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# Toxicity and lethal effects of herbaceous plant crude extracts against *Spodoptera litura*

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## Abstract

**Background:** Tobacco caterpillar, *Spodoptera litura*, attacks various cultivated plants and causes significant yield losses. In this study, an eco-friendly approach like using botanical insecticides was adopted to examine the toxicological effect of six herbaceous plants (*Phyllanthus niruri*, *Cyperus rotundus*, *Euphorbia hirta*, *Boerhavia diffusa*, *Parthenium hysterophorus* and *Cynodon dactylon*) against *S. litura*.

**Results:** Six herbaceous plants crude extract showed a definite level of toxicity against *S. litura* larvae (3rd instar) using the surface diet contamination method. Among tested herbaceous plants crude extract at 5 µg/ml concentration, *C. dactylon* showed significant high larval mortality (75%) against *S. litura* larvae compared to *P. niruri* (39%), *C. rotundus* (36%), *P. hysterophorus* (26%), *B. diffusa* (22%) and *E. hirta* (22%) treatments. A cent percent *S. litura* larval mortality was achieved at lower concentration from crude extract of *C. dactylon* (11.2 µg/ml) compared to *E. hirta* (18.6 µg/ml), *B. diffusa* (24.39 µg/ml), *P. hysterophorus* (31.4 µg/ml) crude extracts. The lethal concentration ($LC_{50}$) of *C. dactylon, P. hysterophorus, C. rotundus, P. niruri, B. diffusa* and *E. hirta* crude extracts was estimated to be 1.45, 4.09, 5.74, 6.75, 10.92 and 13.62 µg/ml, respectively.

**Conclusion:** The study results suggested that *C. dactylon* crude extract possesses the potential to reduce the *S. litura* damage on crops as a natural alternative to the chemical insecticides with no toxicity to beneficial species.

**Keywords:** *Cynodon dactylon*, Discriminating dose, Ethyl acetate, Insecticidal, Larvicidal

## Background

Insect pest damage is one of the major reasons for enormous field crop losses. Tobacco cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), is a serious polyphagous insect pest that damages around 115 plant species (Atwal & Dhaliwal, 2009). Out of 115 globally recorded host plants of *S. litura*, 60 are known from India (Garad et al., 1984). During 2008–09, an outbreak of *S. litura* on soybean in Rajasthan and Maharashtra states of India caused losses up to 64 million and 300 million USD, respectively (Dhaliwal & Koul, 2010). To prevent crop losses, the application of chemical insecticides is the most adopted pest management strategy; however, improper use of insecticides in many nations results in insecticides resistance (Vasantha-Srinivasan et al., 2017). Insecticide pest resistance to different synthetic insecticides is a growing problem, and field-level resistance of *S. litura* against insecticides was reported by Gandhi et al. (2016) and Senthil-Nathan (2013). Present limitations associated with the insecticides necessitated for searching selective and eco-friendly insecticides.

Botanical insecticides are instrumental in the discovery and development of synthetic chemical products. Thus, continued discovery and development of botanical-based products exemplify the direction for designing new and better pesticides (Senthil-Nathan, 2013). *Phyllanthus niruri* mostly found in the tropical and subtropical regions has a long history in the traditional medicinal system to prevent or treat various diseases (Bagalkotkar et al., 2006). *Cyperus rotundus* spread in temperate, tropical and sub-tropical regions

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of the world (Nagarajan et al., 2015), and its essential oil affects the insect pest behavior (Zandi-Sohani & Ramezani, 2015). *Euphorbia hirta* extracts showed a broad range of biological properties, including antimicrobial, antifungal, anti-inflammatory, antioxidant, anticancer and antidiabetic activities (Almosnid et al., 2018). *Boerhavia diffusa* is used in traditional medicine for the treatment of diabetes, stress, dyspepsia, abdominal pain, inflammation, jaundice, enlargement of spleen, heart diseases, bacterial infections and impotence (Nalamolu et al., 2004). *Parthenium hysterophorus* is used in the treatment of skin diseases, malaria, rheumatism and headache (Raghu et al., 2014). *Cynodon dactylon* possesses various medicinal properties such as antiviral, antimicrobial (Dhar et al., 1968), anti-inflammatory (Biswas & Mukherjee, 2003) and immunomodulatory activity (Santhi & Annapoorani, 2010).

Talukder and Miyata (2002) reported plant-derived materials are safe to natural enemies, compatible with biological control agents for Integrated Pest Management (IPM) and non-toxic to the environment. Plant products can be easily produced by farmers in small-scale industries and are less expensive (Talukder & Howse, 1995). Therefore, a century-old practice of protecting crops using plant derivatives, which are known to resist insect attack (Ewete et al., 1996) is considered in this study and planned an in vitro evaluation of dose-dependent toxicity of *P. niruri*, *C. rotundus*, *E. hirta*, *B. diffusa*, *P. hysterophorus* and *C. dactylon* crude extract against *S. litura* larvae.

## Methods

### Collection and extraction of phytochemicals from herbaceous plants

Six different herbaceous plants were collected (entire plant with intact roots) from legumes growing fields (Fig. 1). A weed scientist from the Indian Institute of Pulses Research, Kanpur (Dr. Chaitanya Prasad Nath) identified the herbs up to species viz., *P. niruri*, *C. rotundus*, *E. hirta*, *B. diffusa*, *P. hysterophorus* and *C. dactylon*. Since these are commonly available and well characterized herbs the specimens were not submitted to the repository. In this experiment, heat energy was used to rupture the cell membrane and extract the thermostable bio-compounds from different herbaceous plants. One kilogram (wet weight) of each herbaceous plant was brought to the laboratory in a collection bag; shade dried for a week to remove the moisture from the plant parts. The moisture free herbaceous plants were grinded to powder and stored in PVC bottles at 28 ± 2 °C. The respective herbaceous plant (*P. niruri*—10 g, *C. rotundus*—10 g, *E. hirta*—10 g, *B. diffusa*—10 g, *P. hysterophorus*—10 g and *C. dactylon*—5 g) was weighed in flat bottom round flask along with 50 ml of organic solvent Ethyl Acetate (EtoAc). The contents of the flask were alternatively heated (flasks placed in a hot water bath at 100 °C) and agitated (flasks placed in an orbital shaker


**Fig. 1** Herbaceous plants used for crude extraction

at 150 rpm for 2 h). The heating and agitating step were repeated thrice. The filtrate (organic phase) was collected by passing the contents in the flasks through Whatmanno.1 filter paper. The filtrate was air-dried (24 h) in a glass petri plate, and the remaining residue was suspended in 5–8 ml of Methanol (MeOH). Subsequently, the filtrate was passed through a 0.45µ syringe filter to remove the macroparticles from the crude extract. The crude extract of each herbaceous plant was stored at 8°C for the bioassay experiment. Aforesaid protocol for extraction of bioactive compounds from herbaceous plants against lepidopteran insect pests was developed and standardized in this study. Crude extract appearance and percent yield (i.e., estimated by comparing mass of crude extract to the mass of fresh material) of individual herbaceous plants are presented in Table 1.

### Insect rearing

Test insect, *Spodoptera litura*, was procured from live insect repository of National Bureau of Agricultural Important Insects, Bengaluru, India, that was reared according to Bapatla et al. (2021). Obtained *S. litura* pupae were placed in a glass container for adult emergence. Emerged adult moths were released in a glass container (15 cm diameter and 25 cm height) for mating, which was provided with paper sheets for oviposition and cotton swab with 20% honey solution as a source of food to adult moths. From oviposition within 3–4 days, eggs were hatched to neonates that are reared on tender castor leaves for initial 12 days. Subsequently, larvae were individually reared in 12 well insect culture plates on the semi-synthetic diet (chickpea flour—100 g, methyl-*p*- hydroxybenzoate sodium salt—2 g, 2,4-hexadecenoic acid—1 g, yeast extract powder—10 g, agar–agar—12.75 g, L-Ascorbic acid—3.25 g, multivitamin—2 capsules, vitamin E—2 capsules, Streptomycin—0.25 g, 10%, formaldehyde—5 ml, distilled water—780 ml) until pupation (14–16 days) (Babu et al., 2014). Within 10–12 days adults emerged from the pupa and the biological life cycle (35–40 days) was continued. Test insect was reared in an incubator at 28 ± 1 °C temperature, 70 ± 2% relative humidity and 14:10 light and dark period for six generations in the laboratory and, further generations were used in bioassay experiments.

### Toxicity of herbaceous plant crude extracts

Oral toxicity of the crude extracts of herbaceous plants was evaluated against third instar larvae of *S. litura* using the surface contamination method (Micheal et al., 1997). Initially, a semi-synthetic diet was prepared and poured (3 ml) into each well of 12 well insect rearing plates. Later, 100 µl of crude extract from each herbaceous plant was applied to the diet surface ($3.8cm^2$) in each well and swirled the plate to ensure a uniform spread of the extract over the diet. The surface of the diet was allowed to dry in an aseptic condition for three hours to evaporate the solvent residue. Simultaneously, the diet surface was also treated with distilled water (control), MeOH (negative control) and Flubendiamide 39.35SC (positive control) separately. On to the treated surface of the diet, healthy (active), equal-sized (3rd instar) and pre-starved (2 h) test insect larvae were released individually. Each treatment was replicated thrice with ten larvae per replication. The same set of experiments was repeated in three episodes. Larval mortality was taken on every day for three days. Larvae showing moribund symptoms were also considered dead larvae. The number of dead larvae on 72 h after treatment were considered as final larval mortality and presented as percent larval mortality. The experiment was conducted at 28°C ± 2°C and 70 ± 2% temperature and relative humidity, respectively.

### Lethal toxicity of herbaceous plant extracts

To find out the toxic concentration of each herbaceous plant extract a range-finding test was conducted (at which 20–80% larval mortality can be achieved). For this initially, 5 µg/ml concentration of all herbaceous plant extracts was tested and observed larval mortality. Based

**Table 1** Yield of crude extract and appearance of six herbaceous plants

| Herbaceous plant | Fresh material mass (g) | Crude extract mass (µg) | MeOH (ml) | Concentration of crude extract (µg/ml) | Percent yield of crude extract (%w/w) | Appearance* |
|---|---|---|---|---|---|---|
| *Phyllanthus niruri* | 10 | 100 | 5 | 20 | $1 \times 10^{-5}$ | Olive gum |
| *Cyperus rotundus* | 10 | 120 | 8 | 15 | $12 \times 10^{-6}$ | Olive gum |
| *Euphorbia hirta* | 10 | 100 | 5 | 20 | $1 \times 10^{-5}$ | Olive green gum |
| *Boerhavia diffusa* | 10 | 275 | 5 | 55 | $275 \times 10^{-7}$ | Olive brown semisolid |
| *Parthenium hysterophorus* | 10 | 175 | 5 | 35 | $175 \times 10^{-7}$ | Dark green gums |
| *Cynodon dactylon* | 5 | 75 | 5 | 15 | $15 \times 10^{-6}$ | Bluish green gum |

*Inter-Society Color Council-National Bureau of Standard (ISCC-NBS), *MeOH* methanol

on larval mortality at 5 µg/ml concentration, further, five concentrations were adjusted until the targeted 20–80% larval mortality was achieved. Five lethal concentrations were estimated for *P. niruri* (0.72, 2.43, 6.08, 12.16, 15.0 µg/ml), *C. rotundus* (0.66, 2.21, 5.54, 11.09, 13.87 µg/ml), *E. hirta* (12.31, 13.98, 15.66, 16.74, 18.6 µg/ml), *B. diffusa* (7.27, 11.55, 15.83, 20.11, 24.39 µg/ml), *P. hysterophorus* (1.5, 5.02, 12.56, 25.12, 31.4 µg/ml) and *C. dactylon* (0.6, 2.01, 5.04, 10.08, 11.2 µg/ml) extracts against *S. litura* larvae, respectively. Each treatment was replicated thrice with ten larvae per replication. Larval mortality was taken on every day for three days, and after 72 h final larval mortality was considered.

### Statistical analysis

Descriptive statistics showed percent larval mortality data was positively skewed (1.434). Therefore, the data were transformed ($\log_{10}$) to reduce the skewness to 0.903, i.e., within the range (from $-1$ to $+1$). The transformed data were used for conducting a One way analysis of variance (ANOVA) to observe the significant differences ($p \leq 0.05$) between treatments using SPSS software ver.25. Percent larval mortality data in Table 2 were shown in mean ± standard deviation format. The mean of ranks for percent larval mortality attained by crude extracts, water and methanol were compared using Tukey's HSD post hoc test. To estimate the lethal concentrations of crude extracts from herbaceous plants, probit analysis was carried out using SPSS software ver.25 (IBM Corp., 2017).

## Results

### Crude extract from herbaceous plants

The maximum yield of crude extract was obtained from *B. diffusa* (275 µg), followed by *P. hysterophorus* (175 µg), *C. rotundus* (120 µg), *P. niruri* (100 µg), *E. hirta* (100 µg) and *C. dactylon* (75 µg). The concentration of crude extract from *B. diffusa*, *P. hysterophorus*, *C. rotundus*, *P. niruri*, *E. hirta* and *C. dactylon* was estimated to be 20, 15, 20, 55, 35 and 15 µg/ml by dissolving the semisolid or gum type crude extract with MeOH, respectively. Crude extracts from *P. niruri* and *C. rotundus* are olive in color, whereas *E. hirta*, *B. diffusa*, *P. hysterophorus* and *C. dactylon* extracts were olive green, olive-brown, dark green and bluish green colors, respectively (Table 1).

### Toxicity of herbaceous plant extracts

One way ANOVA table [$F(6,29) = 383.84$, $p < 0.05$] revealed statistically significant differences for *S. litura* larval mortality between herbaceous plants crude extracts at 5 µg/ml concentration. Larval mortality ranged from 22 to 90%; whereas MeOH and water treatments showed no larval mortality. The insecticide flubendiamide 39.35SC treatment resulted in 90% highest and significant larval mortality. Among herbaceous extracts, *Cynodon dactylon* crude extract showed significant and higher *S. litura* larval mortality (75%). The *S. litura* larval mortality from *P. niruri* (39%) and *C. rotundus* (36%) crude extracts were statistically at par; whereas, *P hysterophorus* crude extract resulted in only 26% larval mortality and significant from other treatments. Lower *S. litura* larval mortality (22%) was noticed from *E. hirta* and *B. diffusa* crude extract (Table 2).

### Lethal concentrations of crude extracts

Based on range-finding test results five different lethal concentrations of six herbaceous plants crude extract were tested against 3rd instar larvae of *S. litura*. Five concentrations (15.2, 12.16, 6.08, 2.43 and 0.72 µg/ml) of crude extract from *P. niruri* resulted in 80.00, 60.00, 46.67, 23.33 and 3.33 percent *S. litura* larval mortality, respectively. The crude extract concentrations from *C. rotundus* (13.81, 11.09, 5.54, 2.21 and 0.66 µg/ml) resulted in 80.00, 70.00, 40.00, 20.00 and 10.00 percent *S. litura* larval mortality, respectively. Similarly, 100.00, 80.00,

**Table 2** Toxicity of crude extracts of herbaceous plants against *Spodoptera litura* larvae

| Herbaceous plants | Concentration (µg/ml) | Dosage (µl/3.8cm$^2$) | Larval mortality (%) |
|---|---|---|---|
| *Phyllanthus niruri* | 5 | 100 | $39 \pm 1.00^{b}$ (1.59) |
| *Cyperus rotundus* | 5 | 100 | $36 \pm 1.00^{b}$ (1.56) |
| *Euphorbia hirta* | 5 | 100 | $22 \pm 0.58^{d}$ (1.34) |
| *Boerhavia diffusa* | 5 | 100 | $22 \pm 1.53^{d}$ (1.35) |
| *Parthenium hysterophorus* | 5 | 100 | $26 \pm 1.53^{c}$ (1.42) |
| *Cynodon dactylon* | 5 | 100 | $75 \pm 1.00^{a}$ (1.88) |
| Methanol (99.9%) | | 100 | $0^{e}$ |
| Control (autoclaved water) | | 100 | $0^{e}$ |
| S.Em ± | | | 0.04506 |
| Variance | | | 0.037 |

Values in parenthesis are $\log_{10}$ transformed values and means values followed by same letter are significant with each other

70.00, 56.67 and 33.33 percent *S. litura* larval mortality was observed at 18.6, 16.74, 15.66, 13.98 and 12.31 µg/ml crude extract concentrations from *E. hirta*, respectively. *Boerhavia diffusa* crude extract at 24.39, 20.11, 15.83, 11.55 and 7.27 µg/ml concentrations showed 100.00, 93.33, 70.00, 56.67 and 20.00 percent *S. litura* larval mortality, respectively. Five concentrations (31.4, 25.12, 12.56, 5.02 and 1.5 µg/ml) from *P. hysterophorus* crude extract demonstrated 100.00, 80.00, 66.67, 50.00 and 33.33 percent *S. litura* larval mortality, respectively. Larva of *S. litura* showed 100.00, 86.67, 76.67, 50.00 and 33.33 percent mortality when treated with different concentrations of *C. dactylon* crude extract like 11.2, 10.08, 5.04, 2.01 and 0.6 µg/ml, respectively. Though *E. hirta* (18.6 µg/ml), *B. diffusa* (24.39 µg/ml), *P. hysterophorus* (31.4 µg/ml) crude extracts provided 100% larval mortality, they are at higher concentrations compared to *C. dactylon* (11.2 µg/ml).

Dose–response probit analyses envisaged a nine times differences in $LC_{50}$ values between six herbaceous plant crude extracts against *S. litura* larvae. The lowest $LC_{50}$ was shown by *C. dactylon* crude extract (1.45 µg/ml) followed by *P. hysterophorus* (4.09 µg/ml), *C. rotundus* (5.74 µg/ml), *P. niruri* (6.75 µg/ml), *B. diffusa* (10.92 µg/ml) and *E. hirta* (13.62 µg/ml). Similarly, a five-time variation was observed between herbaceous plant extracts for $LC_{99}$ values. The lower $LC_{99}$ was observed from *E. hirta* (21.56 µg/ml) crude extract followed by *B. diffusa* (30.31 µg/ml), *C. dactylon* (45.67 µg/ml), *P. niruri* (130.42 µg/ml), *C. rotundus* (143.34 µg/ml) and *P. hysterophorus* (213.91 µg/ml) (Table 3).

## Discussion

At the international level incorporation of integrated pest management tactics using biopesticides is being encouraged (Edwin et al., 2016). The herbaceous plants, *P. niruri*, *E. hirta*, *B. diffusa*, *P. hysterophorus* and *C. dactylon* are well known for their diversified medicinal value and therapeutic uses (Dhar et al., 1968; Huang et al., 2012; Lim & Murtijaya, 2007; Nalamolu et al., 2004; Raghu et al., 2014). Insecticidal action of *C. dactylon* and *C. rotundus* was reported against red flour beetle (Islam & Talukder, 2005) and ants, aphids, flies and cockroaches (Bañez & Castor, 2011), respectively; however, *P. niruri*, *E. hirta*, *B. diffusa* and *P. hysterophorus* plants were not studied for their insecticidal activity. Our study is the first to explore the larvicidal potential of crude extract from *P. niruri*, *E. hirta*, *B. diffusa*, *C. rotundus*, *P. hysterophorus* and *C. dactylon* against *S. litura* larvae. However, earlier studies showed plant families like Apiaceae (Roman Pavela & Naděžda Vrchotová, 2013), Asteraceae (Ashokaraj & Mahadev, 2012; Rathi & Gopalakrishnan, 2006; Romo-Asunción et al., 2016), Lamiaceae (Romo-asunción et al., 2016), Rutaceae (Arivoli & Tennyson, 2013; Barakat, 2011; Kumar et al., 2014; Thodsare et al., 2014), Solanaceae (Jeyasankar & Elumalai, 2012), Meliaceae (Dinesh-Kumar et al., 2018), Fabaceae (Bermúdez et al., 2009; Dang Hoa et al., 2017) and Lamiaceae (Rajput et al., 2018) demonstrated anti-larval and antifeedant activity against *Spodoptera* species.

In this study, *C. dactylon* crude EtoAc extract showed higher toxicity (75% larval mortality) compared to other herbaceous plants, whereas Islam and Talukder (2005) reported water extract from *C. dactylon* showed only 37% red flour beetle mortality. Following chemical constituents like cynodin, hydrocyanic acid, triticin, proteins, carboydrated and beta-carotene were reported from *C. dactylon* (George et al., 1997; Kao et al., 2005). Lopez (1903) reported hydrocyanic acid as an insecticidal, which might be attributed to the highest *S. litura* larval mortality from *C. dactylon* crude extract. Limited previous studies pressed us to compare the present results with the efficacy of other plant extracts against *Spodoptera* species. In the present study, *C. dactylon* crude extract at 0.0005% (5 µg/ml) concentration showed 75% larval mortality of *S. litura*; however, 15% crude aqueous

**Table 3** Lethal and sub-lethal toxicity of herbaceous extract against *Spodoptera litura* larvae

| Herbaceous Plants | $LC_{50}$ (µg/ml) | 95% Confidence limits | | $LC_{99}$ (µg/ml) | Regression equation ($Y = a + bX$) | Fit of probit line | | | *n* |
|---|---|---|---|---|---|---|---|---|---|
| | | Lower bound | Upper bound | | | Heterogeneity factor ($\chi^2$) | Slope (±S.E) | *df* | |
| *Phyllanthus niruri* | 6.75 | 5.01 | 9.27 | 130.42 | $Y = 1.5 + 1.809X$ | 1.55 | 1.809 ± 0.296 | 3 | 90 |
| *Cyperus rotundus* | 5.74 | 4.18 | 8.07 | 143.34 | $Y = 1.264 + 1.665X$ | 2.843 | 1.665 ± 0.271 | 3 | 90 |
| *Euphorbia hirta* | 13.62 | 12.72 | 14.27 | 21.56 | $Y = 13.239 + 11.671X$ | 3.196 | 11.671 ± 2.071 | 3 | 90 |
| *Boerhavia diffusa* | 10.92 | 9.51 | 12.19 | 30.31 | $Y = 5.451 + 5.249X$ | 3.202 | 5.249 ± 0.764 | 3 | 90 |
| *Parthenium hysterophorus* | 4.09 | 2.313 | 6.063 | 213.91 | $Y = 0.829 + 1.345X$ | 6.425 | 1.354 ± 0.242 | 3 | 90 |
| *Cynodon dactylon* | 1.45 | 0.87 | 2.09 | 45.67 | $Y = 0.2551.555X$ | 4.877 | 1.555 ± 0.256 | 3 | 90 |

*df* degree of freedom, $\chi^2$ values were not significant for all the assays

leaf extract of *Eupatorium triplinerve* showed 92% *S. litura* larval mortality (Ashokaraj & Mahadev, 2012); *Synedrella nodiflora* showed 100% *S. litura* larval mortality (Rathi & Gopalakrishnan, 2006); *Senecio salignus* (500 µg/ml) and *Salvia microphylla* (500 µg/ml) demonstrated 52.5% and 65.0% *Spodoptera frugiperda* larval mortality, respectively (Romo-asunción et al., 2016); *Zanthoxylum armatum* showed strong larvicidal properties against *S. litura* (Kaleeswaran et al., 2018); *Solanum pseudocapsicum* seeds extract showed 75.3% *S. litura* larval mortality; *Strychnos nuxvomica* (88.98%), *Vitex negundo* (86.41%), *Murraya koenigii* (81.46%), *Zanthoxylum limonella* (80.58%) and *Abrus precatorius* (78.61%) crude extracts showed larvicidal activity against *S. litura* larvae.

Senthil-Nathan et al. (2008) reported dose-dependent mortality was a common phenomenon, which was emphasized in this study. It was noticed that *C. dactylon* crude extract at 1.45 µg/ml reduced *S. litura* larval population to 50%, dose-dependent responses depend upon the concentration of the active ingredient, as well as the mixture of chemicals (Dinesh-Kumar et al., 2018). Lethal concentrations or doses ($LC_{50}$ or $LD_{50}$) were estimated from *S. nodiflora*—0.003 µg/ml (Rathi & Gopalakrishnan, 2006), *A. archangelica*—0.32 µg/ml (Pavela & Vrchotová, 2013), *Swietenia mahagoni*—31.04 µg/ml (Dinesh-Kumar et al., 2018) and *Casimiroa edulis*—79.47 µg/ml (Barakat, 2011) against *Spodoptera* species.

## Conclusion

The crude extracts are easy to prepare and handle in comparison to commercially available chemical insecticides. The present investigation showed the amicable solution for the management of *S. litura* using *C. dactylon* crude extract. Further, the development of a stable and consistent product from *C. dactylon* crude extract may provide a low cost and eco-friendly approach to manage the *S. litura*.

### Abbreviations

USD: United States Dollars; IPM: Integrated Pest Management; SPSS: Statistical Package for Social Sciences; EtoAc: Ethyl acetate; MeOH: Methanol; µg: Microgram; ANOVA: Analysis of variance; µg/ml: Micrograms per milliliter; $LC_{50}$: Lethal concentration; $LD_{50}$: Lethal dose.

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### Authors' contributions

All authors have read and approved the manuscript. DS conducted the experiment and analyzed the data; KGB designed and drafted the manuscript.

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### Availability of data and materials

Not applicable.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Consent was taken from the competent authority.

### Competing interests

Authors declare that there was no conflict of interest.

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