The Valorization of Spent Coffee Grounds Extract as A Prospective Insecticidal Agent Against Some Main Key Pests of *Phesolus Vulgaris* in Laboratory and Field

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Abstract

Exploiting massive amounts of food and agro-waste represents a severe social, economic, and environmental issue. Under the growing demand toward food products free of toxic synthetic insecticides, the methanolic extract of spent coffee grounds (SCGs), which represent the main by-product of coffee production, was applied in the current study as a bioinsecticide against the main pests of the green bean; *Spodoptera littoralis*, *Agrotis ipsilon*, *Bemisia tabaci*, *Empoasca fabi* and *Aphis craccivora*. Deterrent assay, contact bioassay, and lethal concentration analysis were performed to reveal the repellent, antifeedant, and oviposition deterrent effects. Parallel to the above-performed bioassays, the phytochemical composition of the SCGs methanolic extract was investigated via High-Performance Liquid Chromatography (HPLC) analysis. Fourteen phenolic acids and five flavonoids in addition to caffeine (alkaloid) have been identified in the extract. Cinnamic, rosmarinic, and gallic acids were the predominant phenolics, while apigenin-7-glucoside was the main flavonoid, followed by naringin, catechin, and epicatechin. The extract of SCGs showed an insecticidal effect, with mortality between 27.5-76% compared to the control (7.4%) and based on the concentration of the extract used. In the same trend, oviposition efficiency revealed different laid egg batches (0.67, 2.33, 7.33, and 8.67 batch/jar) for 100, 50, 25% of the SCGs extract and control. Finally, the major components of SCGs extract were docked into insecticide acetylcholinesterase enzyme to explore their potential of inhibition, where apigenin-7-glucoside showed a higher binding affinity, followed by catechin, compared to the control (lannate). Obtained findings could be a starting point to develop novel bioinsecticides from SCGs.

Introduction

Many insects have greatly affected food production, human health, and consequently, the global economy. However, the inappropriate use of synthetic insecticides to control such insects is related to the development of resistance in pests, human diseases, and contamination of both the food and the environment¹. Therefore, natural products with insecticidal activity constitute a vital alternative that could be described as safe for humans and the environment. Plants are a rich source of bioactive metabolites such as phenolic acids, which are well known for antioxidant, anticancer, and antimicrobial activities². Previous studies showed that *J. regia* L. phenolic extract showed inhibition in fecundity and development of the grain aphid (*Sitobion avenae* L.) using lab bioassays³. In the same context, Czerniewicz *et al.*⁴ indicated that phenolic acids from *H. perforatum* L., *J. regia* L., *M. piperita* L., and *S. nigra* L. have negatively affected the development, deterrent, and aphidicidal activity of the peach-potato aphid, *Myzus persicae* Sulz., and the bird cherry-oat aphid, *Rhopalosiphum padi* L.

Agricultural and food-industrial solid wastes are accumulated in a vast quantity, represent a global problem for health, the environment, and could be a source for bioactive phenolics. So, the exploitation of these wastes as raw materials for the bio-economy became a target for food sustainability policy⁵. For instance, more than 6.5 million tons of spent coffee grounds (SCGs) were obtained from roasted coffee beans during instant coffee production in 2020/2021 worldwide⁶. SCGs showed concerning alkaloids

and phenolics with superior antioxidant and anti-inflammatory activities⁷⁻⁹. Up to our knowledge, coffee grounds and their waste were used as repellents for the cockroach and gravid *Aedes albopictus* females^{10,11}; however, nothing was reported concerning the insecticidal activity of SCGs extracts.

Common bean (*Phesolus vulgaris*) is a mean grain legume crop present worldwide and third in importance after soybean and peanut. Of this crop, can lose cause to insect pests alone have been estimated to be from 35% to 100% annually¹². Insect pests, including aphids, caterpillars, leafhoppers, and whiteflies, are responsible for this crop's excessive percent of the loss. Several pests like lepidopteran and sucking pests prefer to attack *P. vulgaris* plants¹³.

This study investigates the insecticidal efficacy and the repellent effect of SCGs methanolic extract against five distractive pests of *P. vulgaris* crop, Aphid; *Aphis craccivora*, leafhopper; *Empoasca fabi*, whiteflies; *Bemisia tabaci*, cotton leafworm; *Sopdoptera littoralis*, and cutworm; *Agrotis ipsilon*. Toxicity effects and deterrence were assessed by lethal concentration, deterrent assay, and contact bioassay analysis. Following the screening and identification of SCGs methanol extract using HPLC, molecular docking and dynamic simulation were performed to evaluate compounds with potential insecticidal activity through predicting the binding modes of the major components of the extract at the insect acetylcholinesterase (AChE), which control the synaptic transmission and is essential for neurotransmitter hydrolysis (Acetylcholine). The valorization of SCGs as a novel environmentally friendly insecticide seems to be a business opportunity to have high value-added products, which can be established as an approach enclosed in the general idea of sustainability.

Methods

Materials and chemicals

SCGs were kindly supplied by Eng. Khaled El-Naggar, Misr Cafe (10th. of Ramadan Ind. City, Cairo, Egypt). The material was dried using a hot air oven at 38±2°C until 5% moisture content and stored for further extraction. Phenolic standards and methanol were purchased from Sigma (Sigma–Aldrich GmbH, Sternheim, Germany). All other chemicals used were of analytical grade and obtained from either Sigma–Aldrich or Merck (Darmstadt, Germany).

Solvent extraction procedure

The extraction was carried out as optimized by Mussatto *et al.*⁸. SCGs were extracted with 60% methanol concentration in a solvent/solid ratio of 40 ml/g SCGs, during 90 min. at 60–65°C in a water bath with magnetic agitation. Subsequently, the total content was centrifuged (2500g, 4°C, 20 min), and the supernatant (SCGs extract) was filtered through 0.22 µm filters and stored at -20°C in darkness until analyses or use. The volume of extract recovered was quantified and used for calculations.

Determination of phenolic acids and flavonoids

The phenolic acids and flavonoids profile and content in SCGs methanolic extract were analyzed using high-performance liquid chromatography (HPLC). The 2 mL of the extract was injected into HPLC apparatus (Agilent 1100, G1329A ALS, Milford, MA, USA), equipped with photodiode array detector PDA 2998 (Waters, Milford, MA, USA), quaternary pump, and autosampler. Separation was performed on a Symmetry C18 (4.6 × 150 mm, 3.5 µm) column (Waters, Milford, MA, USA) at 20 °C. The mobile phase was solvent A (2.5% acetic acid, v/v) and solvent B (acetonitrile). The following gradient was applied: 3–9% B (0–5 min), 9–16% B (5–15 min), 16–36.4% B (15–33 min), followed by an isocratic run at 100% of B (5 min) and reconditioning of the column (3% of B, 10 min). The flow rate was 1.0 mL/min. The solvent mixture was degassed in an ultrasonic bath before being used as a mobile phase. The concentration of the phenolic acids was determined from standard curves made with known concentrations of each compound. Different wavelengths were used to retrieve peak areas at 280 nm, 320 nm, and 350 nm to maximize the generated signal and reduce detection and quantitation limits.

Oviposition deterrent activities of SCGs extract against female moth *Spodoptera littoralis*

Twelve plastic jars capability 3 Kg (15cm diameter x and 30cm high) were divided into four groups according to the applied concentrations (100, 50, 25%, and control), with three replicates. Ten pupae are chosen at the same age, five males and five females put inside each jar, and *Nerium oleander* plants (*Nerium oleander*) have been selected for oviposition. For adults who emerged from pupae, three plants of *Nerium oleander* treated with each extract concentration have been added inside jars covered by marshaling cloth and tight by rubber bands. Adults were left till copulation and inspected daily to count how many batches of eggs had been laid on plant per 5 females until all insects died.

Insecticidal effect of SCGs extract on the percentage mortality of *Spodoptera littoralis*

This test was performed using 16 Petri-dishes (15cm in diameter) divided into four groups for three concentrations of SCGs extract (100, 50, and 25%) in addition to the control (water). Twelve leaves of the same size of the *P. vulgaris* plant were sprayed with the extract for each concentration. Five cotton leaf worms at the beginning of the fifth larval instar were added to each plate. The number of survival in those treated dishes is inspected daily to record the percent of mortality.

Survey and population density of insects of *P. vulgaris* according to the treated field by SCGs extract

Field studies were performed at Bheira Governorate from the middle of February 2021 till the end of May 2021. The experimental area has been chosen at the agricultural experiment station of the National Research Centre, Nubaria region, Egypt (latitude 30.8667 N, and longitude 31.1667 E, and mean altitude 21 m above sea level), 140 Km away from Cairo. The field was rectangular (17.5m, width, 29m, length), and the total area of this field was 507.5m. The field has been divided into two parts in width of the land,

each part 8.4 m., separated by line 70cm. Within every two plots, in the side of length, barrier space is 50 cm. Each plot was 8.4 m in width and 3.20m in length. Bronco cultivar of green bean seeds are provided by the crops and seed propagation department of the agricultural research Centre of the Ministry of Agriculture, (Giza, Egypt). Experimental and field studies were carried out following relevant institutional, national, or international guidelines or regulations.

Two seeds were planted per hole and thinned after three weeks after seedling emergence. Manual weeding was controlled, and herbicide was applied. The seeds of the Green bean, *P. vulgaris*, were sown in plots at the mid of February and harvested in June. Surveying of the main key pests of *P. vulgaris* has been done by different methods, yellow-colored sticky traps, sweep nets, and direct counting. The total numbers of each pest were recorded, and the mean number was calculated for various concentrations on every pest.

Molecular Docking

The crystal structure of AChE (PDB ID: 1QON) was obtained from the Protein Data Bank (PDB) (<https://www.rcsb.org/>), prepared as a receptor by removing waters and co-crystallized ligands and ions then protonated using Pymol software (Ver. 2.5.1)¹⁴. Meanwhile, the 3d structure of ligands downloaded from the PubChem database, accessed on 6 October 2021 (<http://pubchem.ncbi.nlm.nih.gov/>), were optimized using the MMFF94 force field by Avogadro Software (Ver. 1.2.0)¹⁵. According to the specified recommendations, the docking process was performed using combined validated AutoDock tools 1.5.6 with Vina¹⁶. Briefly, polar hydrogen atoms and Kollman charges were assigned for the receptor protein, while the addition of hydrogens and protonation with set torsions automatically have been performed for ligands. The grid box dimension was fixed at 40 × 40 × 40 Å, at x, y, and z coordinates with 0.375 Å spacing, and the grid center (32.5, 68.3, and 11.2) based on DeepSite, which is a binding pocket predictor using neural-networks (<https://playmolecule.com/deepsite/>) accessed on 6 October 2021¹⁷. The genetic algorithm was used to evaluate parameters with the default setting, and Lamarckian GA (4.2) was employed for docking simulations. The lowest Gibbs free binding energy (estimated as ΔG in kcal/mol) conformers were selected for post-dock analysis. Discovery Studio software (Ver. 21.1.0.20298) was used to visualize and analyze interactions of the best-docked complexes.

Statistical analysis

The results were expressed as mean values standard deviation from at least three replicates. The data collected from the insecticidal activity was performed for statistical analysis by using the Microsoft Excel program. Compared with one-way ANOVA and Least Significant Difference ($p < 0.05$) test, the significant difference among extract concentrations was followed to differentiate individual mean significant difference at 0.05% level.

Results And Discussion

Characterization of phenolic acids, flavonoids, and caffeine using HPLC

Based on HPLC analysis, fourteen phenolic acids and five flavonoids in addition to caffeine (alkaloid) have been identified in the SCGs methanolic extract (Table 1). Cinnamic (979.38 µg/g), rosmarinic (163.10 µg/g), and gallic (32.51 µg/g) acids were the predominant phenolic acids, followed by sinapic (10.10 µg/g), chlorogenic (8.74 µg/g), salicylic (7.61 µg/g), and caffeic acids (6.41 µg/g) (Table 1). Many researchers have investigated the polyphenolic constituents of coffee waste; however, the yield and type of bioactive compounds extracted from SCGs depend on many factors like the coffee species, instant coffee production technique and efficiency, storage conditions, and the extraction method, which is based on polar solvents. For example, increased the intensity of coffee roasting from medium to dark reduced phenolic acids like chlorogenic due to isomerization, gallic, *p*-coumaric, ferulic acids, increased ellagic acid without affecting caffeic acid and ruten contents⁹. Andrade *et al.*¹⁸ have extracted phenolic compounds from SCGs and coffee husks using different techniques like ultrasonic, Soxhlet, and SFE based on ethanol, ethyl acetate, and CO₂ solvents. They identified chlorogenic acid as abundant among others in all extracts. Meanwhile, gallic acid, *p*-hydroxybenzoic, protocatechuic, vanillic, and tannic were also detected in lower concentrations. In contrast, Okur *et al.*¹⁹ identified both chlorogenic and caffeic acids as minor components, which agrees with our findings. The focus on chlorogenic acid is due to its potential health benefits described in previous studies, including free radical scavenging capacity, anti-inflammatory, antidiabetic, and anticancer effects²⁰.

Table 1 Contents of phenolic acids, flavonoids, and caffeine determined in SCGs isopropanol extract

Compound	Phenolic acids (µg/g of the SCGs extract) ^a	Compound	Flavonoids (µg/g of the SCGs extract)
Gallic acid	32.51±2.08	Catechin	14.55±1.47
Protocatechuic acid	2.07±0.74	Epicatechin	10.08±2.37
<i>p</i> -Hydroxybenzoic acid	4.37±0.88	Naringin	86.94±3.15
Gentisic acid	0.26±0.05	Apigenin-7-glucoside	1534.22±7.74
Chlorogenic acid	8.74±1.05	Chrysin	1.01±0.14
Caffeic acid	6.41±0.74	-	-
Syringic acid	3.41±0.41	-	-
Vanillic acid	2.08±0.56	-	-
Sinapic acid	10.1±1.24	-	-
Rosmarinic acid	0.53±0.22	-	-
Ferulic acid	0.37±0.14	-	-
Salicylic acid	7.61±1.05	-	-
<i>p</i> -coumaric acid	0.16±0.11	-	-
Cinnamic acid	979.38±4.78	-	-
Rosmarinic acid	163.1±3.74	-	-
Compound	Alkaloid (µg/g of the SCGs extract)		
Caffiene	1322.2±5.71		
^a Values represent averages ± standard deviations for triplicate experiments.			

Cinnamic acid, which is predominant in the present study in agreement with Vamanu *et al.*²¹, has generally been reported to be phenolic constituents in SCG in previous research but in much lower concentrations with respect to the extraction technique²². As explained above, the variations might be due to many reasons, including solvent, solvent/sample ratio, extraction time, extraction method, and temperature. To our knowledge, rosmarinic acid, an ester of caffeic acid reported in *Coffea arabica* L.²³, is newly identified in the present study and has never been mentioned before in SCGs. Compared to ethanol and methanol, water was reported as the most efficient solvent for the extraction of gallic acid; therefore, it was not always detected in SCGs²⁴. However, gallic acid was identified in the current study with a median concentration, maybe due to the detection and quantification sensitivity of the analysis²². On the

other hand, coumaric (0.16 µg/g), gentisic (0.26 µg/g), and ferulic (0.37 µg/g) acids were the lowest compared to the identified constituents, which are in agreement with Angeloni *et al.*²⁵ (Table 1).

Apigenin-7-glucoside was the main flavonoid identified in SCGs methanolic extract, followed by naringin, catechin, epicatechin, and chrysin (Table 1). Up to our knowledge, only four flavonoids have been identified in SCGs using ethanol and microwave extraction technique; epicatechin, catechin, rutin, and quercetin; however, nothing was reported about apigenin-7-glucoside or naringin⁹. Generally, both apigenin-7-glucoside and naringin were present in green coffee beans, with an increase in their concentrations upon roasting using either microwave or oven techniques²⁶. It is well-known that flavonoids serve many beneficial health functions and elicit protective effects, including anti-inflammatory, antioxidant, antiviral, and anti-carcinogenic⁹.

Caffeine is one of the essential bioactive components found in SCGs in a remarkable concentration (Table 1), consistent with Kovalcik *et al.*⁷. It is a methylxanthine alkaloid, which showed deleterious effects on the nervous system, on the sensitization of DNA to damage, on the delayed entry of cells into mitosis, and on other aspects of cell division on the development of organisms, on fertility, and on chromatin structure, to mention but a few²⁷.

Oviposition deterrent activities of SCGs extract against female moth *Spodoptera littoralis*

Daily inspection showed that eggs laid by females in jars treated with the maximum SCGs extract (100%) were rarely observed ($0.67 \pm \text{S.D } 0.58$ batch/jar). Although the mean number of eggs is minimal, females have been putting their eggs on the inner wall of jars, not on the treated leaves, and on the opposite, females in non-treated jars have laid their eggs entirely on the leaves. Meanwhile, the mean number of egg batches laid in each treatment $\pm \text{S.D}$ were 2.33 ± 0.56 , 7.33 ± 1.15 , and 8.67 ± 2.57 in each treatment 50, 25, and control, respectively (Fig. 1). The present investigation results agree with Borges *et al.*²⁸, showed oviposition deterrent and larvicidal activities of the phenolic extracts of *T. avellanedae* against 3rd instar larvae *Aedes aegypti*. In the same context, Kovanci²⁹ revealed the feeding and oviposition deterrent activities of microencapsulated cardamom oleoresin and eucalyptol against *Cydia pomonella*, while Basukriadi and Wilkins³⁰ studied the effect of yam bean seed extract and coumarin, which partially deterred the moth *Plutella xylostella*.

Insecticidal effect of SCGs extract on the percentage mortality of *Spodoptera littoralis*

Results showed that SCGs extract (100%) caused mortality with 76% for larvae after four days of treatment. While with 50% extract concentration, the mortality reached 54.5%, and the lowest extract concentration, 25%, recorded 27.5% of mortality compared to control; 7.4% mortality in larvae (Fig. 2). The main remark from our observations during this test was that there was no consumption for treated leaves compared with control dishes where the larvae fed and consumed many parts of leaves. This observation

reveals that SCGs extract can act as a repellent or antifeedant agent against larvae of *Spodoptera littoralis*, which died without feeding on treated leaves.

These results are agreed with Pavela³¹, who showed antifeedant and larvicidal effects for 12 simple phenols and nine phenolic acids against *Spodoptera littoralis* (Boisd.). Along the same lines, *Malpighia emarginata* DC. bagasse acetone extract, which contains many phenolic compounds like gallic acid, epigallocatechin gallate, catechin, *p*-coumaric acid, salicylic acid, and quercetin, have prolonged the pre-pupal stage and increased the mortality of caterpillars of fall armyworm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae)³².

Survey and population density of insects of *P. vulgaris* in the field treated with SCGs extract

Data presented in (Fig. 3) showed the mean number of the main insects found in the field, where aphid, *Aphis craccevora* specimens were 4.5, 10.7, 13.5, and 17.3 at plants treated with 100, 50, 25% of SCGs extract and control, respectively. Jassed, *Empoasca fabii* recorded 0.75, 4, 9, and 12.7 with treatments 100, 50, 25% of SCGs extract and control, respectively. In the case of the whitefly, *Bemisia tabaci*, the data obtained were 2.5, 5.7, 10.7, and 15.5 with concentrations of SCGs extract 100, 50, 25%, and control (water). The number of cutworms, *Agrotis ipsilon* larvae, has been collected during the earlier 35 days of plant age because those larvae after third larval instar sheltered in soil and challenging to be on the foliage part of plants. At the same time, it was accessible to collect larvae of cotton leafworms from the field during all of the season. Both of these two pests are responsible for damage to the foliage of this crop.

Data have been recorded with The mean number of larvae caught from each plot according to concentration for *spodoptera* was 1.7, 5, 6.7, and 8.0 with 100, 50, 25% of SCGs extract and control, respectively. In earlier plant age, larvae of *Agrotis ipsilon* were collected from plants according to different treatments was mean number 2, 5, 8.5, and 11.8 specimens/plant with concentrations 100, 50, 25% of SCGs extract and control, respectively. These results show that a high concentration of 100% SCGs extract gave a highly significant difference compared with other lower concentrations of 50, 25%, and much higher than the control.

The decrease in the population number of *Spodoptera* and *Agrotis* larvae collected from the field has been supported by the results obtained twice a week. The mean number of leaves damaged by those insects was very low in plots treated by high concentrations of 100% and 50% of SCGs extract compared with the lower concentration 25% and control (Fig. 4). These findings reinforce the assumption; the SCGs extract acts as an antifeeding and repellent agent. The above results are in harmony with many previous works; for example, Pavela³¹, who showed the antifeedant effect of phenolic on *Spodoptera littoralis* (Boisd.), and Rahayu *et al.*³³ studied the antifeedant activity of leaf phenolic extract of two cultivars, *Carica papaya* L. on *Spodoptera -litura* F. Larvae.

Evaluation Of Molecular Docking

Evaluation for the potential interaction between the predominates of the SCGs methanolic extract, and insecticide AChE enzyme (1QON) was conducted through a molecular docking study. The intermolecular interactions between the ligand and the target receptor were evaluated. The ideal pose was validated by aligning the X-ray bioactive conformer with the best-fitted pose of the same compound for the enzyme. The perfect pose of each molecule was selected according to the energy score, and validation is considered satisfactory when the RMSD is smaller than 2.0 Å regarding the crystallographic pose of a respective ligand³⁴.

The binding free energies (ΔG) for the extract predominates docked at AChE are shown in Fig. 5, revealing the best poses obtained in the molecular docking analyses. The larger the peaks, the lower the ΔG and consequently the more significant the interaction between the receptor and the ligands with insecticidal ability. Apigenin 7-glucoside displayed the best binding affinity compared to other ligands or the control (Lannate; -5.49 kcal/mol), with high docking scores (-9.16 kcal/mol), followed by catechin and epicatechin; -8.58 and -8.21 kcal/mol, respectively. Rosmarinic and chlorogenic acids showed median scores with -7.69 and -6.99 kcal/mol, while gallic acid was the least (-3.61 kcal/mol) (Fig. 5). The above results follow Tundis *et al.*³⁵ and Zengin *et al.*³⁶, where flavonoids, according to the former, especially apigenin 7-glucoside, showed a potential anti-cholinesterase effect, while the latter proved an enzyme inhibitory effect for the total methanolic extracts of both SCGs and coffee silver skin.

Figure 6a–d shows the interaction of apigenin 7-glucoside, rosmarinic acid, caffeine, and lannate (control) with the AChE receptor. These compounds represent the highest binding affinity from different phenolics, flavonoids, and alkaloids identified in SCGs extract. The higher binding affinity of apigenin 7-glucoside is attributed to the crucial conventional hydrogen bonds formed with GLU237 and ASP375, C-H interaction with GLY481 and HIS480, and finally, Pi-Pi interaction with TYR71, TYR370, and TYR374 (Fig. 6a). The number of Pi-sigma interactions (Pi-alkyl), which primarily involves charge transfer, helps bind rosmarinic acid and caffeine firmly with the receptor residues LEU479, TYR370 TYR374, and TRP83. Again, similar Pi-Pi interactions with TYR370, TRP83, and PHE371 are shown for both, but caffeine has a unique conventional hydrogen bonding with TRP472 and carbon-hydrogen bonding with ASP482 and GLY481, while rosmarinic acid showed unparalleled hydrogen bonds with SER238 and HIS480. Types of bonds and their distances were the main reasons for the differences in binding affinity and free energy between each ligand and the control (Lannate), indicating an insecticidal ability of the tested molecules. The unique Pi-Pi interactions, C-H bonding, and distances of the bonds between ligands and enzyme moieties could make remarkable differences in binding affinity. For example, distances of conventional H-bonding between Apigenin 7-glucoside and AChE moieties are between 1.81-2.04Å, shorter than the same between control (Lannate) and receptor (2.04-2.24 Å). The same trend could be noted for the remaining bonds, as shown in Fig. 7.

According to the literature, the molecular docking method used here identified a conformation that allows the ligand to bind to the residues of the 1QON active sites, around the α -helix between amino acid residues TYR370–TYR374 and around the β -sheet between amino acid residues VAL478–HIS480. For the ligand, it is possible to see hydrogen bonds in common with residues TYR370 and HIS480. There was

also a hydrophobic interaction with residues TYR71, TRP83, TYR370, PHE371, and LEU479³⁷. The interactions obtained after molecular docking of the compounds with the amino acid residues TRP83, TYR370, PHE371, and HIS480 of AChE are similar to those reported in the literature³⁸.

Generally, the main task of the insecticidal agents like the potential compounds examined during the current study is to irreversibly inhibit the production of the AChE enzyme, which is responsible for acetylcholine's hydrolysis, consequently terminating acetylcholine the nerve impulse. The previous concept represents the initial mechanism for an extract or compound to be considered an insecticide in the larval phase³⁹. Therefore, molecular docking represents an essential tool to observe interactions formed inside the active site of the AChE and the mechanism of elucidation of biological action of the potential ligands applied.

Conclusions

Deterrent assay, contact bioassay, and lethal concentration analysis were performed to reveal the repellent, antifeedant, and oviposition deterrent effects of SCGs methanolic extract against the mean pests of green bean (*Phesolus vulgaris*). The extract rich in phenols and flavonoids showed a promising insecticidal impact and oviposition deterrent efficiency based on the concentration of the extract applied compared to the control. A molecular docking study revealed that flavonoids and phenolic acids display higher activity through interaction with insecticidal AChE, which agrees with laboratory and field studies. The above findings open prospects toward exploiting enormous amounts of agro-wastes like SCGs as eco-friendly bioinsecticide.

Declarations

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Author's contributions: Conceptualization, Project administration, Methodology, Investigation, and in silico; Writing—original draft, A.F. and H.H.; Lab and Field insecticidal experiments, H.H. and W.A.; Extraction, Formal analysis, statistical and Data curation, H.A., M.E.. and H.Y.; revising and editing, H.A. and H.H.

Availability of data and materials: All data generated or analyzed during this study are included in this article.

Competing interests: The authors declare no competing interests.

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Figures

Figure 1

Effect of SCGs extract on oviposition of *Spodoptera littoralis* females

Figure 2

Percentage of mortality in *Spodoptera littoralis* larval fed on leaves treated with SCGs extract

Data have been recorded with The mean number of larvae caught from each plot acco

Figure 3

The mean numbers of insects collected from plots of each treatment

Figure 4

Effect of SCGs extract on the mean number of damaged leaves by chewing insects

Figure 5

Binding free energy values were calculated by molecular docking the major constituents identified in SCGs methanolic extract and AChE receptor.

A

B

C

D

Figure 6

Interactions of (A) Apigenin 7-glucoside, (B) Rosmarinic acid, (C) Caffeine, and (D) Lannate (control) with AChE receptor

The ORTEP diagram shows the molecular structure of compound 6, which consists of two phthalonitrile units linked by a biphenyl group. The molecule exhibits several key structural features:

- Phthalonitrile Units:** Each unit contains a benzene ring substituted with two nitrile groups ($\text{C}\equiv\text{N}$). The C-N bond lengths are approximately 1.39 Å and 1.87 Å.
- Biphenyl Linkage:** Two phenyl rings are connected at their para positions. The C-C bond length between the linkage carbons is 1.38 Å.
- Nitrile Groups:** The nitrile carbon atoms are bonded to nitrogen atoms. One N-C bond length is 1.39 Å, and another is 1.87 Å.
- Other Bond Lengths:** Other labeled bond lengths include 0.81 Å, 0.88 Å, 0.79 Å, 1.13 Å, 1.43 Å, 1.75 Å, and 1.85 Å.
- Angles:** Several angles are labeled, such as 120°, 121°, 122°, 123°, 124°, 125°, 126°, 127°, 128°, 129°, 130°, 131°, 132°, 133°, 134°, 135°, 136°, 137°, 138°, 139°, 140°, 141°, 142°, 143°, 144°, 145°, 146°, 147°, 148°, 149°, 150°, 151°, 152°, 153°, 154°, 155°, 156°, 157°, 158°, 159°, 160°, 161°, 162°, 163°, 164°, 165°, 166°, 167°, 168°, 169°, 170°, 171°, 172°, 173°, 174°, 175°, 176°, 177°, 178°, 179°, 180°.

Types and distances of interactions between AChE and **A**-Apigenin 7-glucoside and **B**-Control (Lannate)