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Development of an emulsifiable concentrate formulation using *Sophora alopecuroides* and *Nicotiana tabacum* crude alkaloidal extracts for the sustainable management of wheat aphids

[*Desenvolvimento de uma formulação concentrada emulsionável utilizando extratos brutos de alcaloides de* Sophora alopecuroides *e* Nicotiana tabacum *para o manejo sustentável de pulgões do trigo*]

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ABSTRACT

Wheat aphids are a critical pest, threatening cereal production through rapid reproduction, damage, and disease transmission, and have developed resistance to synthetic insecticides, necessitating the development of eco-friendly alternatives. However, the broader-scale application of plant extracts is limited by their rapid biodegradability, limited persistence, and low yields of bioactive compounds. Moreover, traditional extraction and isolation methods for bioactive compounds require substantial amounts of plant material, resulting in minimal yields, which further constrain their feasibility. To overcome this, our study develops a simplified method for crude alkaloidal extraction, enhancing yields and providing a more efficient and sustainable approach. The presence of alkaloids in the crude extract was confirmed using the Wagner reagent test, Fourier Transform Infrared Spectroscopy (FTIR), and liquid chromatography-mass spectrometry (LC-MS) analysis. The crude alkaloidal extract emulsifiable concentrate formulation (CAE-EC) demonstrated potent insecticidal activity against the tested aphid species, with LC_{50} values ranging from 12.54 to 17.75 mg/mL. Overall, there was concentration, and time-dependent mortality was observed, with a mean mortality of 98.66% at 30 mg/mL 48 hours after application. Additionally, CAE-EC exhibited significant herbicidal activity against *Amaranthus retroflexus*, inhibiting root and shoot elongation by 33% and 91%, respectively. The electrical penetration graph (EPG) analysis suggested potential antifeedant activity of CAE-EC against wheat aphids. Furthermore, dynamic light scattering was used to characterize the polydispersity index and particle size of the emulsifiable concentrate. Notably, CAE-EC exhibited no phytotoxic effects on wheat plants, ensuring safe application. Given its minimal use of organic solvents and remarkable aphicidal and herbicidal activity, our EC formulation offers an up-and-coming eco-friendly alternative for managing wheat aphid populations.

Keywords: botanical pesticides, alkaloids, emulsifiable concentrate, aphids, toxicity, EPG

RESUMO

Os pulgões do trigo são uma praga crítica, ameaçando a produção de cereais por meio de sua rápida reprodução, dos danos e da transmissão de doenças, e desenvolveram resistência a inseticidas sintéticos, o que torna necessário o desenvolvimento de alternativas ecologicamente corretas. No entanto, a aplicação em larga escala de extratos vegetais é limitada por sua rápida biodegradabilidade, baixa persistência e baixos rendimentos de compostos bioativos. Além disso, os métodos convencionais de

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isolamento de compostos bioativos requerem quantidades substanciais de material vegetal, resultando em baixos rendimentos e limitando ainda mais sua viabilidade. Para superar esse obstáculo, o presente estudo desenvolve um método simplificado para extração de alcaloides brutos, aumentando os rendimentos e proporcionando uma abordagem mais eficiente e sustentável. A presença de alcaloides no extrato bruto foi confirmada pelo teste com reagente de Wagner, espectroscopia de infravermelho com transformada de Fourier (FTIR) e análise por cromatografia líquida acoplada à espectrometria de massas (LC-MS). A formulação concentrada emulsionável do extrato alcaloidal bruto (CAE-EC) demonstrou potente atividade inseticida contra as espécies de pulgões testadas, com valores de CL50 variando de 12,54 a 17,75mg/mL. De modo geral, observou-se mortalidade dependente da concentração e do tempo, com uma mortalidade média de 98,66% a 30mg/mL, após 48 horas. O CAE-EC também exibiu atividade herbicida significativa contra Amaranthus retroflexus*, inibindo o alongamento da raiz e da parte aérea em 33% e 91%, respectivamente. A análise do gráfico de penetração elétrica sugeriu potencial atividade antialimentar do CAE-EC contra pulgões do trigo. A dispersão dinâmica de luz foi utilizada para caracterizar o índice de polidispersão e o tamanho das partículas do concentrado emulsionável. O CAE-EC não apresentou efeitos fitotóxicos em plantas de trigo, garantindo sua aplicação segura. Considerando o baixo uso de solventes orgânicos e a sua expressiva atividade aficida e herbicida, a formulação EC oferece uma alternativa ecológica promissora para o controle de populações de pulgões do trigo.*

Palavras-chave: pesticidas botânicos, alcaloides, concentrado emulsionável, pulgões, toxicidade, EPG

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the 2nd most widely cultivated cereal for human use worldwide (Chen *et al*., 2024; Gupta *et al*., 2023). The World Food Organization estimates that 778.6 million tonnes of wheat grain were produced worldwide in 2021–2022 (Hu *et al*., 2023). Its global production still needs to be enhanced to meet the growing demand worldwide (Rockström *et al*., 2017). The wheat aphids *Rhopalosiphum padi, Sitobion miscanthus, and Schizaphis graminum* (Hemiptera: Aphididae) are considered among the most critical pests and often pose a considerable threat to cereal production directly by feeding on plant sap and indirectly by transmitting plant diseases (Li *et al*., 2023a; Xu *et al*., 2022). Aphids are soft-bodied insects of the Aphidinae family that usually occur in colonies, feeding on the undersides of new shoots and leaves (Shih *et al*., 2023). These are included as one of the most devastating pests for sustainable cereal production after abiotic stress, and cause heavy yield losses annually across the globe (Bass and Nauen, 2023). The wheat aphid caused 35-40% direct yield losses by sucking the sap and 35-40% indirect losses by spreading the fungal and viral diseases (Faheem *et al*., 2019).

Pakistan ranks 8th among the world's wheat-growing countries, with an average annual production of 24.946 million tons and a 1.7% contribution to the country's gross domestic product (GDP). Wheat is growing as a rabi (winter) crop in Pakistan, sowing from October to December and harvesting from March to May (Hussain *et al*., 2024). Wheat aphids were not considered a significant pest in Pakistan, and the plant protection department recommended no insecticidal sprays (Saleem *et al*., 2023). However, the world has faced climate change in the recent decade, with Pakistan being one of the most affected countries (Ali *et al*., 2023). These changes threaten sustainable wheat production by changing the pest ecology, biology, and behavior (Bajwa *et al*., 2020). Similarly, in 2020, three wheat aphid species, *S. miscanthus*, *R. padi*, and *S. graminum*, were listed as class I wheat insect pests due to their extensive damage to the wheat crop in China (Li *et al*., 2023b). Therefore, selecting new, safe, less persistent products for eco-friendly and sustainable pest management is timely (Silva *et al*., 2023).

Botanical pesticides are considered a novel alternative to synthetic pesticides because they are less hazardous to mammals and the environment (Benelli *et al*., 2021). These include alkaloids, terpenoids, glucosinolates, limonoids, phenylpropanoids, lipids, and nonprotein amino acids, which are naturally occurring substances that act as a first line of defense against pest attacks (Howe and Jander, 2008). Among the bioactive compounds isolated from plants, alkaloids are the most studied as insecticidal candidates (Fowsiya and Madhumitha, 2020). Alkaloids can interfere with various insect biological activities, such as inhibiting acetylcholinesterase enzymes, redox imbalance, protein synthesis inhibition, and hormonal imbalance (Chowański *et al*., 2016).

The insecticidal potential of *Sophora alopecuroides* and *Nicotiana tabacum* is well-studied, and several commercial products have already been created based on the bioactive compounds found in the *S. alopecuroides* (Zhang *et al*., 2023; Zou *et al*., 2020), and *N. tabacum* (Akbar *et al*., 2023; Voula *et al*., 2023). Typically, plant extracts exhibit greater toxicity than isolated bioactive compounds (Enke and Nagels, 2011). Plant extracts contain hundreds of bioactive compounds with multiple modes of action, which can synergistically affect insect pests (Khorrami *et al*., 2018). The synergistic effects between the mixture compounds in the plant extracts can enhance the overall insecticidal activity and offer a wider range of pest control (Oparaeke *et al*., 2005). Furthermore, the joint combination of aloperine, sophocarpine, oxymatrine, and cytisine, isolated from *S. alopecuroides*, showed significant synergistic activity against *Aphis craccivora* compared to the individual alkaloid toxicity (Ma *et al*., 2018). However, isolating bioactive compounds requires substantial amounts of plant biomass, and the yield of these compounds is exceptionally low (Stéphane *et al*., 2021). Plant extracts contain hundreds of bioactive compounds with diverse modes of action and typically exhibit greater bioactivity than individual isolated bioactive compounds (Enke and Nagels, 2011). Moreover, the conventional methods for isolating bioactive compounds often require substantial amounts of plant material, resulting in minimal yields. To overcome this limitation, our current study focuses on extracting simple crude alkaloidal extracts with higher yields, providing a more efficient and sustainable approach. The primary objective of this investigation was to develop a simple and rapid extraction method for crude alkaloids from *S. alopecuroides* and *N. tabacum*, with the ultimate goal of creating eco-friendly biopesticides for managing wheat aphids, thereby bridging the gap between botanical pesticide research and product development. Furthermore, a crude alkaloid extract emulsifiable concentrate (CAE-EC) formulation was subsequently prepared and evaluated for its biological activities. The effect of CAE-EC on the feeding behavior of *S. graminum* was assessed using the insect electrical penetration graph (EPG). Additionally, the herbicidal activity of CAE-EC was evaluated against *Amaranthus retroflexus*, a common weed.

ETHICAL ASPECTS

Ethical approval was not required for this study as it involved only insect subjects.

MATERIALS AND METHODS

The aphid colony was regularly maintained on potted wheat plants at the Insect Pest Management Program (IPMP) laboratory, Pakistan Agricultural Research Centre (NARC), Islamabad, Pakistan. The culture was kept under controlled conditions at 26 ± 2°C, 60% relative humidity, and a photo period of 14-16 hours. For bioassays, 2^{nd} or 3^{rd}-instar nymphs were used.

The crude alkaloids from *S. alopecuroides* and *N. tabacum* were extracted using a modified method formerly described by (Wang *et al*., 2022; Xiao *et al*., 2019), focusing on simple, crude alkaloidal extraction with higher yields. The plant material identification details were given in our previously published paper (Rizvi *et al*., 2019). Briefly, the *S. alopecuroides* whole plants (5 kg), collected before flowering from Skardu, Baltistan, in June 2022, were defatted with 90% n-hexane for 24 hours under room temperature to remove the steroidal compounds, oily substances, and fats, then macerated with 70% ethanol for 72 h in dark, and finally concentrated at 55°C under reduced pressure using a rotary evaporator (RE100-Pro Digital, Nanbei Instrument, China). In contrast, *N. tabacum* leaves, purchased from a local market in Peshawar, Pakistan, were macerated in 0.5 mol/L NaOH for 3 days, filtered, fractioned with 99% chloroform, and concentrated (Figure 1). The presence of alkaloids in the crude extracts was confirmed using the Wagner reagent test and further validated by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

A simple mixing method was used to formulate the CAE-EC emulsifiable concentrate. Briefly, the oven-dried crude alkaloidal extracts were ground into a fine powder. Our preliminary studies indicated that a combination of crude alkaloidal extract of *S. alopecuroides* and *N. tabacum* has a significant synergistic *effect on overall toxicity*. Therefore, we used a 1:1 combination to create the emulsifiable concentrate formulation. To achieve a 40% w/v CAE concentration, 120 g of the powder was first dissolved in a minimal quantity of absolute ethanol. This mixture was then combined with 180 mL of Methyl Oleate (60% v/v), which served as the primary organic carrier. A surfactant blend of Tween 80 and Span 80 was integrated to reach a hydrophile-lipophile balance (HLB) of 12. The mixture was stirred for 50 minutes using a magnetic stirrer to ensure homogenization. Finally, the total volume of the formulation was adjusted to exactly 300 mL by adding the remaining necessary volume of Methyl Oleate or co-solvent. An identical formulation without the CAE was prepared as a blank control.

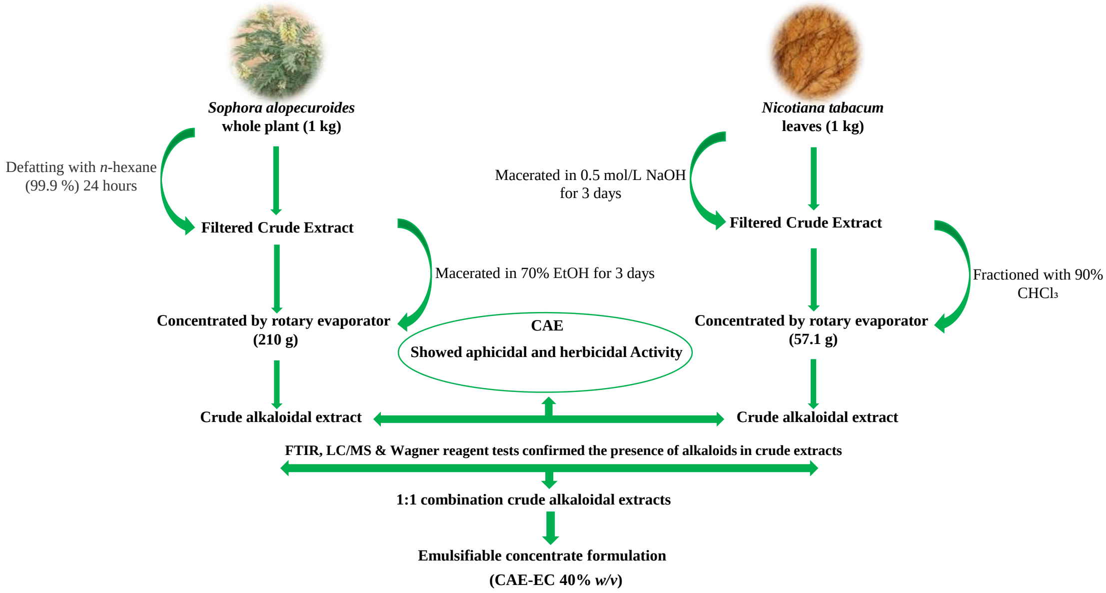

Figure 1. Flow chart of the extraction process.

The Zetasizer Nano ZES (Malvern, UK) was used to measure the zeta potential, polydispersity index, and particle size of CAE-EC. A digital pH (Hanna Instruments, USA) and a viscometer (Movel Scientific Instrument Co., Ltd., China) were used to measure the physical parameters of CAE-EC. The CAE-EC was diluted in double-distilled water at a 1:20 *v/v* ratio.

The FTIR spectroscopy was used to confirm the presence of alkaloid functional groups in the crude extracts of *S. alopecuroides* and *N. tabacum*. The analysis was performed by mixing the dried powder of the crude extract with potassium bromide (KBr) to form a pellet. The FTIR spectra were recorded using an ABB FTLA 2000-100 spectrometer (Quebec, Canada) at a resolution limit of 16 cm^{-1}.

The confirmation of alkaloids in crude extracts of *S. alopecuroides* and *N. tabacum* was carried out using LC-ESI-MS (Liquid Chromatography-Electrospray Ionization-Mass Spectrometry), Agilent Technologies, Santa Clara, CA, USA (Kaur *et al*., 2022). The crude extracts were centrifuged at 12000rpm for 15min before analysis to avoid any plant residues. Briefly, the HPLC system consisted of two pumps and an automated injector, and separation was achieved using a C-18column (Agilent Eclipse, 5 µm, 15 cm, 4.6 mm). The two mobile phases used were named A=0.1% formic acid in water and B=90% acetonitrile in water, with a flow rate of 500 µL/min. The LC conditions were 5% at 0−3 min in B, a linear increase from 5 to 20% between 3 and 25 min, 20 to 40% during 25–40 min, and from 40 to 50% between 40 and 55 min, finally, it reached 50 to 95% at 55–63 min. The water UPLC-TQD Mass spectrometer was utilized in a positive ionization mode. For the MS analysis, the data-dependent automatic switching between MS and MS/MS acquisition modes was used. The primary identification of the compounds was performed by comparing the MS/MS data with the mz Cloud database.

The contact toxicity of CAE-EC was evaluated by using the spraying method. Briefly, t*he* CAE-EC was dissolved in tap water to achieve the following concentrations: 0.5, 1.5, 2, and 3 (w/v %) for contact toxicity assessment against selected aphid species, corresponding to concentrations of 5, 15, 20, and 30 mg/mL, respectively. Each infested with 50 aphids, potted wheat plants were sprayed with 3 mL of CAE-EC at the desired concentration using a Potter tower at a pressure of 0.5kg/cm², resulting in a uniform deposit of 1.5µL/cm² on each plant. The mortality data were recorded after 24 hours of treatment. In contrast, EC without CAE was applied in the control group. A probit analysis was used to construct the lethal concentrations of CAE-EC against the selected aphid species. To assess the toxicity of CAE-EC against the selected aphid species over different time intervals, the same method used for contact toxicity was employed, with 5, 15, and 30mg/mL concentrations of CAE-EC. Mortality data were recorded at 12, 24, and 48 hours post-treatment.

For evaluating the residual contact toxicity of CAE-EC, a 30mg/mL concentration was selected against selected aphid species, as this concentration resulted in the maximum mortalities being recorded in the contact toxicity test. Potted wheat plants were sprayed with 30 mg/mL and allowed to dry for 5 hours. Fifty aphids per plant were then released onto the dry residue (Bett *et al*., 2017). Acetamiprid (3mg/L), a widely used neonicotinoid insecticide for managing sucking insects, served as the positive control. Mortality data were recorded at 24, 48, and 72 hours post-treatment. All toxicity bioassays were conducted under controlled laboratory conditions, maintained at 26±2°C and 50-80% relative humidity. Each treatment, consisting of a single concentration, was replicated five times.

The herbicidal activity of CAE-EC at 0.25, 0.5, 1, 2, and 5mg/mL concentrations was evaluated against the common dicot weed *A. retroflexus* in a Petri dish (9 cm in diameter) by following the procedure previously stated by (Zhou *et al*., 2021). Briefly, different concentrations of CAE-EC were added to Petri dishes having twenty seeds of *A. retroflexus* and were incubated at 25°C±2°C in the dark. The average growth in response to each treatment was calculated as the percentage change in root and shoot length measured five days after treatment compared to the control. The phytotoxicity of CAE-EC at concentrations of 5, 10, 15, 20, and 30 mg/mL was evaluated against wheat plants at three growth stages: seedling, tillering, and grain-filling. For the assessment of phytotoxicity measurements, a damage rating scale previously described by (Rizvi *et al*., 2019). The mean necrosis (%) per leaf per plant was rated as follows: "–" (0% for no significant visible injury), slight "+" (1–25% damage), medium "++" (25–50% damage), and high "+++" (> 50% damage) (Rizvi *et al*., 2019). Each treatment, consisting of a single concentration, was replicated five times.

The feeding behavior of 2 to 3-day-old (postfinal-molt) adult apterous females of *S. graminum* was used for the EPG experiments. The feeding behavior was recorded using an EPG system (Giga-8 Model, Wageningen, The Netherlands) with adjustable voltage and an amplifier with a 10^9 **Ω** resistance (Gao *et al*., 2023). A single *S. graminum* specimen was attached to the system probe with a thin piece of gold wire (0.18 µm in diameter, 4-5 cm long) attached to its thorax with water-based silver glue, and the other end to a bronze nail directly inserted into the system probe. Adults of *S. graminum* were starved for one hour before the recording began and placed on the adaxial side of the third leaf of each plant. To evaluate the feeding behavior and pattern, *S. graminum* was exposed to three concentrations of CAE-EC (0.1, 1, and 5mg/mL). A copper plant electrode was inserted into the potted soil to configure the setup. All wiring was connected to the GIGA amplifier, allowing simultaneous recording during the prolonged experiment. The EPG system was directly connected to a computer, where waveform signals from 8 different channels were displayed on Stylet+d software, and the waveform data analysis was performed using Stylet+a software (Seo *et al*., 2009). The gain was adjusted to 76x from 50x on each channel when any activity was observed. All the treatments were replicated three times, and the means of successful waveform records were from *S. graminum* adults.

Probit analysis was used to construct the LC_{50} and LC_{90} of CAE-EC using SPSS 17.0. One-way analysis of variance (ANOVA) was used to compare toxicity among treatments at different time intervals. Tukey's test was used to calculate the significant difference among the treatments, and P-values less than 0.05 were considered statistically significant. The SPSS 17 software package was used to perform all the statistical analyses. Stylet+d software was used for EPG data analysis and non-parametric analysis, and the Kruskal-Wallis test was employed.

RESULTS

The FTIR analysis of the crude extract of *S. alopecuroides* and *N. tabacum* is presented in Figure 2. The results indicated characteristic FTIR peaks that serve as evidence for the presence of alkaloids in the crude extract. Specifically, the spectra exhibited N-H stretching vibrations at 3,361.36-3,461.69cm^{-1}, C-H stretching at 2,877.09cm^{-1}, and C=C and C=N stretching at 1,665.48cm^{-1}. These findings confirm the presence of alkaloids and suggest that the crude extract is rich in alkaloids, validating the effectiveness of our simple alkaloid extraction method.

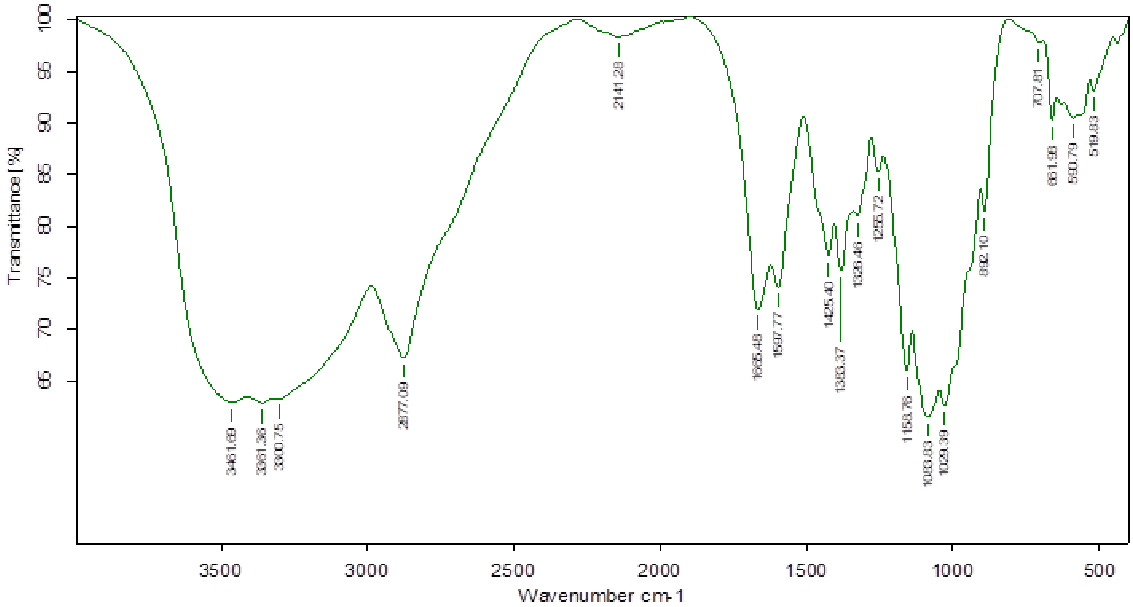

Figure 2. FTIR absorption spectra of KBr pellets of *Nicotiana tabacum* and *Sophora alopecuroides* crude extract powder.

Confirmation of alkaloids in the crude extracts of *S. alopecuroides* and *N. tabacum* is presented in Table 1. The LC-MS analysis revealed the presence of several prominent alkaloids, including nicotine metabolites and derivatives from *N. tabacum*, such as anatabine, cotinine, anatalline, and nicotine-N-glucuronide. Conversely, *S. alopecuroides* extract yielded a distinct set of alkaloids, including 5,6-dehydrolupanine, sophoraflavanone G, kurarinone, oxysophoridine, kurarinol, spheroidenone, oxysophocarpine, and sphingosine. The extraction method employed in our study for alkaloids proved effective, with substantial amounts detected in the extracts. The crude alkaloid extract yields were notably high, with *S. alopecuroides* and *N. tabacum* yielding 21% w/w and 5.7% w/w, respectively. The presence of alkaloids in both extracts was confirmed through positive Wagner reagent tests and LC-MS analysis. The chemical structures of alkaloids identified in the crude extract by LC-MS are consistent with previous reports (Fig. 3).

The dynamic light scattering (DLS) analysis revealed that the mean diameter of the EC was 267.2±3.86nm, with a PDI of 0.144, indicating a moderately monodisperse size distribution. In contrast, the CAE-EC had a mean diameter of 293.2±3.12nm, with a PDI of 0.106, suggesting a relatively monodisperse size distribution. Overall, the results indicate that the addition of crude alkaloids increases the mean particle size while maintaining a relatively uniform size distribution (Fig. 4B a-b). The physical appearance of the emulsifiable concentrate formulation diluted in distilled water is presented in (Fig. 4Aa- d). Upon dilution to 1:20, the EC formulation rapidly dissolved upon minor agitation. A minimal creamy layer developed at the surface of the beaker 24 h after dilution. However, this creamy layer was easily dispersed upon minor agitation. The pH of the CAE-EC was 6.0, while the pH of the EC without the crude alkaloid extract was 6.35. The viscosity of CAE-EC was 3.43 cP, slightly greater than that of EC without crude alkaloid extracts (4.34 cP).

CAE-EC was acutely toxic to adults of treated aphid species, with LC_{50} values of 12.54mg/mL against *Brachycaudus helichrysi*, followed by 13.74, 13.96, 14.12, and 17.75mg/mL against *R. padi*, *S. avenae, S. graminum,* and *M. persicae*, respectively. The confidence intervals overlap, indicating no significant difference in CAE-EC toxicity among the tested aphid species. Furthermore, the LC_{50} value of acetamiprid, used as a positive control, was 0.35mg/L, which is significantly lower than the CAE-EC LC_{50} values (Table 2). The toxicity of CAE-EC increased gradually with increasing concentration and exposure time, indicating a concentration- and time-dependent nature of the toxicity effects (Fig. 5A-C). The CAE-EC at 5, 15, and 30mg/mL significantly reduced the aphid population compared to the control. At 30mg/mL, the maximum mean mortality of 98.66% was observed for *S. graminum,* followed by 97.54, 95.53, 95.12, and 94.33% for *R. padi, B. helichrysi, S. avenae,* and *M. persicae,* respectively. In contrast, in the control, only 9.34% mortality was observed 48 hours after application. Furthermore, the residual contact toxicity of CAE-EC against the selected species of aphids was significantly lower than that of contact toxicity (Fig. 6). The dry residue treatment of CAE-EC showed only 3-13 % mortalities after 48 h of treatment. However, acetamiprid caused 42.21%, 76.32%, and 98.2% mortality at 3mg/L at 3, 12, and 24 h after application, respectively. Moreover, the herbicidal activity of CAE-EC against *A. retroflexus* indicated a concentration-dependent effect (Fig. 7 A-B). The CAE-EC at 5mg/mL concentrations inhibited root and shoot elongation by 33% and 91%, respectively. Furthermore, the CAE-EC had no phytotoxic effects on wheat plants at three different growth stages.

Table 1. Confirmation of alkaloids in the crude extract of *S. alopecuroides* and *N. tabacum* by LC/MS positive mode of analysis

S.NO	RT (min)	Mass Error (ppm)	Observed m/z	MW	Formula	Compound
S. alopecuroides						
1	0.8	2.06	247.18	246.35	$C_{15}H_{22}N_2O$	5,6-Dehydrolupanine
2	2.16	2.41	265.19	264.36	$C_{15}H_{24}N_2O_2$	Oxysophoridine
3	5.19	-0.67	457.22	456.5	$C_{26}H_{32}O_7$	Kurarinol
4	6.57	-0.99	437.2	438.5	$C_{26}H_{30}O_6$	Kurarinone
5	7.15	-0.08	423.18	424.5	$C_{25}H_{28}O_6$	Sophoraflavanone G
6	8.58	1.13	285.17	262.35	$C_{15}H_{22}N_2O_2$	Oxysophocarpine
7	8.79	-6.28	600.47	582.9	$C_{41}H_{58}O_2$	Spheroidenone
8	9.12	-3.03	300.29	299.5	$C_{18}H_{37}NO_2$	Sphingosine
N. tabacum						
1	0.74	5.55	177.10	176.21	$C_{10}H_{12}N_2O$	Cotinine
2	1.32	2.10	240.15	239.32	$C_{15}H_{17}N_3$	Anatalline
3	3.30	0.18	183.09	160.22	$C_{10}H_{12}N_2$	Anatabine
4	8.34	-24.33	322.14	338.36	$C_{16}H_{23}N_2O_6^-$	Nicotine-N-glucuronide

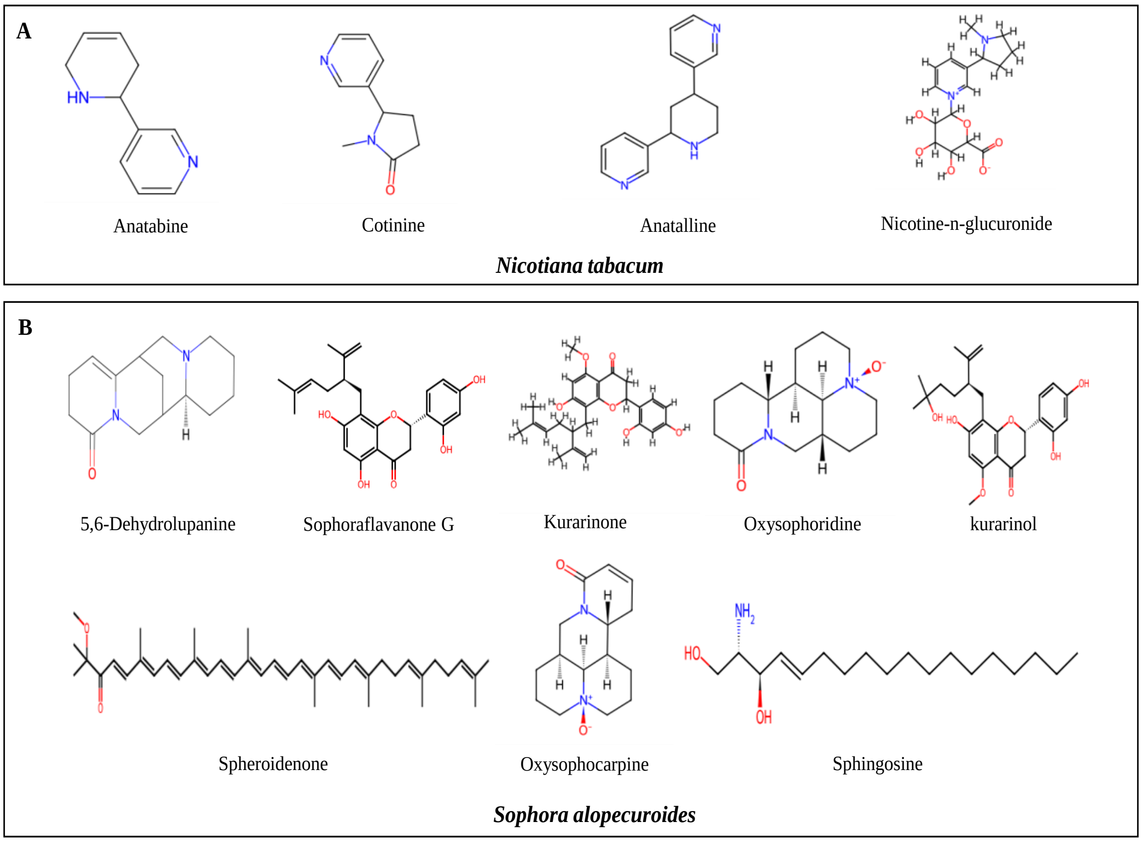

Figure 3. The chemical structures of alkaloids identified in crude extract by LC-MS, consistent with previous reports for (A) *Nicotiana tabacum* (Guo *et al*., 2011; Häkkinen *et al*., 2004; Weber *et al*., 2019; Zou *et al*., 2021), and (B) *Sophora alopecuroides* (Boozari *et al*., 2019; Huang *et al*., 2016; Lu *et al*., 2014; Wei *et al*., 2024; Wu *et al*., 2002).

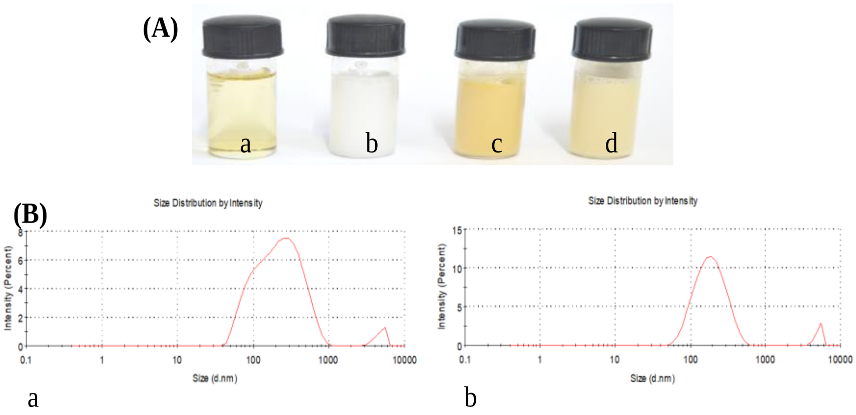

Figure 4. Physical properties of CAE-EC Panel (A), the physical appearance of emulsifiable concentrate (a) EC without crude alkaloids, (b) EC diluted in 50mg/mL distilled water, (c) CAE-EC, (d) CAE-EC diluted in 50mg/mL distilled water. Panel (B), the size distribution intensity of EC in an aqueous solution: (a) EC without crude alkaloids, (b) CAE-EC.

Table 2. Contact toxicity of crude alkaloidal extract emulsifiable concentrate formulations against adults of aphid species

Treatments	N[a]	Slope ± SE	LC_{50}[b] (95% CL) mg/mL	LC_{90}[c] (95% CL) mg/mL	[d]X^2 (*d.f*)
Schizaphis graminum	223	2.58 ± 0.26	14.12 (12.70-15.70)	44.23 (35.66-60.73)	5.28 (3)
Rhopalosiphum padi	245	2.63 ± 0.39	13.74 (09.57-19.84)	42.18 (26.14-150.3)	4.98 (3)
Sitobion avenae	265	2.39 ± 0.21	13.96 (12.21-15.29)	44.53 (35.52-61.34)	6.39 (3)
Brachycaudus helichrysi	254	2.69 ± 0.29	12.54 (8.69-17.30)	30.68 (24.72-99.08)	4.94 (3)
Myzus persicae	233	3.99 ± 0.39	17.75 (16.19-19.09)	37.17 (32.77-44.30)	3.59 (3)
Acetamiprid[e]	251	2.51 ± 0.51	0.35 (0.000156-0.000544)	0.805(0.000311- 0.001299)	10.2 (3)

[a]Total number of aphids treated. [b] 50 % lethal concentration. [c] 90% lethal concentration. [d] $\chi 2$ chi-square, *d.f* degrees of freedom. [e] Acetamiprid (mg/L) was used as a positive control.

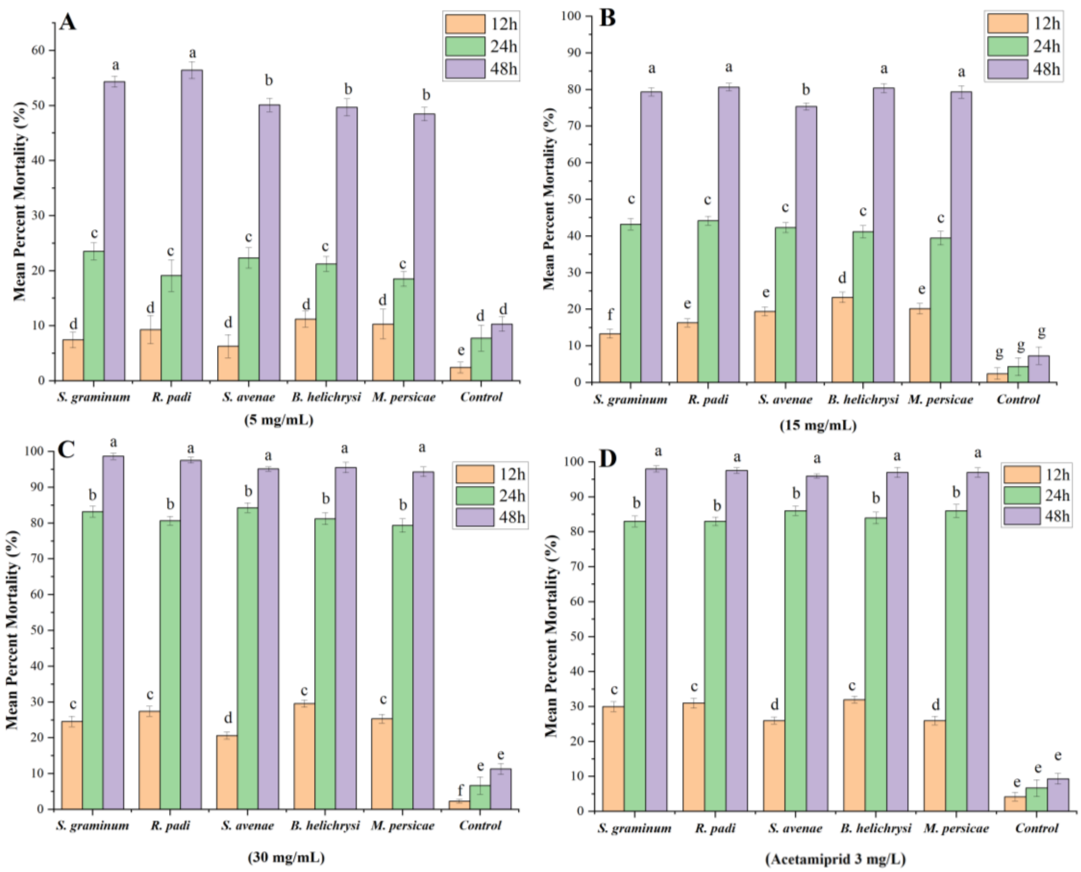

Figure 5. Mean percent mortalities of aphid species after 12, 24, and 48h after treatment caused by CAE-EC at (A) 5mg/mL, (B) 15mg/mL, (C) 30mg/mL, Acetamiprid 3mg/mL (D) as a positive control. Bars within a panel not labeled by the same letter are significantly different according to Tukey's test (*P*<0.05). (D) Acetamiprid 3mg/L was used as a positive control.

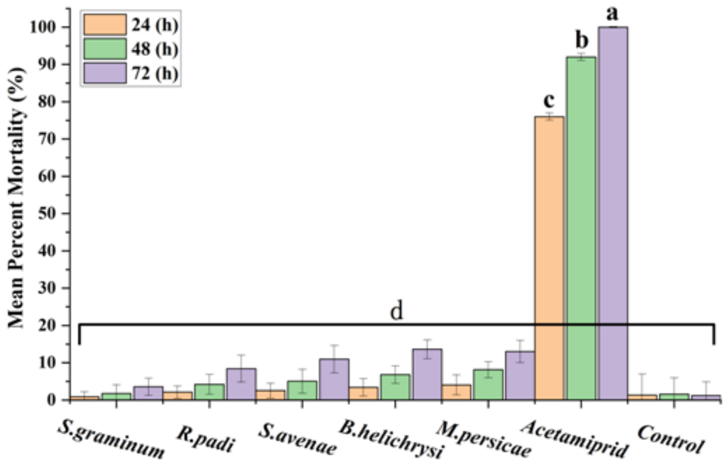

Figure 6. Mean percent mortalities of aphid species released on the dry residues of CAE-EC 30mg/mL concentration after 12, 24, and 48h. Acetamiprid 3mg/mL was used as a positive control. Bars within a panel not labeled by the same letter are significantly different according to Tukey's test (P<0.05).

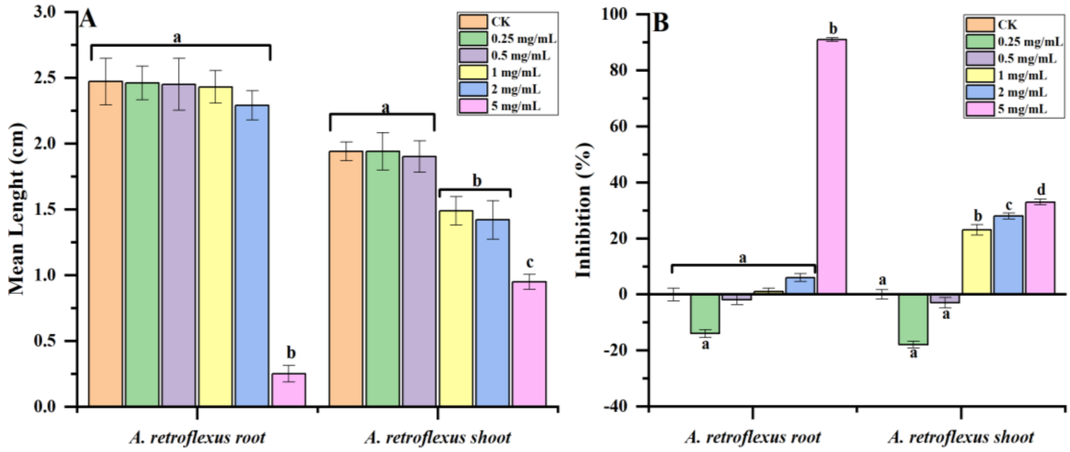

Figure 7. Herbicidal activity of CAE-EC on root and shoot elongation *A. retroflexus* after 7 days of treatments with CK, 0.25mg/mL, 0.5mg/mL, 1mg/mL, 2mg/mL, and 5mg/mL, (A) Mean root and shoot length of *A. retroflexus*, (B) Percent inhibition in root and shoot length of *A. retroflexu*. Bars within a panel not labelled by the same letter are significantly different according to Tukey's test (P<0.05).

The alterations in the feeding behavior and pattern of *S. graminum* exposed to 0.1, 1, and 5mg/mL CAE-EC are presented in Figure 8A-E. The results indicated that *S. graminum* exposed to the desired concentrations of CAE-EC initiated probing behaviors but did not engage in phloem ingestion (E2) or xylem-associated activities (G), with a few insects failing to engage in any probing behavior (PD). In contrast, *S. graminum* feeding on untreated plants showed normal stylet functions, engaging in cell penetration (PD), pathway (C), phloem salivation (E1), phloem ingestion (E2), and xylem-associated (G) activities. The data indicated that the non-probing (NP) time of plants treated with 0.01mg/mL CAE-EC was 51.58 minutes, followed by that of plants treated with 1 or 5mg/mL CAE, which were 56.48 and 94.21 minutes greater than that of the control, respectively, where *S. graminum* took 23.01 minutes only. Similarly, the amount of E2 taken up by *S. graminum* was 288.45 min for the control, while the values at 0.01, 1, and 5mg/mL were 129.71, 143.63, and 163.68 min, respectively (Table 3).

Table 3. Mean (± SEM) duration (in minutes) that adult aphids performed various feeding behaviors on treated and untreated wheat plants during the Electrical Penetration Graph recording period

Treatments mg/mL	NP	C	G	Ingestion		T1st Pr	T1st E2
				E1	E2		
0.1	51.58±5.23a	53.43±5.44a	49.98±3.34a	16.1±6.32a	129.71±5.11a	7.15±1.34a	55.88±7.35a
1	56.48±4.54a	63.21±4.65a	62.83±2.45b	18.96±7.54a	143.63±6.32b	12.64±1.11b	68.11±5.87b
5	94.21±4.76b	95.78±3.11b	95.23±2.76c	29.33±5,21b	163.48±7.21c	19.46±1.98c	95.06±7.45b
Control	23.01±3.45c	43.32±10.33b	16.21±3.22d	6.32.68±4.21a	288.45±9.32d	4.51±2.09c	31.58±4.32c

NP: Non-probing, C: Pathway phase, G: Xylem ingestion, E1: Phloem salivation, E2: Phloem ingestion, T1st Pr: Time to first probe, T1st E2: Time to reach first sustained phloem ingestion bout. According to Tukey's test, the data represent the means of three successful waveforms. Means followed by the same letter are not significantly different *(P<0.05)* across different EPG recording events.

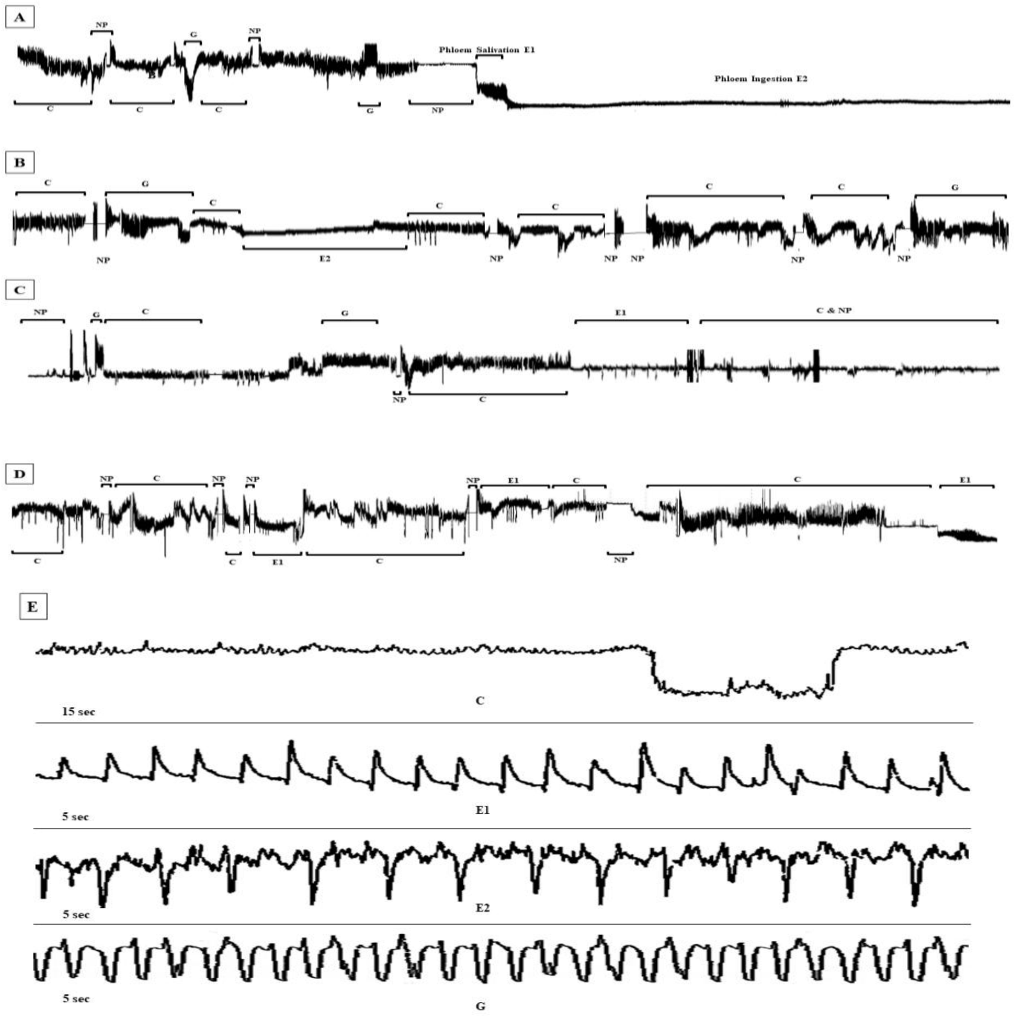

Figure 8. The electrical penetration graphs (EPG) of *S. graminum* feeding on treated and untreated plants. Overview of a four-hour recording by EPG of adult aphids (A) Control, (B) 0.1 (C), 1 and (D) 5mg/mL, (E) Waveforms representation is NP, non-probing; C, pathway phase; G, xylem feeding; E1, active salivation; E2, phloem ingestion; (E) waveforms exhibited by an aphid feeding on potted wheat plants.

DISCUSSIONS

Aphids are soft-bodied insects with suckling mouthparts, and they release honeydew while feeding, which promotes the growth of sooty mold and inhibits normal photosynthesis (Luo *et al*., 2022). In addition to feeding damage, aphids vector several deadly plant pathogens (Shih *et al*., 2023). Managing wheat aphids represents a major challenge due to their rapid reproduction ability and the significant crop losses they cause (Novák *et al*., 2024). Currently, carbamates, neonicotinoids, sulfoximines, and pyrethroids are widely used for their management (Kaleem *et al*., 2023). However, the massive reliance on these synthetic chemicals results in resistance and environmental pollution (Ikbal and Pavela, 2019). Therefore, eco-friendly novel alternatives for their management are needed. In this regard, we have evaluated the aphicidal activity of crude alkaloidal extracts from *S. alopecuroides and N. tabacum* as a novel tool for managing wheat aphids.

Alkaloids are among the most studied potential insecticidal candidates (Chowański *et al*., 2016). However, the isolation of bioactive compounds requires a large amount of plant biomass, and the yield of bioactive compounds is exceptionally low (Stéphane *et al*., 2021). Plant extracts, comprising hundreds of bioactive compounds with diverse modes of action, often exhibit greater bioactivity than individual isolated compounds due to synergistic interactions among multiple constituents, which can enhance overall toxicity (Enke and Nagels, 2011). The results of our current investigation reveal that a rapid and straightforward crude alkaloid extraction method from *S. alopecuroides* and *N. tabacum* achieves remarkably high yields, with *S. alopecuroides* and *N. tabacum* yielding 21% w/w and 5.7% w/w, respectively. The FTIR analysis of crude extract revealed characteristic peaks indicative of heterocyclic compounds, with N-H stretching vibrations around 3,500–3,000 cm^{-1} and C-H stretching around 2800-3000 cm^{-1}, consistent with previous reports (Hu *et al*., 2024; Seçkin and Meydan, 2022; Vajjiram *et al*., 2017). Our study demonstrates the efficacy of a simple extraction process in isolating these alkaloids, as confirmed by FTIR and LC-MS analysis. The LC-MS analysis revealed several prominent alkaloids in *N. tabacum* and *S. alopecuroides* extract, consistent with previous literature that *S. alopecuroides* contains sophocarpine, oxysophocarpine, matrine, oxymatrine, cytisine, and aloperine (Wei *et al*., 2024; Boozari *et al*., 2019; Huang *et al*., 2016; Lu *et al*., 2014; Wu *et al*., 2002). Similarly, from the *N. tabacum* plant extract, predominant metabolites and derivatives of nicotine were reported, including myosmine, anatabine, noricotine, anabasine, nicotine, cotinine, and toosendanin (Zou *et al*., 2021; Weber *et al*., 2019; Guo *et al*., 2011; Häkkinen *et al*., 2004).

Alkaloids serve as plants' first line of defense against arthropod attacks, interfering with various cellular and physiological activities of insects (Ali *et al*., 2019). Plant alkaloids, including matrine, piperine, camptothecin, nicotine, and berberine, have shown considerable insecticidal activity against various pests, including *Diaphorina citri* (Rizvi *et al*., 2024), *Spodoptera exigua*, and *Leptinotarsa decemlineata* (Lim *et al*., 2014). In our study, the aphicidal activity indicated that the CAE-EC formulation was acutely toxic to the selected aphid species, with LC_{50} values ranging from 12.54 to 17.75mg/mL when applied via contact to potted wheat plants. Meanwhile, the residual contact toxicity of CAE-EC was significantly lower than that of the contact toxicity. Overall, time- and concentration-dependent mortalities were observed. The average mortality rate of CAE-EC against the selected aphid species was 96.23%, slightly lower than that of acetamiprid, which caused 100% mortality at 3mg/L at 48 hours post-application. Similarly, (Wu *et al*., 2002) stated that the multiple combinations of anabasine, toosendanin, nicotine, sophocarpine, and aloperine between 0.1%–10% (*w/v*) had shown significant mortalities against *Jacobiasca formosana*, *Lilioceris lilii*, and *Ectropis bhurmitra.* Furthermore, the alkaloids matrine and sophocarpine present in S. alopecuroides have shown considerable mortality against various agricultural pests, and several matrine-based insecticides are currently commercialized in China (Huang *et al*., 2020). Regarding the mode of action of alkaloids against insects, the previous studies indicated neurotoxicity (Adamski *et al*., 2020). Our results align with those of (Puripattanavong *et al*., 2013), who found that an emulsifiable concentrate emulsion containing tobacco extract showed considerable toxicities against aphids and remained stable under various conditions. Similarly, emulsifiable concentrate formulations containing *S. alopecuroides* have a prolonged repellent effect against *D. citri* compared to the ethanolic extract (Rizvi *et al*., 2019). The results indicated that the CAE-EC formulation has potential as a novel alternative to synthetic insecticides against aphid species.

Plant extracts and essential oils at relatively high concentrations have been shown to cause insect mortality, while relatively low concentrations have demonstrated repellent and deterrent effects on arthropods (Gao *et al*., 2021; Liao *et al*., 2016). In our current study, the impact of sublethal concentrations of CAE-EC on the feeding behavior of *S. graminum* was evaluated using electric penetration graph (EPG) recordings. The feeding behavior and pattern indicated that the total duration of phloem sap ingestion and synchronized salivation were significantly shorter in the adult aphids exposed to the wheat plants treated with 0.1, 1, and 5mg/mL of CAE-EC than in the control aphids. The non-probing time of the aphids was greater than that of the control aphids. Furthermore, the salivation (E1) and sap-feeding (E2) durations of *S. graminum* significantly decreased with increasing concentrations of CAE-EC. Our results indicated that CAE-EC alters the feeding behavior of *S. graminum.* Similarly, the crude extracts of *N. tabacum* and *Melia azedarach* have shown antifeedant activity against *Spodoptera litura* (Venkateswarlu *et al*., 2013), and alkaloids from *Sophora alopecuroides* have shown antifeedant activity against *Clostera anastomosi* (Yang *et al*., 2006). Furthermore, sublethal concentrations of flonicamid, imidacloprid, cycloxaprid, and thiamethoxam significantly altered the non-probing time, phloem sap ingestion, salivation (E1), and sap-feeding (E2) activities in *S. avenae* (Aeinehchi *et al*., 2021), *S. graminum* (Gul *et al*., 2023), and *A. gossypii* (Koo *et al*., 2015). Our findings suggest that CAE-EC has a significant impact on the feeding behavior of *S. graminum*, providing a potential mechanism for its aphicidal activity.

CONCLUSIONS

Our current study evaluated the efficacy of crude alkaloid extract emulsifiable concentrate formulation containing *S. alopecuroides* and *N. tabacum*, as a novel alternative to synthetic insecticides for the sustainable management of wheat aphids. However, to fully assess its potential as an eco-friendly pest control candidate, further research is required to study its environmental impact and effects on non-targeted organisms. The simple, crude alkaloid extraction method employed in this study offers a more efficient and sustainable alternative to traditional methods of isolating single bioactive compounds, which typically require large amounts of plant biomass. Our process yields a higher recovery of crude alkaloids. It reduces the risk of insect resistance due to the synergistic activity of multiple compounds present in the crude extract, offering a distinct advantage over individual compounds. Therefore, the findings of our current study indicated that CAE-EC is an eco-friendly alternative for controlling wheat aphids.

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CONTRIBUTORS

Syed Arif Hussain Rizvi: Conceptualization, data curation, visualization, original draft writing, methodology. Shanza Khan: Data curation, formal analysis, methodology, software. Ihsan Ul Haq: Supervision, Project management. Areej A. Al-Khalaf, Mohamed A. Wadaan, Fahd A. Al-Mekhlafi: Review, editing, and funding.

DATA AVAILABILITY STATEMENT

The research data are available within the article itself.

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