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Insecticidal effects of phenolic compounds on the development of polyphagous *Spodoptera litura* larvae

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ABSTRACT

We studied the potential of 4-plant secondary compounds (Phenolic compounds: Hydroquinone, Pyrogallol, Phloroglucinol and Pyrocatechol) as biopesticides against the lepidopteran, *Spodoptera litura* (Fabricius). These compounds at 1, 5, 25, 125, 625, 3125 ppm concentrations had antibiosis influence on 12-days old larvae of *S. litura.* Among these test phenols Phloroglucinol was most toxic to growth and nutritional physiology of the insect. Our study demonstrated that these phenolic compounds may be developed as ecofriendly and safe alternatives to conventional pesticides.

Keywords: Antibiosis, biopesticides, development, insect, larvae, phenolic compounds, phloroglucinol, secondary metabolites, *Spodoptera litura*.

INTRODUCTION

Sustainable agricultural practices are required to ensure food availability for the growing population in developing countries. One of the major constraints to food production are the insect pests which cause huge yield losses. Though yield losses were reduced considerably with the advent of synthetic pesticides, however their persistent use has resulted in evolution of resistant strains of insect pests. Also, increasing level of pesticide residues in food and soil is causing major health hazard leading to neurological dysfunction (5,18), other acute and chronic diseases such as eye irritation, cancer, reproductive and developmental disorders (1,34). Recently, much attention is being given to plant derived compounds as pesticides which are environmentally safe, easily biodegradable and non-toxic to organisms.

Phenolic compounds in plants are crucial for their growth, development and defense (22). In humans some of these compounds have anticancer, anti-inflammatory, antifungal and pharmacological activity (25). However, there is considerable variation in insect's response to phenolics, some deter insects feeding, whereas, others have undesirable physiological effects on insects. This variation in responses of insects to phenolics is partly due to diversity in oxidative conditions in insect digestive system (4). In insects having alkaline midgut, the phenolic compounds are oxidised to hydrogen peroxide and organic hydroperoxides, whose further breakdown produces highly reactive free radicals. These radicals oxidize cellular components and deteriorate the nutrients quality of food in the gut lumen (15,31).

The common cutworm, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is an important polyphagous insect-pest (6,16). Its caterpillars are voracious feeders and defoliate the crops in vegetative stage and also attack the fruits in fruiting stage (2). The crop losses by this pest range from 25-100 % depending on the crop stage and extent of

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infestation (12,14). Hence, this study aimed to determine the bioinsecticidal potential of simple phenolic compounds against the lepidopteran insect pest *S. litura*.

Figure 1. *Spodopteralitura*

METHODS AND MATERIALS

The experiments were done during the month of July, 2018 in Department of Zoology, Guru Nanak Dev University, Amritsar (Latitude and longitude: N 31°38'2.3280", E 74°52'20.1396"). Altitude: 244 m above sea level. Semiarid climate, mean daily temperature: 23.3 °C and mean annual rainfall: 703 mm.

Insect rearing: The culture of *S. litura* was obtained from the fields of our University (Figure 1). These were then reared for subsequent generations in the insect culture room under controlled conditions (25±2 °C temperature and relative humidity 60 ± 5 %). The larvae were fed on fresh castor leaves, in battery jars (15 cm × 10 cm) covered with muslin cloth to prevent their escape. The pupae were transferred to separate jars having moist sterilized sand covered with filter paper. Adults emerged from the pupae were transferred to oviposition jars lined with filter paper to facilitate egg laying in ratio 2:1 (Female:Male). They were fed on a cotton swab soaked in honey solution (1:4 honey/water) and hanged from the mouth of the oviposition jars. The egg batches of the oviposition jar were laid on the filter paper. When the eggs turned into black head stage, the filter paper with egg batches were cut and kept in petri plates until hatching. The newly hatched larvae were transferred into battery jars having fresh castor leaves. The 12 days old larvae were used for the experiments.

Chemicals used: Pyrocatechol, Phloroglucinol, Pyrogallol and Hydroquinone were procured from Sigma Aldrich pvt. Limited Mumbai.

Experiments: Artificial diet was prepared with slight modifications (20) and was amended with different concentrations [1 ppm (0.0001 %), 5 ppm (0.0005 %), 25 ppm (0.0025 %), 125 ppm (0.0125 %), 625 ppm (0.0625 %) and 3125 ppm (0.3125 %)] of phenolic compounds and control (only water). The antibiosis effect was determined by feeding the 12 days old larvae on diet amended with different concentrations of test compounds. The larvae were individually kept in separate sterilized cups with pieces of artificial diet containing various concentrations of test compounds. Every day, the diet was changed and the experimental cups were cleaned to avoid any infection. The various developmental parameters [larval mortality (%), adult emergence (%), females emerged (%), adult longevity, larval period, total development period and pupal weight] were recorded to find the effects of tested compounds on the 12 days old larvae of *S. litura*. The treatments were replicated 6 times, with 5 larvae in each replicate.

A nutritional assay was done to assess the influence of phenolic compounds on the relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion of ingested food (ECI), efficiency of conversion of digested food (ECD) and approximate digestibility (AD) [19,33]. Before the start of experiment, the larvae were starved for 6.0 h to clean their gut. The initial weights of experimental larvae were taken, individual larvae were kept into sterile plastic containers with known weight of diet (treated and untreated). These experimental cups were kept in BOD Incubator [27±2 ºC temperature, 65±5 % humidity, and L16 : D18 photoperiod] for 3 days. After 72 h, final weight of larvae, diet left and the fecal matter were oven dried at 60 ºC for 48 h and were weighed to know their dry weight. The dry weight data showed the water loss under controlled conditions. The nutritional indices were calculated as under:

$$\text{RGR} = \frac{\text{Change in larval dry weight/day}}{\text{Starting larval dry weight}}$$

$$\text{RCR} = \frac{\text{Change in diet dry weight/day}}{\text{Starting larval dry weight}}$$

$$\text{ECI} = \frac{\text{Dry weight gain of insect}}{\text{Dry weight of food ingested}} \times 100$$

$$\text{ECD} = \frac{\text{Dry weight gain of insect}}{\text{Dry weight of food ingested - Dry weight of frass}} \times 100$$

$$\text{AD} = \frac{\text{Dry weight gain of insect - Dry weight of frass}}{\text{Dry weight of food ingested}} \times 100$$

Where, RGR: Relative growth rate, RCR: Relative consumption rate, ECI: Efficiency of conversion of ingested food, ECD: Efficiency of conversion of digested food, AD: Approximate digestibility

Statistical Analysis

The data were represented as their mean ± SE. The one-way analysis of variance (ANOVA) with Tukey’s test was done at $p \leq 0.05$ to check the differences in means, SPSS software for windows version 16.0 (SPSS Inc, Chicago), Microsoft office Excel 2007 (Microsoft Corp., USA) and Assistant software were used for statistical analysis.

RESULTS AND DISCUSSION

Bioassay studies: Ingested phenolic compounds had adverse effects on the growth, development and nutritional physiology of 12 days old larvae of *S. litura*. Phloroglucinol at the highest concentration of 3125 ppm was most toxic to larvae with 93.33 % mortality as compared to16.67 % mortality in control (Figure 2a). While Hydroquinone was least toxic. The adult emergence (%) from the treated larvae was also decreased (Figure 2b). The emergence was reduced in concentration dependent manner in all treatments with Phloroglucinol, showing the maximum inhibitory effects. Phloroglucinol consumption reduced the adult emergence (%) by 96 % at the highest concentration, as compared to control. At 3125 ppm concentration the Pyrocatechol caused 72 % inhibition followed by Hydroquinone (68 %) and Pyrogallol (56 %). Similar findings were reported when 6 days old *S. litura* larvae were fed on Pyrogallol containing diet (8). Thus the higher concentrations had more severe effects on survival of the larvae than lower concentrations, indicating that larval toxicity was associated with phenolics content in the diet. Phenolics in plants provide the resistance to insects. It was reported that wheat containing high levels of total phenolics was resistant to aphids (10). Our findings are in accordance with Manoukas (23) who also noticed decline in pupation (%) and emergence (%) with phenolic compounds viz., Hydroquinone, Phloroglucinol and Pyrogallol in Olive fruit fly, *Bactrocera olea* (Rossi). Insecticidal activities have also been reported for Hydroquinone derivatives obtained from aerial parts of *Roldanobarba-johannis* (J.E Smith) against *Spodoptera frugiperda* (J.E Smith) (7). A significant decline was also observed in pupation (%) and emergence (%) of first and second instar larvae of *B. cucurbitae*, when fed on diet amended with Phloroglucinol (28). At Phloroglucinol concentration of 3125 ppm, no females emerged (Figure 2c). The phenolic compounds present in *Citrus colocynthis* (L), *Rubussanctus* (Rosaceae) and *Lycium barbarum* (L.) are toxic to domestic housefly, *Musca domestica* (L.) was also reported (24).

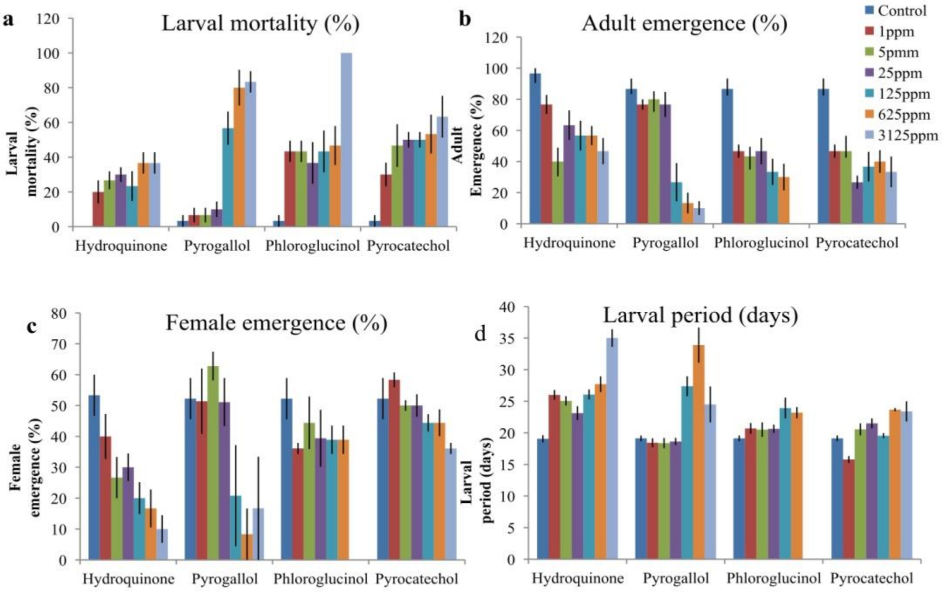

Figure 2. (a) Larval mortality (%) (b) Adult emergence (%) (c) Female emergence (%) (d) Larval period (days) of *S. litura* when 12 days old larvae were fed on different concentration of phenolic compounds

The larval period was also significantly prolonged with the phenolic compounds. At 3125 ppm concentration, the larval period in Phloroglucinol, Pyrocatechol, Hydroquinone and Pyrogallol treated 12 days old larvae increased by 18.77 days. 8.88 days, 8.63 days and 6.11 days, respectively, when compared with control (Figure 2d).

The total development period of 12 days old larvae was delayed significantly with all phenolic compounds (Figure 3a). In Phloroglucinol fed larvae, a delay of 16 days was observed at 3125 ppm than control. Pupae at higher concentration of phenolic compounds weighed much less than control in all treatments (Figure 3b). The longevity of emerged adults was significantly reduced with increase in concentration of phenolic compounds (Figure 3c). At 3125 ppm, Phloroglucinol followed by Pyrogallol had greater effects on longevity of adults which was reduced to 44.31 % and 51.45 % of control, respectively.

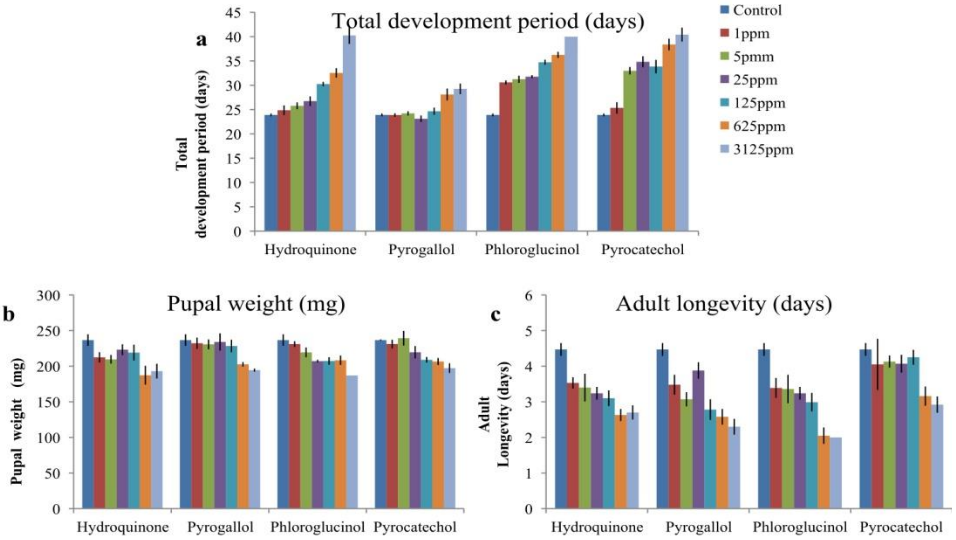

Figure 3. (a) Total developmental period (days) (b) Pupal weights (mg) (c) Adult longevity (days) of *S. litura* when 12 days old larvae were fed on different concentration of phenolic compounds.

A significant prolongation in larval and total development period was observed in the larvae of *S. litura* treated with phenolic compounds as compared to control. Delayed growth could be due to less ingestion of food containing the phenolic compounds. The highest contents of polyphenolic compound, chlorogenic acid occurring in cortex tissue of sweet potato inhibits the larval growth and survival of immature insects (27). Pyrocatechol isolated from leaves of quaking aspen had antifeedant effects on larvae of *Spodoptera eridania* (Cramer) and final instar of *Papilio glaucus* (1). The retarded larval growth increases the mortality of insect due to increased exposure to natural enemies and diseases (9). The phenolic compounds inhibited the larval and pupal growth of *S. frugiperda* (17,32). According to them, the inhibition of insect growth was due to activity of phenolic compounds, which reduces the nutritional value of food. Since the Lepidopteran adults feed very little as adults, all nutrients required for adult survival, reproduction and migration have to be acquired in the larval stages (11). To pupate, the larvae need to attain a critical weight (13). Low food consumption and delayed growth of larvae adversely affects the pupae formation, emergence of adults and their longevity. Moreover, the pupae formed from the treated larvae weighed lower than control. Lower pupal mass causes poor fecundity as pupal mass is related to reproductive potential (11).

Nutritional studies: The Phloroglucinol and Pyrocatechol at higher concentrations significantly affected the RGR and RCR. The Phloroglucinol at 3125 ppm reduced the RGR to 27 % of control. Pyrogallol decreased the RGR with increase in concentration up to 625 ppm, but the increased RGR and RCR of larvae was not enhanced by the Hydroquinone treatment (Table 1,2). ECI and ECD were not significantly influenced by Phloroglucinol and Pyrocatechol. ECI increased significantly with Pyrogallol but ECD was not much affected. On the other hand, Hydroquinone fed larvae showed significant increase in ECI and ECD after treatment (Table 3, 4). AD was reduced in Hydroquinone, Pyrogallol and Phloroglucinol fed larvae, but increased in Pyrocatechol treated larvae (Table 5). Our results thus showed that the growth rate of Phloroglucinol and Pyrocatechol treated larvae decreased notably with increasing concentration of phenolic compounds. Lower consumption of treated diet may account for lower growth rate.

Table 1. Effects of different concentrations (ppm) of phenolic compounds on Relative Growth Rate (mg/mg/day) 12 days old instar larvae of *S. litura*

Phenolic compounds Conc (ppm)	**Hydroquinone**	**Pyrogallol**	**Phloroglucinol**	**Pyrocatechol**
Control	0.33 ± 0.04^{a}	0.89 ± 0.08^{a}	0.36 ± 0.03^{a}	0.97 ± 0.12^{a}
1	0.35 ± 0.39^{a}	0.82 ± 0.46^{ab}	0.29 ± 0.04^{ab}	0.87 ± 0.18^{ab}
5	0.85 ± 0.05^{c}	0.81 ± 0.08^{ab}	0.27 ± 0.04^{ab}	0.90 ± 0.65^{ab}
25	0.89 ± 0.40^{c}	0.80 ± 0.07^{ab}	0.28 ± 0.05^{ab}	0.51 ± 0.05^{bc}
125	0.91 ± 0.03^{c}	0.85 ± 0.05^{a}	0.24 ± 0.04^{abc}	0.44 ± 0.02^{c}
625	0.85 ± 0.06^{c}	0.55 ± 0.05^{b}	0.19 ± 0.04^{bc}	0.39 ± 0.02^{c}
3125	0.64 ± 0.02^{b}	0.70 ± 0.04^{ab}	0.09 ± 0.01^{c}	0.30 ± 0.01^{c}
F-value (df=6)	39.59^{**}	3.36^{*}	5.37^{**}	9.14^{**}

**Significant at 1%, *Significant at 5%. Means followed by the same letter within the columns are not significantly different according to Tukey's test at $P \leq 0.05$.

Table 2. Effects of different concentrations (ppm) of phenolic compounds on Relative Consumption Rate (mg/mg/day) 12 days old instar larvae of *S. litura*

Phenolic compounds Conc (ppm)	**Hydroquinone**	**Pyrogallol**	**Phloroglucinol**	**Pyrocatechol**
Control	2.15 ± 0.06^{a}	2.16 ± 0.09^{a}	2.23 ± 0.09^{a}	2.10 ± 0.05^{a}
1	3.30 ± 0.21^{bd}	3.21 ± 0.17^{bc}	2.02 ± 0.08^{a}	4.63 ± 0.75^{bc}
5	3.46 ± 0.11^{d}	4.01 ± 0.20^{bd}	2.05 ± 0.15^{a}	5.42 ± 0.34^{c}
25	2.81 ± 0.10^{bc}	4.20 ± 0.17^{d}	1.86 ± 0.16^{ab}	3.19 ± 0.23^{ab}
125	2.47 ± 0.10^{ac}	3.79 ± 0.26^{bd}	1.80 ± 0.07^{ab}	3.18 ± 0.27^{ab}
625	2.65 ± 0.15^{ac}	2.87 ± 0.20^{ac}	1.39 ± 0.03^{bc}	2.31 ± 0.08^{a}
3125	2.95 ± 0.15^{bcd}	2.94 ± 0.15^{ac}	1.19 ± 0.05^{bc}	1.92 ± 0.03^{a}
F-value (df=6)	12.23^{**}	15.47^{**}	12.31^{**}	14.95^{**}

**Significant at 1%. Means followed by the same letter within the columns are not significantly different according to Tukey's test at $P \leq 0.05$.

The growth rate of Pyrogallol fed larvae was also reduced despite higher consumption of treated diet by the larvae. Phenolic compounds can negatively affect the development of insects not only by affecting food ingestion but also by causing post ingestive and post digestive changes (29). The ECI of Pyrogallol fed larvae was lower than control, which indicated interference of phenolic compounds in utilization of ingested food for its growth. On the contrary, the nutritional indices viz., RGR, RCR, ECI and ECD increased in the larvae fed on Hydroquinone treated diet. It was observed that larvae fed on phenols diets respond by excreting excess fecal pellets as compared to individuals fed control diet to remove the toxic substances present in the diet (3). The higher ECD of Hydroquinone treated larvae could thus be a physiological adaptation, wherein, the larvae excessively defecates the diet having toxic compounds, thereby less quantity of food material is allocated to body tissues.

Table 3. Effects of different concentrations (ppm) of phenolic compounds on Relative efficiency of Conversion of Ingested Food (in %) 12 days old instar larvae of *S. litura*

Phenolic compounds Conc (ppm)	**Hydroquinone**	**Pyrogallol**	**Phloroglucinol**	**Pyrocatechol**
Control	16.33±2.07^{ad}	44.65±5.39^{a}	17.39±1.66	16.55±2.19
1	10.32±0.77^{d}	26.28±2.14^{b}	16.00±2.46	15.71±1.88
5	25.40±1.51^{ab}	23.41±2.42^{b}	15.46±2.87	17.91±1.83
25	33.49±1.88^{bc}	19.55±2.01^{b}	18.13±3.73	16.00±2.04
125	38.88±2.69^{c}	24.42±3.35^{b}	15.04±2.92	15.13±1.07
625	33.08±3.31^{bc}	19.65±1.37^{b}	15.10±3.83	17.63±1.12
3125	24.30±2.44^{ab}	24.58±0.89^{b}	7.58±1.37	16.50±0.73
F-value (df=6)	20.59**	8.76**	Ns	ns

**Significant at 1%, ^{ns}Non Significant. Means followed by the same letter within the columns are not significantly different according to Tukey's test at P ≤ 0.05.

Table 4. Effects of different concentrations (ppm) of phenolic compounds on efficiency of Conversion of Digested Food (in %) 12 days old instar larvae of *S. litura*

Phenolic compounds Conc (ppm)	**Hydroquinone**	**Pyrogallol**	**Phloroglucinol**	**Pyrocatechol**
Control	28.23±6.79^{a}	68.5±14.40	29.44±6.26	27.78±6.27
1	35.82±17.7^{ab}	65.48±4.85	22.50±3.34	23.70±1.87
5	55.08±5.24^{ab}	42.42±6.49	22.64±5.40	23.65±2.29
25	67.10±13.8^{ab}	50.63±8.30	22.86±4.30	21.59±2.36
125	75.93±4.44^{b}	39.65±6.83	21.85±3.80	20.16±1.22
625	51.57±6.19^{ab}	53.48±8.36	21.75±3.73	21.34±1.16
3125	55.12±6.44^{ab}	58.78±8.83	`17.45±2.63	20.49±0.67
F-value (df=6)	2.83*	ns	Ns	ns

*Significant at 5%, ^{ns} Non-Significant. Means followed by the same letter within the columns are not significantly different according to Tukey's test at P ≤ 0.05.

Among all phenolic compounds, Phloroglucinol proved the most toxic to larvae of *S. litura*. Our findings corroborated the research of Pavela (26) who found Phloroglucinol, among the nine phenols tested, as the most effective compound in preventing the feeding of larvae of Colorado potato beetle, *Leptinotarsa decemlineata* (Say). The findings thus reveal that the simple phenols have considerable potential for use in management of the insect pest and need to be evaluated further for enhancing resistance in plants.

Table 5. Effects of different concentrations (ppm) of phenolic compounds on Approximate Digestibility (%) 12 days old instar larvae of *S. litura*

Phenolic compounds Conc (ppm)	**Hydroquinone**	**Pyrogallol**	**Phloroglucinol**	**Pyrocatechol**
Control	75.35 ± 2.43^{a}	76.24 ± 2.62^{a}	75.99 ± 2.33^{ab}	75.22 ± 2.43^{ab}
1	56.70 ± 2.13^{bc}	44.24 ± 4.48^{cd}	76.36 ± 1.71^{a}	67.21 ± 4.14^{b}
5	56.38 ± 2.20^{bc}	59.91 ± 2.28^{abc}	76.19 ± 3.06^{a}	80.20 ± 2.82^{a}
25	21.94 ± 2.26^{d}	48.82 ± 4.96^{bcd}	80.14 ± 3.51^{a}	76.12 ± 3.63^{ab}
125	54.66 ± 1.27^{c}	64.39 ± 3.11^{ab}	74.67 ± 2.23^{ab}	77.17 ± 1.48^{ab}
625	66.16 ± 1.95^{ab}	42.14 ± 3.45^{d}	63.89 ± 3.70^{b}	82.94 ± 1.35^{a}
3125	58.21 ± 3.50^{bc}	44.74 ± 5.13^{cd}	46.81 ± 2.30^{c}	81.02 ± 2.86^{a}
F-value (df=6)	47.75^{**}	11.01^{**}	17.55^{**}	3.34^{*}

**Significant at 1%, *Significant at 5%. Means followed by the same letter within the columns are not significantly different according to Tukey's test at $P \leq 0.05$.

CONCLUSIONS

Our findings showed that all tested plant based phenolic compounds were insecticidal and significantly reduced the *S. litura* populations by disrupting the developmental process. Among all the tested phenols, phloroglucinol was found most deterrent and toxic to insect larvae. Thus phenolic compounds could be developed as ecofriendly and safe biopesticides.

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Compliance with ethical standards

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest

The authors declare that they have no conflict of interest.

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