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RESEARCH ARTICLE

# Toxicity of fermented ginger waste extract in controlling the common cutworm (*Spodoptera litura*) and beet armyworm (*Spodoptera exigua*) under laboratory conditions

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## ABSTRACT

Ginger (*Zingiber officinale* Roscoe) waste is a residue from industrial factories that has potential for utilization in the production of bio-extract for pest control. Therefore, this study aimed to investigate the toxicity of fermented ginger waste extract through contact toxicity and oral toxicity methods in controlling the common cutworm (*Spodoptera litura* Fabricius) and the beet armyworm (*Spodoptera exigua* Hübner). The experiment was a completely randomized design (CRD) with 10 replicates. Mortality rates were recorded at 48, 72, and 96 h after treatment under laboratory conditions. Fermented ginger waste extract at concentrations of 0%, 5%, 25%, 50%, and 100% (w/v) was used. The results showed that for the contact toxicity method at 100% concentration after 96 h, the common cutworm had the highest mortality rate of 76.00 ± 1.29% ($LC_{50}$ = 14.450 µg larva$^{-1}$), while the beet armyworm had the highest mortality rate of 66.00 ± 0.95% ($LC_{50}$ = 68.426 µg larva$^{-1}$). For the oral toxicity method, the common cutworm had the highest mortality rate of 52.00 ± 1.70% at 100% concentration after 96 h ($LC_{50}$ = 91.275 µg larva$^{-1}$), and the beet armyworm had the highest mortality rate of 58.00 ± 1.02% at 100% concentration after 96 h ($LC_{50}$ = 88.400 µg larva$^{-1}$). Therefore, the results of this study demonstrate the efficacy of fermented ginger waste extract in controlling common cutworm and beet armyworm under laboratory conditions, and it has the potential for further application in field conditions.

**Key words:** Beet armyworm, common cutworm, contact toxicity, fermented ginger waste extract, oral toxicity, *Spodoptera exigua*, *Spodoptera litura*, *Zingiber officinale*.

## INTRODUCTION

Ginger (*Zingiber officinale* Roscoe) waste is a residue from industrial factories. Generally, fresh ginger rhizomes contain approximately 83%-94% moisture and ginger oleoresins comprise around 20%-30% of volatile oils, approximately 10% of fixed oils, and 50%-70% of pungent resinous matter. However, when compared to the total dry weight of ginger waste, the extracted oils account for only 4%-10% of the total weight. As a result, a significant amount of sent industrial waste is to landfills, incinerated, or used as low-value animal feed (Yu et al., 2021). According to Inthalaeng et al. (2023), ginger waste, which accounts for approximately 90% of the by-product from industrial ginger processing plants after oil extraction, is highly promising for further utilization. This is because ginger waste still contains carbohydrates (such as starch, pectin, and cellulose), fats, proteins, sugars, and volatile oils, among other components.

The utilization of ginger waste to produce bio-fermentation for insect control is another promising alternative for valorizing this residue. During the fermentation process, acetic acid, alcohols, and propionic acid are produced, which can be used as insecticides for various insects such as mosquitoes, houseflies, and cockroaches (Neupane and Khadka, 2019). Additionally, Subash and Raju (2014) demonstrated the effective potential of fermented ginger extract in controlling the common cutworm (*Spodoptera litura* Fabricius).

Ginger contains bioactive compounds that can effectively control insect pests due to its pungent taste and volatile oil composition, including gingerols, shogaols, paradols, zingerone, gingerdiones, and gingerdiols (Gao et al., 2022). The bioactive compounds in ginger have been found to inhibit insect growth and inhibit the feeding of *Spilosoma obliqua* F. (Agarwal et al., 2001). Rizali (2020) also reported that a 10% ginger (*Zingiber officinale*) extract could control up to 58% of the armyworm (*Chrysodeixis chalcites*) within 7 d after spraying the extract on green mustard plants grown in a greenhouse. Consistent with these findings, Aryani and Auamcharoen (2016) reported that crude extracts from Zingiberaceae plants were effective in repelling the maize weevil (*Sitophilus zeamais* Motschulsky), with ginger extracts achieving a maximum repellency of 99% at a concentration of 1415 µg $cm^{-2}$ within 8 h after treatment. These preliminary studies indicate that ginger extracts possess insecticidal properties that can be used for controlling various plant pests.

In most vegetable cultivation or monocultures, major insect pests such as the common cutworm (*S. litura* Fabricius), beet armyworm (*S. exigua* Hübner) and (*Spodoptera frugiperda* J.E. Smith) are commonly encountered (Sajid et al., 2020). Infestations by these cutworms and fall armyworm can cause yield losses of 20%-70% (Rodingpuia and Lalthanzara, 2021; Guera et al., 2023). Farmers typically rely on synthetic chemical insecticides for control, leading to insecticide resistance, destruction of natural enemies, and enabling the emergence of new insect pest outbreaks. Furthermore, chemical residues can accumulate in the environment. Therefore, the use of plant extracts for insect pest control can help reduce the reliance on synthetic chemical insecticides.

Therefore, this study aimed to investigate the toxicity of fermented ginger waste extract through contact toxicity and oral toxicity methods in controlling the common cutworm (*S. litura* Fabricius) and the beet armyworm (*S. exigua* Hübner) under laboratory conditions.

## MATERIALS AND METHODS

### Fermented ginger waste extract

Fermented ginger (*Zingiber officinale* Roscoe) waste extract was prepared following the methods of Prishanthini and Vinobaba (2014), Lanjar et al. (2015), and Naik and Naik (2015). Ginger waste from an industrial factory was mixed with molasses and sterilized distilled water in a ratio of 3:1:10 in clean plastic containers (4 L). The containers were tightly sealed, with three replicates prepared. During the fermentation process, the containers were opened once a week to release the gases produced. The fermentation containers were stored at room temperature away from direct sunlight for a period of 3 mo. After fermentation, the ginger fermentation extract was filtered through Whatman No. 1 filter paper and stored at 4 °C before being used for the experiments.

### Determination of 6-gingerol and 6-shogaol content in fermented ginger waste extract

Fermented ginger waste extract was analyzed for 6-gingerol and 6-shogaol content using high-performance liquid chromatography (HPLC Alliance 2695 system, detector 2996, software empowers; Waters, Milford Massachusetts, USA). A 50 mL aliquot of the fermentation extract was transferred into a separatory funnel, and 50 mL ethyl acetate was added. The mixture was gently shaken and allowed to separate into two layers. The lower layer was collected (extraction repeated three times). The combined extracts were evaporated, and the volume was adjusted to 10 mL with methanol. The HPLC analysis conditions were as follows: Column Luna C18 150×4.6 mm, 5 µm; flow rate 1.0 mL $min^{-1}$; injection volume 20 µL; detector PDA 280 nm; mobile phase gradient system with acetonitrile (A), 0.1% phosphoric acid (B), and methanol (C).

### Rearing of common cutworm and beet armyworm for experiments

The rearing procedures for the common cutworm (*Spodoptera litura* Fabricius) and beet armyworm (*S. exigua* Hübner) were adapted from Bullangpoti et al. (2012). Eggs of the common cutworm and beet armyworm were obtained from the National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency, Thailand, and reared at the laboratory of Valaya Alongkorn Rajabhat University under the Royal Patronage Pathum Thani Province, Pathum Thani, Thailand. The common cutworm and beet armyworm eggs were placed in a 16×21×16 cm insect rearing box with aerated lids covered with wire mesh, along with 50 g artificial diet. The rearing was conducted at 26 ± 1 °C and 60 ± 5% RH. The eggs hatched into first-instar larvae within 2-4 d. The artificial diet was changed every 3 d or when it changed color to prevent contamination. When the larvae reached the fourth instar, they were transferred individually into a 27-compartment plastic box (16.7×32.5×2.5 cm) and reared until the pupal stage and adult emergence. Ten pairs of adults were separated and reared in 8×15×10 cm plastic boxes. Cotton wool soaked in a 20% sugar solution was provided and changed every 48 h. Cabbage leaves were placed in the rearing boxes for oviposition by the adult females. The egg masses of the common cutworm and beet armyworm were collected and reared to increase the population for subsequent experiments.

### Fermented ginger waste extract toxicity via contact toxicity

The method by Majeed et al. (2018) was followed. Cabbage leaves were washed with 0.1% (v/v) sodium hypochlorite NaOCl (Clorox) for 5 min and rinsed twice with sterilized distilled water. Cotton wool soaked in water was wrapped around the petioles and covered with plastic to maintain moisture during the experiment. The cabbage leaves were placed in Petri dishes (9 cm diameter, 1.5 cm height). The experimental design was a completely randomized design (CRD) with 10 second-instar common cutworm larvae per Petri dish, divided into five treatments: Water (control), 5%, 25%, 50%, and 100% (w/v) fermented ginger waste extract.

The toxicity of the fermented ginger waste at different concentrations was evaluated by directly spraying the larvae with an air brush sprayer positioned approximately 15 cm away from the larvae on the cabbage leaves. A volume of 0.5 mL was sprayed to evenly cover the leaves. Larval mortality was recorded at 48, 72, and 96 h after spraying. The same procedure was followed for the beet armyworm.

### Ginger waste fermentation liquid toxicity via oral method (oral toxicity)

The experimental design was a completely randomized design (CRD) with 10 second-instar common cutworm larvae per Petri dish, divided into five treatments: Water (control), 5%, 25%, 50%, and 100% (w/v) fermented ginger waste extract.

Cabbage leaf discs (3 cm diameter) were dipped in the respective fermentation liquid concentrations for 1 min, air-dried at room temperature, and then placed in Petri dishes. Larval mortality of the common cutworm was observed and recorded at 48, 72, and 96 h. The same procedure was followed for the beet armyworm. Mortality rates were calculated using the following formula:

$$\text{Mortality \%} = (\text{Number of dead insects}/\text{Total number of test insects}) \times 100$$

The median lethal concentration ($LC_{50}$) was estimated by probit analysis methods. Differences in mortality rates were compared using Duncan's new multiple range test (DMRT) at a 95% confidence level ($P < 0.05$) with SPSS statistical software (IBM, Armonk, New York, USA).

## RESULTS AND DISCUSSION

### Quantity of 6-gingerol and 6-shogaol in fermented ginger waste extract

An examination of the quantities of 6-gingerol and 6-shogaol revealed that the fermented ginger waste extract contained 0.4 and 1.2 mg 100 $mL^{-1}$. The fermented ginger waste extract used for testing appeared as a clear brown liquid with a pungent ginger aroma. In terms of its physical properties, the fermented ginger waste extract had a pH value of 5.26. The study by Ko et al. (2019) found that the quantities of 6-gingerol and 6-shogaol extracted using water were 0.68 ± 0.08 mg $g^{-1}$ and 0.39 ± 0.03 mg $g^{-1}$, respectively. The amounts of these compounds found in ginger may vary depending on the extraction method and the solvent chosen. Additionally, genetic factors and environmental conditions during cultivation can influence their concentrations. The concentration of essential oils in ginger can fluctuate according to the season and growing location.

### Contact toxicity of fermented ginger waste extract on mortality rates of common cutworm and beet armyworm

The results of testing the contact toxicity of fermented ginger waste extract on the mortality rate of common cutworm at concentrations of 0%, 5%, 25%, 50%, and 100% (w/v) revealed that after 48, 72, and 96 h, the 100% concentration caused the highest mortality rate ($P < 0.05$). Specifically, after 48 h, the mortality rate was 54.00 ± 0.36% ($LC_{50}$ = 91.399 µg $larva^{-1}$); after 72 h, 64.00 ± 0.21% ($LC_{50}$ = 22.939 µg $larva^{-1}$); and after 96 h, 76.00 ± 1.29% ($LC_{50}$ = 14.450 µg $larva^{-1}$) (Table 1). It was observed that as the concentration of the fermented ginger waste extract increased, the mortality rate also increased correspondingly. Moreover, the longer the exposure duration, the higher the mortality rate.

Regarding the mortality rate of beet armyworms due to contact toxicity, it was found that after 48, 72, and 96 h, the fermented ginger waste extract at a 100% concentration caused the highest mortality rate ($P < 0.05$) in these larvae. Specifically, after 48 h, the mortality rate was 40.00 ± 0.10% ($LC_{50}$ = 187.054 µg larva$^{-1}$); after 72 h, 53.00 ± 0.70% ($LC_{50}$ = 91.437 µg larva$^{-1}$); and after 96 h, 66.00 ± 0.95% ($LC_{50}$ = 68.426 µg larva$^{-1}$) (Table 1).

**Table 1.** Mortality rates (%) and median lethal concentration ($LC_{50}$) for *Spodoptera litura* and *S. exigua* after treated with fermented ginger waste extract by contact toxicity at 48, 72 and 96 h under laboratory conditions. Means followed by the same letter in a column are significantly different according to DMRT ($P < 0.05$). $LC_{50}$: Lethal concentration at which half the population is killed, and the active ingredient concentration is expressed in µg larva$^{-1}$; LCL: lower confidence limit; UCL: upper confidence limit.

| Fermented ginger waste extracts (%, w/v) | Mortality rates (± SD) (%) | | | | | |
|---|---|---|---|---|---|---|
| | *Spodoptera litura* | | | *Spodoptera exigua* | | |
| | 48 h | 72 h | 96 h | 48 h | 72 h | 96 h |
| Control | 0.00 ± 0.00$^{d}$ | 0.00 ± 0.00$^{d}$ | 0.00 ± 0.00$^{e}$ | 0.00 ± 0.00$^{d}$ | 0.00 ± 0.00$^{d}$ | 0.00 ± 0.00$^{d}$ |
| 5 | 18.00 ± 0.60$^{c}$ | 24.00 ± 1.13$^{c}$ | 29.00 ± 1.37$^{d}$ | 2.00 ± 0.11$^{c}$ | 10.00 ± 0.33$^{c}$ | 17.00 ± 0.08$^{c}$ |
| 25 | 35.00 ± 1.05$^{b}$ | 59.00 ± 1.41$^{b}$ | 61.00 ± 1.70$^{c}$ | 4.00 ± 1.00$^{c}$ | 12.00 ± 1.00$^{c}$ | 23.00 ± 0.22$^{c}$ |
| 50 | 37.00 ± 0.58$^{b}$ | 60.00 ± 0.25$^{b}$ | 70.00 ± 1.01$^{b}$ | 36.00 ± 1.45$^{b}$ | 36.00 ± 1.45$^{b}$ | 46.00 ± 0.88$^{b}$ |
| 100 | 54.00 ± 0.36$^{a}$ | 64.00 ± 0.21$^{a}$ | 76.00 ± 1.29$^{a}$ | 40.00 ± 0.10$^{a}$ | 53.00 ± 0.70$^{a}$ | 66.00 ± 0.95$^{a}$ |
| $LC_{50}$, µg larva$^{-1}$ | 91.399 | 22.939 | 14.450 | 187.054 | 91.437 | 68.426 |
| LCL | 85.278 | 16.241 | 10.864 | 141.454 | 84.182 | 59.438 |
| UCL | 97.520 | 29.638 | 18.036 | 232.653 | 98.691 | 77.414 |
| Slope | 1.951 | 0.925 | 0.842 | 1.724 | 1.621 | 1.327 |

### Oral toxicity of fermented ginger waste extract on mortality rates of common cutworm and beet armyworm

The oral toxicity testing of fermented ginger waste extract on common cutworms at concentrations of 0%, 5%, 25%, 50%, and 100% (w/v) after 48, 72, and 96 h revealed that the different concentrations of the extract significantly affected the mortality rates of the common cutworms ($p < 0.05$). The 100% concentration of the fermented ginger waste extract caused the highest mortality rate, with $LC_{50}$ values of 282.448, 172.715, and 91.275 µg larva$^{-1}$ at 48, 72, and 96 h, respectively (Table 2).

Regarding the oral toxicity of the fermented ginger waste extract on beet armyworms, the results were consistent with those observed for common cutworms. It was found that after 48, 72, and 96 h, the 100% concentration of the fermented ginger waste extract caused the highest mortality rate ($P < 0.05$) in beet armyworms, with $LC_{50}$ values of 191.389, 132.507, and 88.400 µg larva$^{-1}$ at 48, 72, and 96 h, respectively (Table 2).

From the experiments, it can be observed that the mortality rates of common cutworms from oral toxicity were lower than those from contact toxicity (76.00 ± 1.29% and 52.00 ± 1.70%, respectively), and similarly for beet armyworms (66.00 ± 0.95% and 58.00 ± 1.02%, respectively). In the contact toxicity testing, the fermented ginger waste extract was sprayed directly onto the insect bodies, ensuring thorough exposure. After direct exposure, the larvae exhibited abnormal movements, or some became immobile. Comparing the results at 48, 72, and 96 h, mortality rates of both common cutworms and beet armyworms tended to increase over time. This indicates that the fermented ginger waste extract possesses toxicity against these lepidopteran larvae. Studies on the chemical composition of ginger have revealed that the major components in ginger rhizomes are gingerol, shogaol, and zingerone, which belong to the group of secondary metabolites (Sinha and Ray, 2024). These phenolic compounds, comprising approximately 5%-8%, contribute to the pungent aroma and spicy taste of ginger. These substances exhibit toxicity towards lepidopteran larvae (Lepidoptera: Noctuidae) (Hamada et al., 2018). In this study, the quantities of 6-gingerol and 6-shogaol in the fermented ginger waste extract were examined, revealing concentrations of 4.00 and 12 µg mL$^{-1}$, respectively. These findings are consistent with the study by Keosaeng et al. (2022), who performed crude extractions of ground *Z. officinale* rhizomes using hexane and tested the extract against second-instar *S. litura*, *S. exigua*, and *S. frugiperda* larvae using contact toxicity. The ginger rhizome extract exhibited the highest toxicity towards *S. exigua*, with $LC_{50}$ values of 9.92 and 8.40 µg larva$^{-1}$ at 24 and 48 h post-treatment, respectively, followed by *S. frugiperda* with $LC_{50}$ values of 7.68 and 3.96 µg larva$^{-1}$. These results align with the findings of Hasyierah et al. (2020), who reported that the gingerol and shogaol components in ginger extracts exhibited toxicity towards *Nilaparvata lugens* and *Spodoptera* spp., with mortality rates as high as 90%. Thus, gingerol and shogaol possess insecticidal activity. Similarly, previous studies have demonstrated that gingerol is an effective biopesticide for insect control. These findings are consistent with the research by Khadem et al. (2022), who found that fermented ginger extract resulted in over 80% mortality of citrus mealy bugs (*Planococcus citri*) after 120 h exposure ($LC_{50}$ = 10.95 w/v).

**Table 2.** Mortality rates (%) and median lethal concentration ($LC_{50}$) of *Spodoptera litura* and *S. exigua* after treated with fermented ginger waste extract by oral toxicity at 48, 72 and 96 h under laboratory conditions. Means followed by the same letter in a column are significantly different according to DMRT (P < 0.05). $LC_{50}$: Lethal concentration at which half the population is killed, and the active ingredient concentration is expressed in µg larva$^{-1}$; LCL: lower confidence limit; UCL: upper confidence limit.

| Fermented ginger waste extracts (%, w/v) | Mortality rates (± SD) (%) | | | | | |
|---|---|---|---|---|---|---|
| | *Spodoptera litura* | | | *Spodoptera exigua* | | |
| | 48 h | 72 h | 96 h | 48 h | 72 h | 96 h |
| Control | 0.00 ± 0.00$^{d}$ | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{d}$ | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{d}$ | 0.00 ± 0.00$^{d}$ |
| 5 | 0.00 ± 0.00$^{d}$ | 00.00 ± 0.00$^{c}$ | 00.00 ± 0.00$^{d}$ | 0.00 ± 0.00$^{c}$ | 0.00 ± 0.00$^{d}$ | 0.00 ± 0.00$^{d}$ |
| 25 | 3.00 ± 0.48$^{c}$ | 16.00 ± 0.98$^{b}$ | 16.00 ± 0.10$^{c}$ | 0.00 ± 0.00$^{c}$ | 9.00 ± 0.67$^{c}$ | 12.00 ± 0.28$^{c}$ |
| 50 | 13.00 ± 0.30$^{b}$ | 20.00 ± 0.50$^{b}$ | 36.00 ± 0.67$^{b}$ | 15.00 ± 1.20$^{b}$ | 33.00 ± 0.88$^{b}$ | 40.00 ± 0.73$^{b}$ |
| 100 | 27.00 ± 1.00$^{a}$ | 43.00 ± 0.20$^{a}$ | 52.00 ± 1.70$^{a}$ | 34.00 ± 1.73$^{a}$ | 47.00 ± 1.43$^{a}$ | 58.00 ± 1.02$^{a}$ |
| $LC_{50}$, µg larva$^{-1}$ | 282.448 | 172.715 | 91.275 | 191.389 | 132.507 | 88.400 |
| LCL | 267.764 | 146.916 | 84.035 | 184.261 | 125.162 | 81.516 |
| UCL | 297.131 | 198.514 | 98.515 | 198.517 | 139.851 | 95.283 |
| Slope | 1.021 | 1.529 | 0.962 | 1.125 | 1.036 | 0.971 |

## CONCLUSIONS

The 100% concentration of the fermented ginger waste extract was able to effectively control common cutworms and beet armyworms better than other concentrations, through both contact and oral exposure methods, at 48, 72, and 96 h. When considering the application approach, the contact method, where the extract was directly applied to the larvae, resulted in higher mortality rates for both common cutworms and beet armyworms compared to the oral method. This presents an alternative for utilizing ginger waste, which is a by-product from industrial plants. Instead of burning, burying, or using it as low-quality animal feed, the waste can be fermented to produce an effective biopesticide. However, further studies on the toxicity of the fermented ginger waste extract under field conditions are recommended to facilitate the development of an efficient biopesticide for controlling common cutworms and beet armyworms.

### Author contribution

Conceptualization: N.C., J.N. Methodology: J.N. Validation: N.C. Formal analysis: J.N. Investigation: N.C. Resources: J.N. Data curation: J.N. Writing-original draft: J.N. Writing-review & editing: J.N. Supervision: J.N. Project administration: J.N. All co-authors reviewed the final version and approved the manuscript before submission.

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