Article

Insecticidal Activities of *Securidaca longepedunculata* Fresen Extracts and Feeding Behavior of *Schizaphis graminum* Rondani: Electropenetrography Approach

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ABSTRACT: The aphid, *Schizaphis graminum* Rondani (Hemiptera: Aphididae), is one of the most destructive pests of wheat. It is responsible for significant economic losses in the agricultural sector, with an estimated 45% of wheat fields affected. Plant-based insecticides have seen a rapid increase in popularity in recent years due to their efficacy, cost-effectiveness, biodegradability, and lower toxicity compared to synthetic pesticides. The study aimed to evaluate the toxic potential of *S. longipedunculata* extracts against *S. graminum* and investigate the insect's feeding behavior on wheat. Initially macerated in methanol, the different extracts of *S. longipedunculata* organs were fractionated using *n*-hexane, chloroform, ethyl acetate, and butanol. The feeding behavior was analyzed by comparing the waveforms generated by the EPG with the control. After 72 h of treatment, the ethyl acetate fraction extracted from root had the highest toxicity against aphids, with mean 26 mortality of *S. graminum* at LC50 of 330 ppm; 25 mortality *S. graminum* at LC50 of 400 ppm for leaves; and mean 24.5 mortality *S. graminum* at LC50 of 540 ppm in stem bark. EPG analysis indicated that the extract fractions enhanced plant tissue resistance by significantly preventing aphid access to the phloem. The toxic effect of the botanical extracts significantly enhanced the chemical composition of the leaf medium, resulting in a drastic reduction in the number of tissue attacks by *S. graminum*. In summary, besides their toxicity to *S. graminum*, extracts of *S. longipedunculata* reinforce the plant's defense mechanisms, significantly reducing the *S. graminum* population. They also reinforce wheat's defense mechanisms. *S. longipedunculata* can, therefore, be used as a promising agent in the biological control of *S. graminum*.

Pesticidal activities of *Securidaca longipedunculata* Fresen extracts and feeding behavior of *Schizaphis graminum* Rondani from wheat using the electropenetrography (EPG).

INTRODUCTION

Wheat is the world's third most important crop. It is the major ingredient in many of the world's diets, accounting for 20−29% of calories and dietary protein.[1] It has become the world's most economical and nutritious staple food, accounting for around 40% of the global population's diet.[2] The aphid, *Schizaphis graminum*, is a frequent sap-sucking biting insect on cereals such as *Triticum aestivum* L (*Poaceae*) and *Sorghum bicolor* L.[3] It feeds by ingesting phloem and xylem sap from leaves or stems. As it feeds, it siphons off these conductive vessels and other tissues in its path, leading to acute dehydration and even death of the plant. Aphid infestation leads to a significant drop in yield, estimated at 35−45%.[4,5] Annual yield losses caused by green bugs worldwide are estimated at 2 million to 1 billion US dollars.[6]

Nowadays, pest control relies heavily on the use of synthetic pesticides,[7−10] the most widely used being insecticides.[11] A specific class of insecticides, including organochlorines, organophosphates, carbamates, and fungicides, has been identified as neurotoxic to humans. These compounds, such as DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane), lindane, and chlordecone, have been found to persist in the soil for decades.[12] This represents a significant environmental concern. The majority of insecticides act on the central nervous system, disrupting neuronal transmission. The majority of insecticides act on the central nervous system, disrupting neuronal transmission. This is exemplified by
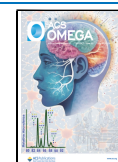
insecticides such as VIPER 46 C, BONIDE "Systemic Insect Control," and SUPECTRACIDE, which are commonly utilized in numerous agricultural settings. The majority of these insecticides contain at least one complex of bioactive compounds, such as acetamiprid and indoxacarbe. The recurrent exposure of green bugs to synthetic chemicals has forced them to develop a form of resistance to these products.[13] Recent studies have demonstrated that the most prevalent defense mechanism is largely based on gene mutation[14] and conformational change of target protein receptors,[15] which complicates control and leads to the use of plants.[16−19]

Numerous medicinal plants have proved highly effective against insect pests and could be a valid replacement for synthetic pesticides.[20,21] Some plant families, such as Solanaceae, Meliaceae, Apocynaceae, and Polygalaceae, have shown interesting insecticidal properties against aphids.[22] S. longipedunculata (Polygalaceae), due to its bioavailability in the zone and its variety of secondary metabolites, notably polyphenols, would be a potential insecticide for S. graminum.

Wheat, S. graminum's main host, has developed defense mechanisms to counter the repeated assaults of S. graminum, sometimes forcing it to abandon the plant.[23] The EPG technique enables us to visualize insects' feeding behavior in the plant's different leaf layers. The technique involves inserting the insect's stylet into the plant tissue as it sucks in sap, thus closing the circuit and generating an electrical signal.[24]

The study aimed to evaluate the effect of our S. longipedunculata extracts against S. graminum and to analyze the insect's behavior toward S. longipedunculata extracts using the electropenetrography technique.

■ MATERIALS AND METHODS

Sampling Site and Collection of S. longipedunculata. The hauts-Bassins region (Bobo-Dioulasso) is in western Burkina Faso. Securidaca longipedunculata were harvested in Nasso (11°10′27.83″ N latitude; long 4°28′184″W), approximately 15 km northwest of Bobo-Dioulasso. Its south-Sudanian climate is characterized by a dry season (October to April) and a rainy season (May to September). The rains are relatively abundant, with about 1100 mm of water per year. Average temperatures oscillate between 24 and 30 °C. The specimens collected were identified by botanist and plant ecologist Dr. Hermann Yempabou Ouoba, of Joseph Ki-Zerbo University, and the species' correct botanical name was confirmed by the International Plant Name Index (www.ip-ni.org).

Extraction of S. longipedunculata. Plant sample extraction was done following the procedure.[24] The different organs of S. longipedunculata are dried under shade and laboratory conditions, then powdered, and passed through methanol 70 for maceration followed by fractionation. Each 500 g sample of S. longipedunculata was dissolved in a jar with methanol and then stored in a dark container for 72 h under laboratory conditions. The mixture was filtered through cotton and then through N°1 Wman paper with three repetitions to get a pure solution. The macerate was rotavaporized at 29 °C for 30 min and then placed in the oven. Fractionation was conducted in the following order: n-hexane, chloroform, ethyl acetate, and butanol. The different fractions were evaporated to dryness using a Rotavapor and concentrated in sterilized test

tubes to a more or less reasonable yield to perform the desired tests within the scope of the study.

Collecting Schizaphis graminum. The green bug, Schizaphis graminum, was initially collected in wheat fields of the variety "Faisalabad-2008." It was continuously reared for over two years at the National Agricultural Research Center (NARC) in Islamabad, Pakistan. The IPMP laboratory served as a test site. The S. graminum population was maintained on wheat seedlings without any exposure to insecticide under laboratory conditions with a temperature of 18 ± 2 °C, $60 \pm 5\%$ RH, and photoperiod of 16:8 L:D. Old or dead wheat plants were automatically replaced by young plants. The 10th generation of S. graminum, after the various feeding operations, was used as a test.

Preparation of Test Solutions. The preparation followed the protocol.[25] The highest concentration stock solution (C1) was prepared using Charles' eq ($C_1V_1 = C_2V_2$). Four solvents were used to extract the crude methanolic extract of n-hexane, chloroform, ethyl acetate, and butanol. To sum up, 100 mL of water with 885 mg per liter of crude extract were divided using an appropriate 500 mL separating funnels. The extract fractions were all diluted to the final concentrations needed for the next bioassays in 1 mL of HLB, tween 12 surfactants.

Bioassays. The test involved spraying S. longipedunculata organ fractions on wheat plants separately using a hand sprayer until runoff (adaxial and abaxial leaf sides). Distilled water was sprayed as a control treatment. All treated pots were kept at room temperature to dry. Each extract concentration was replicated three times, and 20 apterous new adult S. graminum were transferred to each pot. The treated pots having aphids were placed under laboratory conditions (18 °C, $60 \pm 5\%$ RH, and a photoperiod of 16:8 L: D). The mortality was checked at 72 h postexposure to extracts. The aphids were considered dead if they did not move after being gently touched with a soft brush. For further evaluation, the part with the highest activity was chosen and diluted into six other fractions, i.e., 1000, 500, 250, 125, and 62.5 ppm. These dilutions were tested on other plants.

Electropenetrography (EPG) Recording. The testing followed the protocol[26]: Twenty to thirty-day-old plants with a fully developed second leaf were used to record the Electrical Penetration Graph (EPG, DC system). The concentration of the most active organ extract fraction, causing 30% death of the aphid, was chosen for the electropenetrography tests. Thus, five plastic pots containing young wheat plants were infested with 20 S. graminum each. Four of these had been sprayed with the extract fraction of the concentration and kept for 30 min. The remaining pot of wheat served as a control.

After this treatment, a gold wire (20 mm long, 20 μm in diameter) was fixed at one end to the dorsal surface of an adult aphid (2−4 days postmolt using a water-based dye) while the other end was soldered in aluminum to a copper wire, which was connected via a 1GX resistance amplifier to the substrate containing the plant. An aphid, which had fasted for 30 min prior to the test, was then placed on the abaxial side of a leaf, and the complete system was placed inside a 100−100−100 cm closed Faraday cage constructed of wood and wire mesh to minimize interference in wave recording. The circuit was completed when the insect introduced its stylet into the leaf, and details of aphid penetration were tracked in DC current mode over an 8 h. Voltage variations were recorded and analyzed using a computer equipped with STYLET 30 software. All recordings lasted 8 h.

Statistical Analysis. For toxicity, mortality data were analyzed using probit analysis to calculate the lethal concentration 50 (LC50). ANOVA assessed statistical significance, and means were compared using the Tukey test comparison at $P = 0.05$. The EPG recording data were statistically analyzed by one-way analysis of variance with Tukey's post hoc test (IBM, SPSS, Statistics, version 22).

■ RESULTS

Bioassays. *Choice of the Most Lethal Fraction of Plant Extracts against S. graminum.* The results of treatment with the different fractions of *S. longipedunculata* extracts were all toxic to *S. graminum* after 72 h of treatment (Table 1), with the

Table 1. Mortality Rates of 1000 ppm *S. longipedunculata* Leaf Extract Fractions on *S. graminum* after 72 h Exposure[a]

solvent	time	mean ± SD	L. 95% CI, U. 95% CI
n-hexane	24 h	16 ± 8.485[a]	−60.24, 92.24
	48 h	18.5 ± 7.778[b]	−51.38, 88.38
	72 h	19.5 ± 7.78[b]	−50.38, 89.38
chloroform	24 h	20 ± 9.89[b]	−68.94, 108.9
	48 h	22 ± 9.89[b]	−66.94, 110.9
	72 h	22.5 ± 10.61[b]	−72.8, 117.8
ethyl acetate	24 h	24.5 ± 12.02[ab]	−83.5, 132.5
	48 h	25 ± 11.31[b]	−76.65, 126.6
	72 h	26 ± 11.31[ab]	−75.65, 127.6
butanol	24 h	15.5 ± 9.19[a]	−67.09, 98.09
	48 h	17.5 ± 7.78[a]	−52.38, 87.38
	72 h	19 ± 8.48[b]	−57.24, 95.24
negative control (distilled water)	24 h	3 ± 2.83[a]	−22.41, 28.41
	48 h	5.5 ± 3.54[a]	−26.27, 37.27
	72 h	6 ± 2.83[a]	−19.41, 31.41
	24 h	20.9 ± 10.3 [a]	−53.21, 180.16
positive control (acetamiprid)	48 h	22.5 ± 9.42 [b]	−77.7, 137.27
	72 h	24 ± 11.86 [b]	−70.64, 133.45

[a]SD: Standard deviation; U. 95% CI: upper 95% confidence intervals of the mean; L. 95% CI: lower 95% confidence intervals of the mean. Letters indicate significant differences among the treatments ($P < 0.05$).

Table 2. Mortality Rate of 1000 ppm *S. longipedunculata* Stem Bark Extract Fractions on *S. graminum* after 72 h Exposure[a]

solvent	time	mean ± SD	L. 95% CI, U. 95% CI
n-hexane	24 h	10.5 ± 6.36[a]	−48.68, 67.68
	48 h	12 ± 5.66[a]	−38.38, 62.82
	72 h	14 ± 7.07[a]	−49.53, 77.53
chloroform	24 h	14 ± 8.48[a]	−62.24, 90.24
	48 h	17.5 ± 6.36[b]	−39.68, 74.68
	72 h	18.5 ± 4.95[a]	−25.97, 62.97
ethyl acetate	24 h	23 ± 11.31[b]	−78.65, 124.6
	48 h	24 ± 11.31[b]	−77.65, 125.6
	72 h	25 ± 11.31[ab]	−76.65, 126.6
butanol	24 h	11 ± 7.07[a]	−52.53, 74.53
	48 h	15 ± 4.24[a]	−23.12, 53.12
	72 h	15.5 ± 3.54[a]	−16.27, 47.27
negative control (distilled water)	24 h	7.5 ± 3.54[a]	−24.27, 39.27
	48 h	9 ± 4.24[a]	−29.12, 47.12
	72 h	9.5 ± 4.95[a]	−34.97, 53.97
positive control (acetamiprid)	24 h	20.9 ± 10.3 [a]	−53.21, 180.16
	48 h	22.5 ± 9.42 [b]	−77.7, 137.27
	72 h	24 ± 11.86 [b]	−70.64, 133.45

[a]SD: Standard deviation; U. 95% CI: upper 95% confidence intervals of the mean; L. 95% C I: lower 95% confidence intervals of the mean. Letters indicate significant differences among the treatments ($P < 0.05$).

Table 3. Mortality Rate of 1000 ppm *S. longipedunculata* Root Extract Fractions on *S. graminum* after 72 h Exposure[a]

solvent	time	mean ± SD	L. 95% CI	U. 95% CI
n-hexane	24 h	16.5 ± 9.19[b]	−66.09	99.09
	48 h	17 ± 8.48[a]	−59.24	93.24
	72 h	18 ± 8.48[b]	−58.24	94.24
chloroform	24 h	13.5 ± 7.78[a]	−56.38	83.38
	48 h	15.5 ± 7.78[a]	−44.38	80.30
	72 h	15.5 ± 7.78[a]	−54.38	73.23
ethyl acetate	24 h	22.5 ± 10.61[b]	−72.8	117.8
	48 h	23 ± 11.31[b]	−78.65	124.6
	72 h	24.5 ± 10.61[b]	−70.8	119.8
butanol	24 h	11.5 ± 6.36[a]	−45.68	68.68
	48 h	13.5 ± 6.36[a]	−43.68	70.68
	72 h	15 ± 7.07[a]	−48.53	78.53
negative control (distilled water)	24 h	7.5 ± 4.95[a]	−24.27	51.97
	48 h	6 ± 0.2[a]	−46.12	26
	72 h	8.5 ± 0.71[a]	−36.97	12.85
positive control (acetamiprid)	24 h	20.9 ± 10.3[a]	−53.21	180.6
	48 h	22.5 ± 9.42[b]	−77.7	137.27
	72 h	24 ± 11.86[b]	−70.64	133.45

[a]SD: Standard deviation; U. 95% CI: upper 95% confidence intervals of the mean; L. 95% C I: lower 95% confidence intervals of the mean. Letters indicate significant differences among the treatments ($P < 0.05$).

highest mortality rate for the ethyl acetate leaf fraction (26 ± 11.31) followed by the chloroform fraction (22.5 ± 10.61), then the *n*-hexane fraction (19.5 ± 7.78), the butanol fraction (19 ± 8.48), and finally the water residue (6 ± 2.83).

For treatments with bark extract fractions (Table 2), the ethyl acetate fraction (25 ± 11.31) showed good toxicity compared with the chloroform fraction (18.5 ± 4.95), then the butanol fraction (15.5 ± 3.54), then the *n*-hexane fraction (14 ± 7.07), and finally the residual water (9.5 ± 4.95) after 72 h of treatment.

As for the root extract fractions (Table 3), the ethyl acetate fraction (24.5 ± 10.61) was always the most toxic to aphids, compared with the other less toxic fractions in the following order: *n*-hexane fraction (18 ± 8.48), chloroform fraction (15.5 ± 7.78), butanol fraction (15 ± 7.07), residual water (8.5 ± 0.71).

Thus, for all three organs, the ethyl acetate fraction of leaves proved most lethal to aphid populations, followed by the ethyl acetate fraction of stem bark and the ethyl acetate fraction of root. Consequently, the ethyl acetate fraction may be wise for controlling *S. graminum* L in wheat. It would, therefore, be

necessary to determine the concentration that would kill 50% of the aphid population and possibly 30%.

Selection of the Low Concentration of Plant Extracts Most Lethal against *S. graminum*. All the ethyl acetate concentrations chosen for the tests impacted aphid mortality (Figures 1−23). However, specific concentrations of *S.*

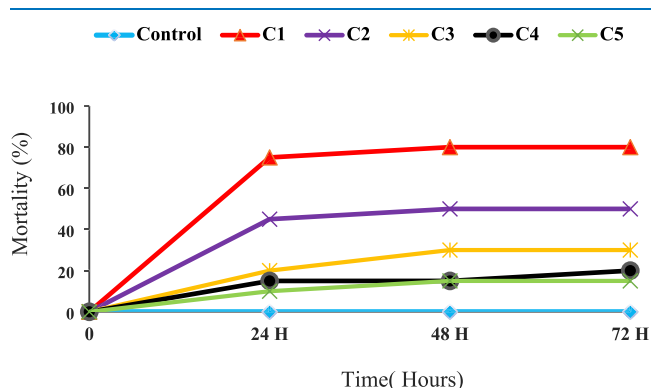*longipedunculata* extracts had a particular impact on insect mortality.

Figure 1. Mortality rate of the lethal concentration for 72 h of *S. longipedunculata* leaf extracts on *S. graminum*.
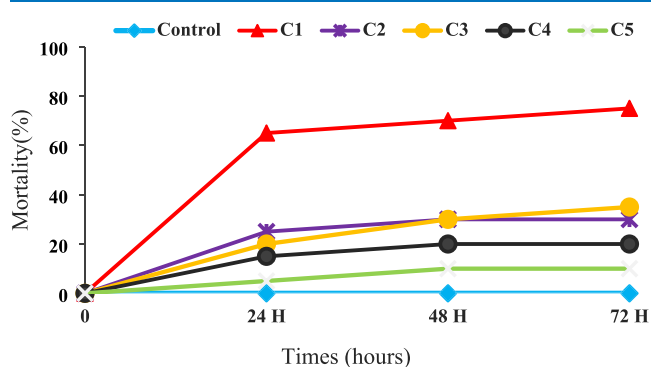

Figure 2. Mortality rate of the lethal concentration for 72 h of *S. longipedunculata* stem bark extracts on *S. graminum*.
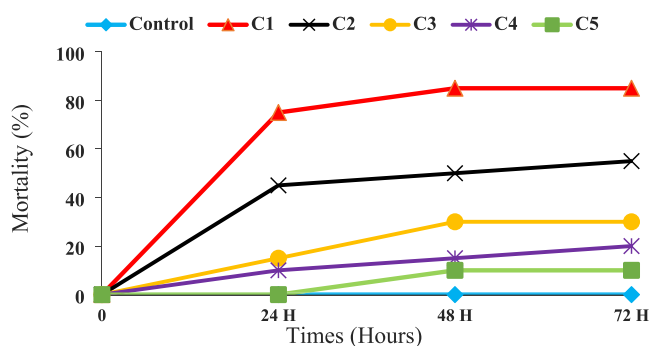

Figure 3. Mortality rate of the lethal concentration for 72 h of *S. longipedunculata* root extracts on *S. graminum*.

Concentrations tested were C1= 1000 ppm; C2 = 500 ppm; C3 = 250 ppm; C4 = 125 ppm; C5 = 62.5 ppm. Treatment was conducted for 72 h.

The ethyl acetate fraction of leaves with these different variable concentrations had a less significant impact on aphids ($\alpha = 0.0259$; $P < 0.05$). A concentration of 440 ppm was sufficient to neutralize half of the aphids on the wheat after 72 h of treatment.[27]

Concentrations tested were C1= 1000 ppm; C2 = 500 ppm; C3 = 250 ppm; C4 = 125 ppm; C5 = 62.5 ppm. The treatment lasted 72 h.

The results obtained showed that aphid mortality was significantly related to the concentration of the ethyl acetate fraction ($\alpha = 0.0379$; $P < 0.05$), for a concentration of 540 ppm decimated half of the aphids infesting the plant after a 72 h treatment.

The concentrations evaluated were C1= 1000 ppm; C2 = 500 ppm; C3 = 250 ppm; C4 = 125 ppm; C5 = 62.5 ppm.

The treatment lasted 72 h. Results showed that aphid mortality was significantly related to concentration ($\alpha = 0.0636$; $P > 0.05$). Results showed that aphid mortality was significantly related to concentration ($\alpha = 0.0636$; $P > 0.05$). The fraction concentration of 330 ppm was the one that caused the death of half of the green bugs infested with wheat after 72 h of treatment. From these results, it appeared that the ethyl acetate leaf fraction was the most lethal at 400 ppm to decimate 50% of the *S. graminum* population followed by the bark fraction at 540 and 330 ppm for the ethyl acetate root fraction.

2-Electropenetrography (EPG) Recording. EPG recordings of aphid feeding behavior revealed the appearance of six (06) waveforms, namely, waveform Np (nonprobing), waveform C (routing phase), waveform F (mesophyll passage difficulties), waveform G (xylem ingestion), waveform E1 (phloem salivation), and waveform E2 (phloem ingestion). The appearance of these different waveforms shows that *Triticum aestivum* has opposed the passage of the *S. graminum* stylet through the leaf tissue layers from the epidermis to the phloem.

The E2 wave is associated with the aphid's ingestion of phloem sap, which is essential for its survival. Inaccessibility of sap-conducting tissue (G wave; E2 wave) or the toxicity of this sap represents a significant turning point in the life of the insect. The aphid's access to sap-conducting tissue is reduced as a result of host reinforcements, and conversely, increased access to toxic sap leads to dehydration and death. Table 4 demonstrates that the population of *S. graminum* was significantly disrupted in their probing of the various leaf layers by *S. longepedunculata* extracts.

The impact of these extracts was strikingly evident in the phloem layer (wave E2), which exhibited a moderate degree of unprobing by the *S. graminum* population in comparison to the negative control. The total number of *S. graminum* that had access to the leaf layers was low, as was the average probing time compared with the negative control.

The average time taken for the waveforms corresponding to the tissue layer soundings to appear was relatively shorter for the extract-treated feet than for the neutral control. The different waveforms took longer to appear in wheat plants treated with plant extracts than in the negative control. These observations suggest that the *S. graminum* population had more difficulty probing extract-treated plants than untreated plants (Figure 4). *T. aestivum* plants treated with *S. longepedunculata* extracts exhibited a reduction in the time required for *S. graminum* to abandon its leaf layers in comparison to the negative control. The toxic effect of *S. longepedunculata* extracts enabled *T. aestivum* to resist the onslaught of *S. graminum*.

■ DISCUSSION

The different extracts of *S. longepedunculata* were all effective against *S. graminum*. For all fractions used, ethyl acetate from the roots was the most active followed by the chloroform fraction. Exposure to *S. longepedunculata* root extracts at LC50 (330 ppm) resulted in a significant reduction of the *S. graminum* population compared to the different controls. The other parts of the plant, i.e., leaves and bark, were inhibitory to

Table 4. Comparison of EPG Parameters of *S. graminum* Probing *Tritium aestivum* under *S. longipedunculata* Extracts[a]

feeding behavior parameter	treatment				Kruskal−Wallis
	control (s)	leave 1 (s)	bark 2 (s)	root 3 (s)	P value
1. time (in second) from the start of recording to the first Probe (t-1Pr)	535.78 ± 179.3[a]	659.188 ± 72.29[a]	236.318 ± 29.78[a]	750.438 ± 188.14[a]	0.06
2. time (in second) from the start of recording to the first xylem phase (t-1G)	159988 ± 3812.62[a]	76198 ± 1952.25[a]	183898 ± 3195.03[a]	75318 ± 2171.8[a]	0.02
3. time (in second) from the start of recording to the first phloem phase (t-1E)	39418 ± 1315.51[b]	123148 ± 2496.91[a]	100908 ± 1733.14[a]	115288 ± 2240.07[a]	0.03
4. time (in second) from the start of recording to the first phloem salivation phase (t-1E2)	41618 ± 1351.51[b]	138918 ± 2675.95[a]	180578 ± 3069.89[a]	148868 ± 2363.15[a]	0.0001
5. % duration of nonprobing (Np)	8.57 ± 1.07	24.67 ± 3.96	31.52 ± 1.93	25.53 ± 3.64	0.0001
6. % duration of phloem salivation (E2)	45.24 ± 7.65	14.95 ± 4.97	7.34 ± 1.92	10.95 ± 3.02	0.0001
7. average duration (in second) of nonprobing (a-Np)	524.76 ± 86.95[a]	718.07 ± 106.31[a]	834.27 ± 121.84[a]	747.32 ± 120.78[a]	0.26
8. average duration (in second) of xylem phase (a-G)	2766.3 ± 505.59[a]	2297.9 ± 804.92[a]	1255 ± 412.8[a]	2612.7 ± 293.3[a]	0.25
9. average duration (in second) of phloem phase (a-E1)	235.33 ± 69.36[a]	450.36 ± 48.1[a]	327.08 ± 54.91[a]	389.54 ± 54.36[a]	0.07
10. average duration of phloem Salivation phase (a-E2)	11455 ± 2894.69[a]	1493 ± 215.62[b]	1027 ± 113.76[b]	1494 ± 276.75[b]	0.0001

[a]All times are in second. All numbers are averages per 8 h observation period. Values in one row followed by different letters differ significantly ($P < 0.05$). Letters indicate significant differences among the treatments.
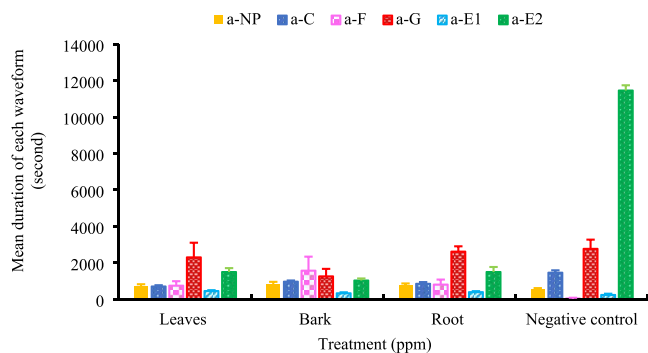

Figure 4. Mean duration of each waveform after *S. longepedunculata* 250 ppm treatment.

aphid reproduction but relatively weak compared to roots at LC50 of 340 and 540 ppm, respectively. This efficacy of the extracts indicates the potency of the bioactive compounds present in the extracts. The high mortality caused by the ethyl acetate fraction to the detriment of the chloroform fraction could be justified by the polar nature of the extracts. The ethyl acetate fraction, which is more polar than the chloroform fraction, therefore had the property of capturing more polar and apolar compounds such as alkaloids, saponins, flavonoids, glycosides, and terpenoids compared to the chloroform fractions, thus increasing the spectrum of action on the insect (Table 1).[28]

The same results were reported by Hassan et al.,[29] who highlighted the insecticidal potential of *S. longepedunculata*'s ethyl acetate fractions. This species is renowned for its medicinal virtues due to its diverse array of bioactive compounds. Indeed, pharmacophores such as octadecanoic acid, n-hexadecanoic acid[30] and *p*-coumaric acid, gallic acid, chlorogenic acid, and many others[31] isolated from *S. longepedunculata* root extracts have demonstrated clear insecticidal activity. The active compounds present in the fractions are involved not only in insect cytotoxicity but also in the neutralization of toxins set up by the insect for its feeding.[32,33] Previous research[34] demonstrated that this

cytotoxicity was due to a concerted action between the combination of jasmonic acid, salicylic acid, and the pharmacophores of *S. longepedunculata*, which significantly reduced the population of *S. graminum*. Consequently, for aphid feeding, approximately 76 proteins present in its saliva are indispensable, including the expression of the Sg2204 protein, which acts as a suppressor of wheat defense. The activation of *S. longepedunculata*'s bioactive compounds resulted in the inhibition of the protein's expression in the insect's saliva, rendering the medium indigestible to the green bug.[35,36]

The extract was sprayed on the aphids, which then infiltrated the cuticle or the walls of the aphid's digestive tract via a systemic effect. This reached the central nervous system, where the target receptors are located.[37] The ligands bind to the target receptors, thereby interacting with the proteins or enzymes. This disruption of the cholinergic transmission system ultimately leads to the insect's death.[38]

During the process of feeding, *S. graminum* employs its stylet via a callose to penetrate the various leaf layers and reach the sap-conducting vessels of the host plant, where the aphid punctures the vessel to obtain its food. In other work,[39,40] it was asserted that aphid saliva, which is rich in hydrolyzing proteins, totally destroys the host plant's defense system when expressed. The exposure of *S. longepedunculata* extracts to aphids revealed a similar condition through dietary disruption to the normal state (Table 4). The aphids found it difficult to gain access to the leaf layers, particularly the phloem and xylem. The time required for *S. graminum* to make contact with wheat leaves (t-Pr) was significantly longer than that of the control (535.78 ± 179.3 s; 750.438 ± 188.14 s). The host plant exhibited a more defensive response to the insects' onslaught, likely in conjunction with the *S. longepedunculata* extracts. Recent studies[41−43] have validated the hypothesis that the host plant reacts physically by increasing the quantity of trichomes on the cuticle and chemically by expressing the callose synthase and *β*-glucanase genes. Similarly, the average duration of contact with the cuticle was found to be very low (Figure 4) in comparison to the control, which suggests that

the green bugs were expelled by the volatile compounds released by the host leaf. This hypothesis is supported by the findings elsewhere,[44,45] which indicated that plants concentrate their volatile compounds as a deterrent to invaders. Moreover, this technique has been identified as the most aggressive and dissuasive.[46] Furthermore, partial resistance was observed at the phloem level, characterized by an increased number of probes before the first phloem ingestion (t-1E2) (148868 ± 2363.15 s) compared with untreated plants (41618 ± 1351.51 s). A greater number and duration of salivation events without subsequent phloem feeding, as well as a shorter time spent by the phloem feeding on plants with reduced sensitivity, were observed (Table 4). These different characteristics provide evidence of the efficacy of *S. longepedunculata* extracts.

Recent research has elucidated the resistance of wheat to access its sap-conducting vessels, which ensures its survival by accumulating reactive oxygen species along the veins,[47] narrowing the width of vascular bundles,[48] and obstructing sieve cells.[49] This phloem feeding has a significant impact on the fate of the insect, even reducing its population due to the various metabolites introduced by wheat.[50] In addition, Greenslade et al.[51] had found that when wheat was infested with aphids, plant tissues located close to the feeding site accumulated biologically active compounds such as asparagine and octopamine, as well as threonine, glutamine, succinate, trehalose, glycerol, guanosine, and choline in response to the infestation.

The presence of these chemical and molecular deterrents, in addition to the barriers established by *T. aestivum* via *S. longepedunculata* to prevent access to the various leaf tissues, demonstrates the effectiveness of *S. longepedunculata* in protecting our fields against *S. graminum*. Consequently, it can be employed as a large-scale biopesticide.

CONCLUSIONS

This study has shown that fractions of the various organs of *S. longipedunculata* control aphids in wheat and reinforce the plant's foliar defenses against aphids. The efficacy proved by these extracts makes it a reference biopesticide in integrated pest management for wheat. In addition to its bioavailability and ease of use, this biopesticide has no impact on the environment compared with synthetic pesticides. To improve the quantity of this biopesticide, it would be essential to explore the molecular side better to select effective ligands, proteins, or even genes, using the molecular clustering technique and to simulate the various protein−ligand interactions.

ASSOCIATED CONTENT

Data Availability Statement

Data is contained within the article.

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Author Contributions

R.G. performed the methods and investigation. N.A.S., R.N.M., F.A., M.F.R., E.Z., and H.G were responsible for conceptualization and funding acquisition and provided supervision in analysis while I.U.H., P.S., A.Y., E.Z., S.A.H.R., and S.H.T. were responsible for original drafting of the manuscript. R.G. writing—original draft preparation, E.Z. and M.F.R. writing—review and editing, and E.Z. and F.A. visualization.

Notes

The authors declare no competing financial interest.
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