50^{a}±0.09
2	40 µg	13.17^{ab}±0.41
3	100 µg	12.17^{ab}±0.34
4	200 µg	11.12^{c}±0.23
5	400 µg	4.5^{d}±1.2
6	*p* value	0.00
7	F value	41.99

Means followed by the same letter in a column are not significantly different at $p<0.05$; (One-way ANOVA followed by Tukey test

∗Average of five replicates, 10 insects per replicate

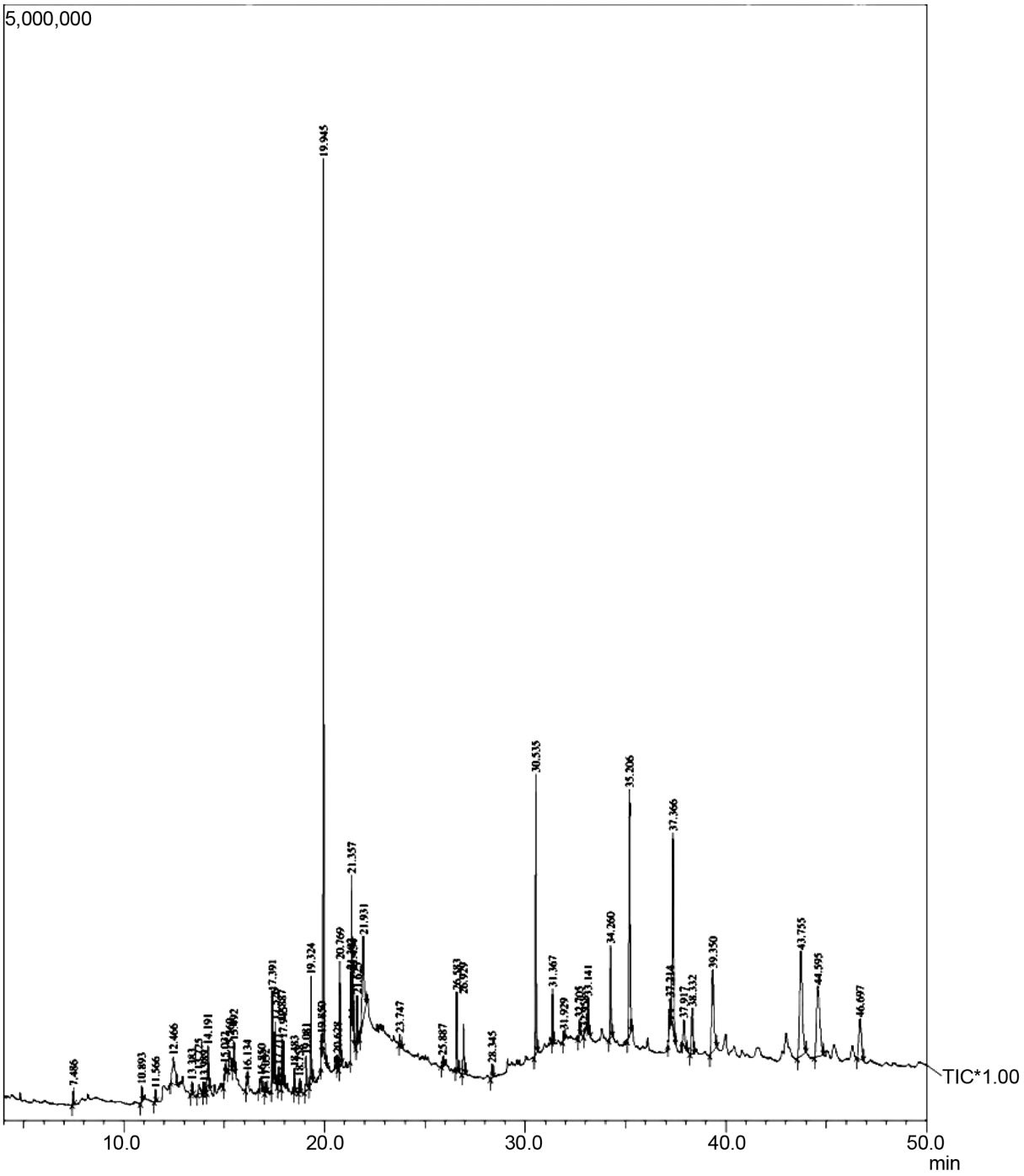

Fig. 6 — GC–MS Chromatogram of the *C. roseus* leaf ethanol extract.

observed after seven days, especially in the treatment doses of 200 and 400 µg per insect.

A similar result was observed when *D. koenigii* were subjected to *Lantana camara* leaf hexane extract treatment[37]. The per cent survival till moulting was higher in the control group. Fifth-instar nymphs treated with *Cr*-LEE showed decreased survival at doses 100 and 200 µg/Insect compared to the control group; at 400 µg/Insect dose, the percentage of survival till moulting was minimum. Adult emergence was significantly lower in the treated groups. The least number of normal adults were formed at a dose of 200 µg/Insect; only 4% of normal adults were formed at this dose. Treatment at doses 40, 100, and

Table 3 — GC-MS analysis of *C. roseus* leaf ethanol extract showing phytochemicals which were present in abundance or showed some biological activities

S. No.	R/T*	Peak Area (%)	Name of the Compound	Molecular Weight	Molecular Formula	Compound Nature	Activity**	References
1	19.945	16.24	Hexadecanoic acid, trimethylsilyl ester	328	$C_{19}H_{40}O_2Si$	Ester compound	Larvicidal Insecticidal	17,18
2	20.769	2.15	Phytol	296	$C_{20}H_{40}O$	Diterpene	Antioxidant Antimicrobial Anticancer Anti-inflammatory and diuretic Antidiabetic activity in type-II diabetic patients Insecticidal	19–22
3	21.357	2.29	Ethyl oleate	310	$C_{20}H_{38}O_2$	Ethanolic fatty acid oleic	Insect repellent and oviposition deterrent Antibacterial	23,24
4	30.535	6.07	Geranylgeraniol	290	$C_{20}H_{34}O$		Bactericidal and precursors of Mevalonic acid pathway Insecticidal and repellent	25–30
5	31.367	1.05	Hexacosane	366	$C_{26}H_{54}$	Hydrocarbon	Pesticidal Insecticidal	31
6	34.260	2.64	Tetracontane	562	$C_{40}H_{82}$		Antimicrobial Antitumor and anticancer	32
7	35.206	7.89	Vitamin E	430	$C_{29}H_{50}O_2$	Vitamin compound	Antiageing Analgesic Antidiabetic Anti-inflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor and anticancer, Hepatoprotective, Hypocholesterolemic, Antiulcerogenic, Vasodilator, Antispasmodic, Antibronchitic,	21
8	37.917	1.11	Stigmasta-5,22-dien-3-ol	412	$C_{29}H_{48}O$	Steroidal	Antioxidant, Antibacterial, Anti-inflammatory, Antiarthritic Antiasthmatic, Diuretic	33
9	38.332	1.86	Hexatriacontane	506	$C_{36}H_{74}$		Antioxidant; Insecticidal	32,34
10	39.350	4.81	Stigmast-5-en-3-ol, (3.β)	414	$C_{29}H_{50}O$	Steroidal	Antimicrobial, Antioxidant, Anti-inflammatory, Antiarthritic, Antiasthmatic, Diuretic, Antioxidant, Antimicrobial, Analgesic, Antidiabatic	33
11	44.595	5.97	Methyl commate b	470	$C_{31}H_{50}O_3$	-	-	-
12	46.697	2.68	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296	$C_{20}H_{40}O$	Terpene alcohol	Antimicrobial	20

* Retention Time

** Activities of the compound as reported cited in the references

400 µg/Insect showed a decrease in number of normal adult emergence from the treated fifth instars, which were 64, 10.66, and 27.61%, respectively.

Earlier reports showed that *Ocimum sanctum* leaf hexane extract decreased the survival of *D. koenigii* in a dose-dependent manner by dry film residue method[14]. Also, the insecticidal effect of *Sida acuta* leaf methanol and ethyl acetate extract had shown an insecticidal effect against *D. cingulatus*[38]. One p.p.m. concentration of α-amyrin acetate from *C. roseus*

showed larvicidal and insect growth regulator effect against the malaria vector *Anopheles stephensi* Liston; at this concentration, 100% mortality was observed in first and second instars larvae, and 94% mortality was observed in third and fourth instars larvae. The larval duration and developmental time were also increased[39]. The aqueous extracts of *C. roseus* at a dose of 1000 ppm have shown larvicidal activity against larvae of gram pod borer *Helicoverpa armigera*[40]. Ursolic acid isolated from the *C. roseus* leaves was reported to have larvicidal activities with LC_{50} and LC_{90} values of 40.09 ppm and 189.15 ppm, respectively, against *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles stephensi*[9].

The *Cr*-LEE was reported to increase the nymphal period of fifth-instar nymphs of *D. koenigii.* In the case of a control group, it was 6.9 days; it was increased to 10.85 days in the treatment with a dose of 400 µg/insect. At the treatment doses of 100 µg/Insect and 200 µg/Insect, the nymphal duration was moderately increased to 7.73 days, 7.80 days. Treatment with the doses 40 µg/Insect, the nymphal period increased marginally. In general, fifth-instar nymphs of *D. koenigii* moulted into adults after 6-7 days under optimal conditions. The fifth instar nymphs mortality was negligible at optimal conditions. The effect of *Cr*-LEE on the growth of fifth-instar nymphs of *D. koenigii* was analyzed by evaluating the growth index of the control and treated nymphs. The results indicated the adverse effect of *Cr-LEE* on the growth index of fifth-instar nymphs of *D. koenigii*. An increase in the developmental period and a decrease in adult emergence were observed in the nymphs treated with *Cr-LEE,* which resulted in a decrease in the growth index in a dose-dependent way. The growth index significantly declined in the treatment with a dose of 400 g/Insect. The most apparent decrease in the growth index in these treatments was associated with a large reduction in the emergence of normal adults.

Treatment of fifth-instar nymphs with *Cr*-LEE resulted in developmental anomalies in the adults that emerged. These anomalies were supernumerary, wing deformed, adultoid, adults with exuviae attached, shrunk abdomen and aborted moulting adults. Similar abnormalities were reported in *D. koenigii* in response to Beta-Cyfluthrin[41] and hexane extract of *Ocimum sanctum* in dry film residual method[14].

In our study on the GC-MS analysis of *C. roseus*, the presence of various chemical compounds was shown. The abundantly present chemical compounds were 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester, Hexadecanoic acid, ethyl esterstigmast-5-en-3-ol, geranylgeraniol, methyl commate B, hexacosane and tetracontane and caryophyllene. The other chemical compounds were in less abundance and covered an area of less than 1%. In ethanol extracts of *C. roseus*, the abundantly present chemical compounds were Hexadecanoic acid, ethyl ester vitamin E, Phytol, bicyclo heptane, 1h-indolizino[8,1-cd]carbazole, geranylgeraniol, methyl commate b, stigmast-5-en-3-ol, alpha.-linoleic acid, 2, 20-cycloaspidospermidine-3-carboxylic acid, 3,7,11,15-tetramethylhexadec-2-en-1-ol, tetracontane, ethyl oleate, phytol, cis-z-.alpha.-bisabolene epoxide, 1,2-benzenedicarboxylic acid and caryophyllene. The results were in agreement with the earlier study on *C. roseus*[42]. This study also reported the presence of 1, 2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester, Hexadecanoic acid ethyl ester, 3, 7, 11, 15-tetramethylhexadec-2-en-1-ol, dodeconoic acid methyl ester, phytol in the extract. Arulvendhan *et al*.[43] reported that the principal bioactive compounds present in *C. roseus* leaves were 3-methylmannoside, squalene, pentatriacontane, and 2,4,4-trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene. Pinho *et al*.[44] reported the presence of 88 chemical compounds in the leaf and flower extracts of *C. roseus.* The compounds were like Hexadecanoic acid, ethyl ester, Phytol, methyl commate b, stigmast-5-en-3-ol, α-linoleic acid, 3, 7, 11, 15-tetramethylhexadec-2-en-1-ol, tetracontane, ethyl oleate, phytol, cis-z-.alpha.-bisabolene epoxide and 1, 2-benzenedicarboxylic acid. The results were similar to our findings. Similarly, Lawal *et al*.[45], in their study, reported chemical constituents of the *C. roseus* were linolenic acid ethyl ester, stearic acid, phytol, Hexadecanoic acid ethyl ester, linolenic acid ethyl ester, dodecyl alcohol, geraniol and citral and dotriacontane. Similarly, 52 compounds were reported in the GC-MS analysis of *C. roseus* leaf oil. The main constituents were (E, E)-2, 4-hexadienal, citronellol, geraniol, p-cresol, (Z, E)-pentadecanal, Hexadecanoic acid ethyl ester, palmitic acid and phytol, heneicosane, tricosane and tetracosane[46]. This indicated that plant extracts contain certain components like Hexadecanoic acid, Phytol, Ethyl oleate, Geranylgeraniol, Hexacosane, Hexatriacontane, etc., which shows the juvenoid activity. These juvenoid compounds present in the plant extract may disrupt the juvenile hormone level

in the insects. The change in the JH level in the insect may affect their survival and development[47,48].

Insect survival, growth, and development are controlled by multiple factors. These factors can be external or internal. In crickets, *Modicogryllus siamensis* photoperiod and temperature separately regulate nymphal development through JH and insulin/TOR signalling pathways[49]. The duration of the nymphal period is also under the control of insect hormones[50,51]. This increase in the nymphal periods has suggested the presence of insect growth regulators in the phytochemicals extracted from the *C. roseus* leaves extract[11,39].

Conclusion

The present study revealed that treatment of *D. koenigii*, the fifth-instar nymphs, with the *Cr*-LEE affected the survival of the insects. The treatment with *Cr*-LEE increased nymphal period and decreased growth index indicated its growth suppressive activities. Developmental anomalies observed in the adults developed from treated nymphs suggested the impact of *Cr*-LEE on development. GC-MS studies of the extract showed the presence of Hexadecanoic acid, Phytol, Ethyl oleate, Geranylgeraniol, Hexacosane, and Hexatriacontane, which has insecticidal and insect growth regulatory activities. Therefore, it is apprehended that the phytoconstituents of *Cr*-LEE singly or synergistically affect the survival, growth and development of *D. koenigii*.

Conflict of interest

The authors declare no conflict of interest.

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