International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 25-06-2024, Accepted: 26-07-2024, Published: 13-08-2024
Volume 9, Issue 8, 2024, Page No. 85-90

In silico screening identified scopolamine as a potent anti-termite agent against *Odontotermes obesus* through ROS activation and enzymatic inhibition

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Abstract
Termites, particularly *Odontotermes obesus* pose a significant threat to agricultural and structural integrity worldwide. Traditional chemical pesticides often cause resistance and cause environmental concerns. Therefore, identifying novel, effective, and eco-friendly anti-termite agents is crucial. This study aimed to explore scopolamine as a potential anti-termite agent through in silico screening, focusing on reactive oxygen species (ROS) activation and enzymatic inhibition mechanisms. In silico molecular docking was used to predict the binding affinity of scopolamine to key termite enzymes. superoxide dismutase (SOD) inhibition assays were conducted to assess the ability of scopolamine to disrupt antioxidant defense. The H_2DCFDA assay was used to measure intracellular ROS levels, whereas the NADPH oxidase assay was used to evaluate the impact of scopolamine on ROS producing enzymes. Molecular docking results indicated a strong binding affinity of scopolamine to Endo-1,4-β-glucanase, Exo-1,4-β-glucanase, and Endo-1,4-β-Xylanase, suggesting significant inhibitory potential. SOD inhibition assays confirmed that scopolamine effectively impaired the antioxidant defense mechanisms in termites. H_2DCFDA assays revealed elevated ROS levels in scopolamine-treated termites, indicating oxidative stress. NADPH oxidase assays further support the role of scopolamine in enhancing ROS production, contributing to cellular damage and termite mortality. The dual mechanism of ROS activation and enzymatic inhibition by scopolamine is a robust approach for termite control. This strategy not only ensures higher efficacy, but also mitigates the risk of resistance development. This study underscores the potential of scopolamine as a novel and sustainable solution for termite management, paving the way for eco-friendly pest control strategies.

Keywords: Termites, scopolamine, anti-termite, reactive oxygen species (ros), molecular docking, enzymatic inhibition, oxidative stress

Introduction

In silico screening has become a crucial method for identifying potential anti-termite agents, with natural compounds showing significant efficacy. Scopolamine, a tropane alkaloid from *Datura stramonium*, has proven effective against *Odontotermes obesus* by activating reactive oxygen species (ROS) and inhibiting critical enzymatic activities. Its complexation with cyclodextrins enhances solubility and bioavailability, improving its effectiveness in agrochemical formulations (Monica *et al*., 2021) [5]. This is consistent with other studies demonstrating the anti-termite potential of plant-based chemicals, such as essential oils from *Tagetes erecta*, which alter biochemical parameters like glycogen, protein, and lipid levels (Susheel *et al*., 2023) [7]. Additionally, plant extracts from *Grevillea robusta* have reduced termite survival rates and inhibited gut flagellates, further supporting the use of natural compounds in termite management (Asma *et al*., 2020) [2]. Computational and biological screening platforms have also identified natural products like sesamin, which exhibit antifungal properties and enzymatic inhibition capabilities, potentially repurposable for anti-termite use (K. Wadhwa *et al*., 2023) [4]. Low-dose pesticides such as chlorantraniliprole have effectively managed termites by causing behavioral symptoms like intoxication and ataxia, which could be synergistically enhanced with natural compounds like scopolamine (Asad *et al*., 2023) [1]. The identification of anti-schistosomal compounds from beetles highlights the untapped potential of insect-derived molecules in pest management (Tom, L, Gallinger *et al*., 2022) [8]. In breast cancer research, in silico screening of FDA-approved drugs has demonstrated that compounds like thioridazine can modulate intracellular targets, a strategy applicable to identifying anti-termite agents (Claudia *et al*., 2018) [3]. Screening peptide bond-containing compounds for antiviral activity against HCV protease further underscores the versatility of in silico methods in drug discovery, extendable to termite control (Tuyet *et al*., 2019) [9]. Additionally, plant latex-based formulations, such as those from *Calotropis procera*, have shown high toxicity and repellency against termites, offering a sustainable alternative to synthetic pesticides (Ravi *et al*., 2013) [6]. These studies collectively highlight the potential of scopolamine and other natural compounds for termite management via ROS activation and enzymatic inhibition, supported by in silico screening and combinatorial approaches.

Materials and methods

1. Selection of bioactive alkaloids against termiticidal activity

Identifying natural alkaloids requires a thorough search of scientific articles and databases to gather information about their occurrence and quality in various plants. The selection process for the proposed study's alkaloid panel involved using databases like PubMed, Science Direct, and Google Scholar to find research articles, reviews, and publications on the termicidal activity of natural alkaloids. We also consulted ethnobotanical literature and traditional knowledge sources to identify plants traditionally used for termite management or known to contain insecticidal

alkaloids. Additionally, plant databases such as the Plant Metabolic Network, KNApSAcK, and the Natural Products Atlas were examined for comprehensive information on secondary metabolites in different plant species. It is crucial to assess the reliability and relevance of sources during the literature search. Following the latest publications based on these searches, we identified 11 alkaloids for the proposed study.

Selected Alkaloids (11): Nicotine, Anabasine, Caffeine, Pyrethrin, berberine, Aconitine, quinine, Scopolamine, Allethrin, Permethrin, Strychnine.

2. In-silico virtual screening

2.1. Target Identification

The first step in virtual screening is to identify a molecular target relevant to termicidal activity. In this study, we identified three enzymes, namely Endo-1,4-β-glucanase, Exo-1,4-β-glucanase or Cellobiohydrolase, and Endo-1,4-β-Xylanase, which are essential for cellulolytic activity in many termites, including the Indian termite *Odontotermes obesus.*

2.2. Docking

Molecular modeling and docking studies were conducted with Discovery Studio and Auto Dock-assisted Chimera 2017.02 software. Energy minimizations were performed in Chimera to achieve an RMSD gradient of 0.1 kcal·mol−1·Å−1 using the MMFF force field, with partial charges calculated automatically. X-ray crystallographic structures of Endo-1,4-β-glucanase (PDB ID: 1NLR), Exo-1,4-β-glucanase or Cellulose 1,4-beta-cellobiosidase (3P6B), and Endo-1,4-β-Xylanase (PDB ID: 1XYF) were sourced from the PDB website. Water molecules were removed from all target receptors, and proteins were prepared for docking using Discovery Studio with default settings. The active site for docking was defined using 11 alkaloid ligands. The docking protocol was verified through self-docking of 3D optimized ligands near the receptor's active site, resulting in a docking pose with an energy score and the lowest RMSD. Verified docking procedures were then used to examine ligand-enzyme interactions at the active site, predicting binding patterns and analyzing the structure-activity relationship to explain the strong binding affinity of the ligands.

3. Anti-oxidant capabilities of alkaloids

3.1. Anti-oxidant activity of selected alkaloids

3.1.1. Determination of SOD

Superoxide dismutase (SOD) activity was measured using the SOD test kit-WST (Sigma-Aldrich®) for selected alkaloids. The reaction mixtures were combined with 100 µL of the test sample in DMSO, gently agitated, and incubated at 37°C for 20 minutes. SOD's suppressive effect on xanthine oxidase-generated superoxide was assessed using tetrazolium salt, with absorbance measured at 450 nm using a microplate reader. Ascorbic acid served as the positive control. Data are reported as mean ± standard error from two replicates.

4. Termites

Odontotermes obesus termites were collected from the Division of Entomology at Professor Jayashankar Telangana State Agricultural University in Rajendranagar, Hyderabad, and transported to the laboratory in a humid thermocol box. They were placed in a hygrostat chamber, maintaining 70 ± 5% humidity using a wet paper towel. The chamber's temperature was set at 27 ºC ± 2ºC, and minimal light conditions were ensured by covering it with a black cotton cloth.

5. Termiticidal activity

Kang *et al*. (1990) employed the no-choice bioassay to evaluate the anti-termite efficacy of specific alkaloids (pyrethrin, quinine, scopolamine, allethrin, permethrin). Test samples were prepared in three concentrations (1, 2, and 3 mg/mL of solvent) and dissolved in 1 mL methanol. These samples were applied to 1 g filter paper (Whatman No. 3, 8.5 cm diameter). A control was created using filter paper treated with only solvent. After air-drying the treated papers to evaporate the solvent, 100 active termites (90 workers and 10 soldiers) at or beyond the third instar were placed on each filter paper in a 9 cm diameter, 1.5 cm high Petri dish. Sterilized sand in the dishes was intermittently hydrated with water droplets. The experiment was randomized with six replicates per sample, and termite mortality was recorded daily over 14 days.

6. Bio molecular analysis

6.1. Isolation and processing of biological matrices

Worker termites (20 in number) were treated with scopolamine at 3 mg/mL for 5 days. Biological samples were prepared following an established protocol. Whole-body extracts were obtained by slaughtering, homogenizing, and centrifuging the termites. Changes in biomolecule levels were measured at different intervals, such as 3 and 5 days, leading to the identification of several significant biomolecules.

7. ROS Activation studies

7.1. Sample Treatment

A total of 120 fresh termite workers were collected and quickly washed with ice-cold phosphate-buffered saline (PBS) to remove external debris. Scopolamine stock solutions were prepared in ethanol at 100 µg/mL and then diluted with PBS to 10, 25, and 50 µg/mL working concentrations. Termites were divided into six groups of 20: normal control (G1), solvent control (G2), scopolamine treatments (S10: 10 µg/mL, G3; S25: 25 µg/mL, G4; S50: 50 µg/mL, G5), and positive control (fipronil, G6). Each termite received a consistent dosage of the solution, and they were then allowed 12 hours to metabolize the compounds.

7.2. Preparation of Whole-body Extract

After treatment, termites were quickly rinsed with ice-cold PBS to remove external debris. The rinsed termites were placed in a pre-chilled homogenization tube, and 1 mL of ice-cold homogenization buffer with protease inhibitors (PMSF, aprotinin, leupeptin, and EDTA) was added. Using a motor-driven or handheld homogenizer, termites were thoroughly homogenized for 1-2 minutes on ice to prevent protein degradation. The homogenate was then centrifuged at 10,000 × g for 10 minutes at 4°C to pellet the debris. The supernatant, containing soluble proteins and other cellular components, was collected as the termite worker whole-body extract. Protein concentration was immediately measured using the BCA or Bradford assay, adjusted as

necessary for experiments, and aliquoted to prevent degradation from repeated freeze-thaw cycles. Aliquots were stored at -80°C for long-term preservation. This method ensured high-quality extracts for subsequent biochemical analyses, maintaining cellular component integrity.

7.2.1. H_2DCFDA assay

The H_2DCFDA assay (Zhang, W., & Zhang, X. 2022) utilized whole-body termite extracts from all groups to measure reactive oxygen species (ROS) levels. Thawed termite extract aliquots were used to prepare a working solution of H_2DCFDA (2',7'-dichlorodihydrofluorescein diacetate) at 10 µM in PBS. In a black 96-well microplate, 100 µL of the termite extract was mixed with 100 µL of the H_2DCFDA working solution, achieving a final dye concentration of 5 µM. The plate was incubated in the dark at room temperature for 30 minutes. Fluorescence intensity was then measured using a microplate reader at excitation and emission wavelengths of 485 nm and 528 nm, respectively. Controls with only PBS and dye were included to establish baseline fluorescence for background subtraction. Fluorescence data were analyzed by comparing sample well readings to controls, quantifying ROS levels in the termite extracts. This assay sensitively measures oxidative stress in termites to assess the effects of various treatments or conditions on ROS production.

7.2.2. NADPH Oxidase assay

To apply the NADPH Oxidase Assay Pick, E., & Mizel, D. (1981) to whole-body extracts from termites across nine different treatment groups, we first collected and homogenized a sufficient number of termites for each group in ice-cold phosphate-buffered saline containing protease inhibitors. This was followed by centrifugation at 10,000 x g for 10 minutes at 4°C to remove cellular debris, and the supernatants containing soluble proteins were carefully collected. Protein concentrations were then determined using a BCA or Bradford assay to ensure equal protein loading for subsequent analyses. For the assay, we prepared a reaction mixture of NADPH and cytochrome c in PBS and added 100 µL of each termite extract to the wells of a 96-well plate containing this mixture. Control wells contained all reagents except the termite extract to account for background absorbance. The plates were incubated at 37°C for 30 minutes, and the absorbance at 550 nm was measured initially and at the end of the incubation period to calculate changes indicative of NADPH Oxidase activity. Activity was calculated by comparing the initial and final absorbance readings, adjusted for protein concentration and time, to express the activity in terms of nmol of cytochrome c reduced per minute per mg of protein. This setup allowed us to systematically compare enzyme activities across the nine groups, analyzing differences in oxidative stress or enzyme regulation due to various treatments.

Results and discussion

1. Selection of bioactive alkaloids against Termiticidal activity

Based on the data base sources, we identified total 11 alkaloids for the proposed study.

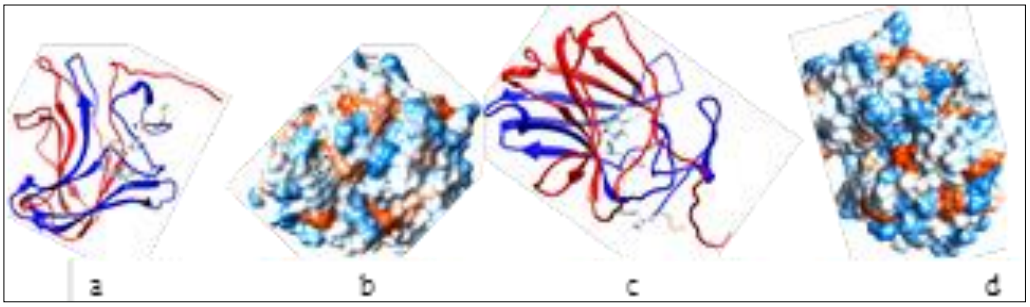
Selected Alkaloids (11): Nicotine, Anabasine, Caffeine, Pyrethrin, berberine, Aconitine, quinine, Scopolamine, Allethrin, Permethrin, Strychnine.

2. In-silico Virtual screening

Table 1: Results for promising ligands against Endo-1,4-β-Glucanase, Exo-1,4-β-glucanase or Cellulose 1,4-beta-cellobiosidase and Endo-1,4-β-Xylanase.

Ligand	Endo-1,4-β-Glucanase (PDB ID: 1NLR)		Cellulose 1,4-β-Cellobiosidase (3P6B)		Endo-1,4-β-Xylanase (PDB ID: 1XYF)	
	Dock score	Gibbs free energy (ΔG) in kCal/mol	Dock score	Gibbs free energy (ΔG) in kCal/mol	Dock score	Gibbs free energy (ΔG) in kCal/mol
Alkaloids						
Nicotine	- 6.24	- 788.23	- 7.48	- 754.89	- 6.39	- 754.89
Anabasine	- 7.59	- 822.36	- 6.53	- 711.05	- 5.96	- 778.14
Caffeine	- 8.55	- 895.23	- 7.99	- 758.34	- 8.07	- 856.39
Pyrethrin	- 9.69	- 951.37	- 10.27	- 992.36	- 9.31	- 955.31
berberine	- 6.38	- 741.51	- 7.38	- 858.21	- 6.38	- 698.23
Aconitine	- 7.21	- 844.69	- 6.89	- 741.69	- 6.93	-756.36
quinine	- 9.29	- 897.34	- 7.83	- 854.64	- 9.68	- 974.20
Scopolamine	- 7.11	- 772.19	- 9.55	- 946.92	- 9.58	- 969.31
Allethrin,	- 9.99	- 988.11	- 9.58	- 948.32	- 9.95	- 987.62
Permethrin	- 10.27	- 996.32	- 9.79	- 969.33	- 9.24	- 969.33
Strychnine	- 7.22	- 854.13	-00206.81	- 769.04	- 7.28	- 852.39

Docking results of Scopolamine



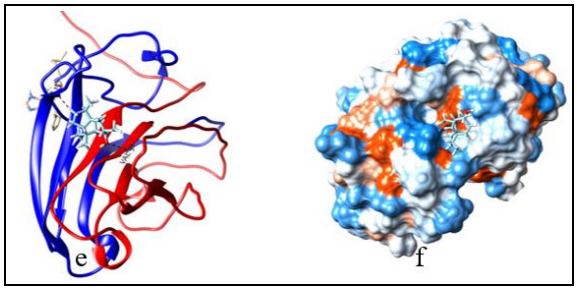


Figure 1: The figure illustrates In silico docking poses of scopolamine with carbohydrate-active enzymes. Panels a c and e showcase the predicted binding modes of scopolamine (depicted in stick representation) within the active sites of Endo-1,4-β-Glucanase (PDB ID: 1NLR), Cellulose 1,4-β-Cellobiosidase (PDB ID: 3P6B), and Endo-1,4-β-Xylanase (PDB ID: 1XYF) respectively, using a 3D wireframe interaction representation. Panels b, d, and f offer a surface view representation of the docking poses of scopolamine for Endo-1,4-β-Glucanase (PDB ID: 1NLR), Cellulose 1,4-β-Cellobiosidase (PDB ID: 3P6B), and Endo-1,4-β-Xylanase (PDB ID: 1XYF) respectively, highlighting the potential interactions between scopolamine and the enzyme surfaces. These visualizations provide valuable insights into the possible inhibitory mechanisms of scopolamine on these crucial enzymes involved in carbohydrate metabolism.

3. *In vitro* Anti-oxidant activity

3.1. SOD Inhibition activity of alkaloids

Table 2: results for SOD Inhibition activity of alkaloids

Conc in µg/mL	Pyrethrin	Quinine	Scopolamine	Allethrin	Permethrin
5	7.520 ± 0.56	8.22 ± 0.91	10.69 ± 1.13	8.57 ± 0.92	9.23 ± 1.04
10	16.320 ± 1.42	13.26 ± 1.45	32.69 ± 3.52	13.96 ± 1.13	21.36 ± 2.31
20	28.210 ± 3.03	24.81 ± 2.65	57.03 ± 5.61	21.35 ± 2.34	35.69 ± 3.67
40	40.690 ± 4.12	35.19 ± 3.31	79.69 ± 8.05	36.85 ± 3.87	56.32 ± 5.42
60	57.330 ± 5.94	50.39 ± 4.89	97.12 ± 9.64*	49.25 ± 4.71	82.34 ± 8.07
80	76.230 ± 7.57	66.27 ± 6.54	99.68 ± 9.84**	61.38 ± 6.23	93.57 ± 9.56*
100	91.270 ± 9.04*	75.09 ± 7.32	99.85 ± 10.03*	75.36 ± 7.62	99.68 ± 10.04*

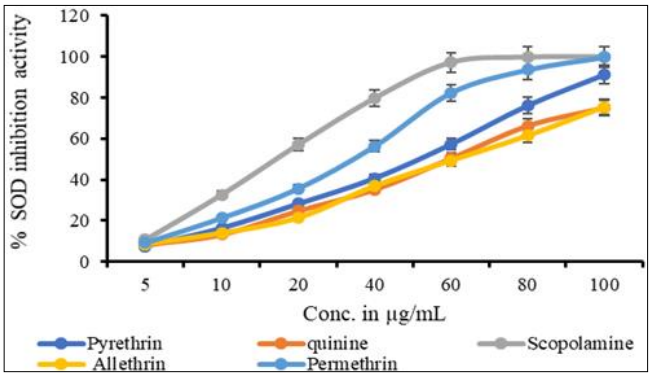


Figure 2: SOD Inhibition activity of alkaloids; Results were represented as mean ± SEM, n=3. ANOVA was used for data analysis; t-test was used to determine the statistical differences between groups. Superscript symbols * and # indicate significant differences observed from either Ascorbic acid or control group. * Significantly different from control group with $p<0.05$ and ** Significantly different from Ascorbic acid group with $p<0.01$.

4. Termites

Fig 3: Indian termite *Odontotermes obesus* grown in humid laboratory condition

5. Anti termite activity using No-Choice Assay

Table 3: Anti-termite activity at different concentrations of alkaloids vs different days

	1 mg/mL at day 01	1 mg/mL at day 5	1 mg/mL at day 10	1 mg/mL at day 14
Pyrethrin	0	3.69 ± 0.23	8.57 ± 0.74	21.36 ± 1.69
quinine	0	2.15 ± 0.17	6.88 ± 0.58	15.61 ± 1.36
Scopolamine	0	7.58 ± 0.69	18.33 ± 1.47	39.54 ± 4.52
Allethrin	0	4.69 ± 0.36	10.77 ± 1.22	25.69 ± 2.13
Permethrin	0	5.99 ± 0.39	15.07 ± 1.69	32.47 ± 3.98
Control	0	0	2.33 ± 0.32	3.97 ± 0.28
Fipronil	0	12.36 ± 1.26	21.99 ± 2.41	43.67 ± 4.85
	2 mg/mL at day 01	2 mg/mL at day 5	2 mg/mL at day 10	2 mg/mL at day 14
Pyrethrin	0	6.78 ± 0.52	15.28 ± 1.47	31.68 ± 2.69
quinine	0	3.85 ± 0.47	10.23 ± 1.33	21.47 ± 1.47
Scopolamine	3.65 ± 0.26	26.38 ± 1.69	51.37 ± 4.25	82.37 ± 6.35
Allethrin	0	13.22 ± 1.08	34.12 ± 2.69	63.15 ± 5.89
Permethrin	2.14 ± 0.18	19.33 ± 1.65	39.65 ± 3.55	72.33 ± 6.74
Control	0	0	2.69 ± 0.17	5.12 ± 0.42

Fipronil	3.23 ± 0.29	19.68 ± 1.58	52.38 ± 5.22	87.14 ± 7.96
	3mg/mL at day 01	3 mg/mL at day 5	3 mg/mL at day 10	3 mg/mL at day 14
Pyrethrin	3.74 ± 0.23	16.35 ± 1.23	29.33 ± 2.35	51.98 ± 4.78
quinine	2.86 ± 0.17	11.37 ± 0.36	24.13 ± 2.17	42.31 ± 3.56
Scopolamine	11.74 ± 0.69	45.69 ± 2.58	81.34 ± 5.69	100 ± 8.56
Allethrin	7.89 ± 0.58	19.65 ± 1.52	35.12 ± 3.22	87.12 ± 8.12
Permethrin	9.55 ± 0.74	38.56 ± 3.28	74.12 ± 5.84	100 ± 9.23
Control	0	0	4.78 ± 3.12	6.35 ± 0.54
Fipronil	12.36 ± 1.03	46.98 ± 4.13	81.37 ± 6.59	100 ± 8.54

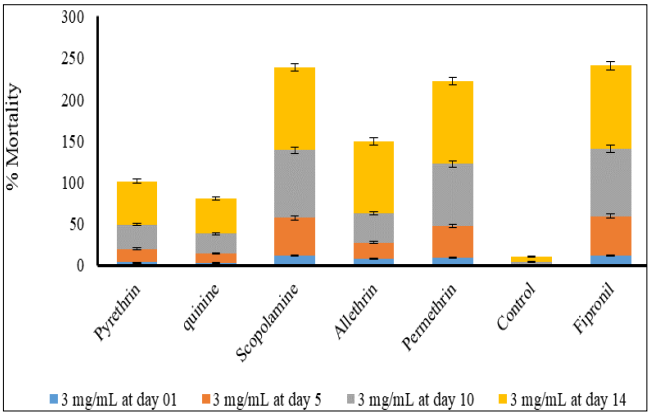


Figure 4: % Mortality of Indian termite in presence of various doses of alkaloids such as 3 mg/mL of selected compounds; Results were represented as mean ± SEM, n=3. ANOVA was used for data analysis; t-test was used to determine the statistical differences between groups. * Significantly different from control group with $p<0.05$ and ** Significantly different from Ascorbic acid group with $p<0.01$.

6. Enzymatic analysis

Table 4: Fold inhibition of enzyme activities in response to scopolamine and allethrin exposure. (a) Cellulase activity, (b) β-glucosidase activity, (c) 3-mercaptopyruvate sulfurtransferase (3MST) activity

Cellulase activity			
	Day 3	Day 5	Day 10
Control	1	1	1
Scopalamine	0.59 ± 0.06	0.38 ± 0.05	0.25 ± 0.04
Allethrin	0.67 ± 0.04	0.45 ± 0.07	0.38 ± 0.05
Beta glucosidase			
	Day 3	Day 5	Day 10
Control	1	1	1
Scopalamine	0.52 ± 0.04	0.31 ± 0.06	0.22 ± 0.04
Allethrin	0.69 ± 0.05	0.48 ± 0.03	0.41 ± 0.02
3 MST			
	Day 3	Day 5	Day 10
Control	1	1	1
Scopalamine	0.67 ± 0.08	0.48 ± 0.05	0.39 ± 0.02
Allethrin	0.81 ± 0.09	0.65 ± 0.03	0.53 ± 0.04

7. Results of ROS Activation studies

7.1. H_2DCFDA assay

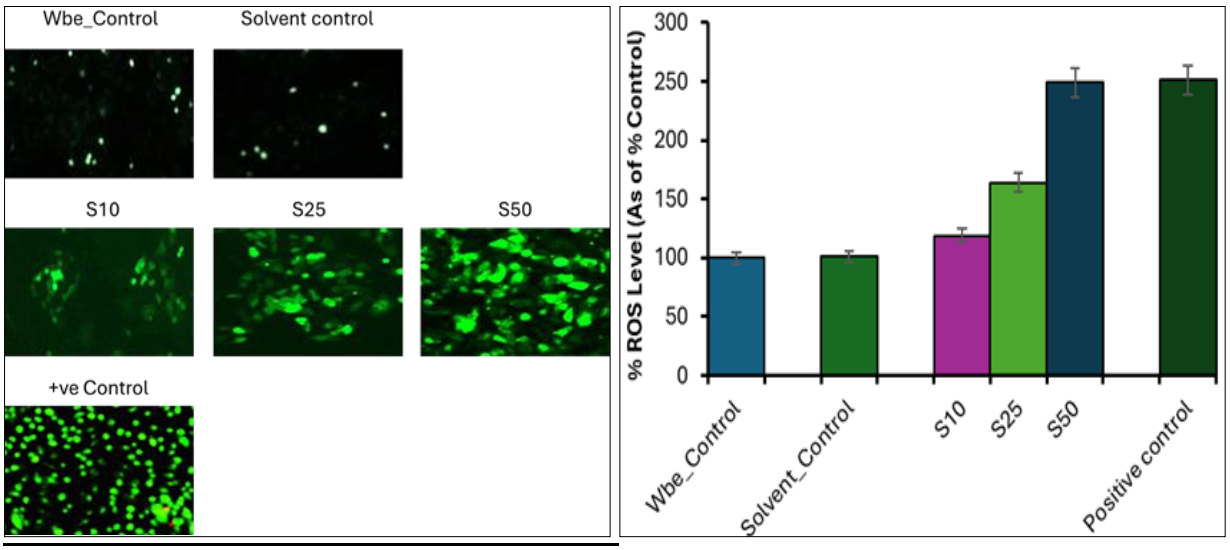


Figure 5: This figure shows the results of the H_2DCFDA assay for six groups. (a) Fluorescent images indicating ROS determination and (b) quantitative representation of ROS levels; Termites were divided into six groups of 20 termites each and assigned to normal control (G1), solvent control (G2), scopalamine treatments (S10: 10 mg/L, G3; S25: 25 mg/L, G4; S50: 50 mg/L, G5), and a positive control (fipronil, G6). The H_2DCFDA assay was performed to measure the relative levels of reactive oxygen species (ROS) in the termites. Higher fluorescence intensity indicates higher ROS levels.

7.2. NADPH Oxidase assay % ROS levels NADPH Oxidase Activity (nmol/min/mg protein)

Table 5: % ROS levels, NADPH oxidase activity of Scopolamine

	% ROS levels	**NADPH Oxidase Activity (nmol/min/mg protein)**
Wbe_Control	100	0.53 ± 0.03
Solvent_Control	101 ± 9.66	0.52 ± 0.03
S10	119 ± 8.57	0.77 ± 0.06
S25	164 ± 11.31	0.93 ± 0.08
S50	249 ± 15.62	1.37 ± 0.09
Positive control	251 ± 14.75	1.42 ± 0.08

This study identified scopolamine as an effective anti-termite agent against *Odontotermes obesus* using in silico screening. Scopolamine induces reactive oxygen species (ROS), causing oxidative stress that damages termite cells, and inhibits crucial enzymes like Endo-1,4-β-glucanase, Exo-1,4-β-glucanase, and Endo-1,4-β-Xylanase essential for termite digestion. *In vitro* assays confirmed significant enzyme activity reduction. Scopolamine's dual action ROS activation and enzyme inhibition makes it a potent alternative to synthetic pesticides, potentially offering lower environmental and health risks. Further research is needed to optimize delivery methods and evaluate its efficacy in field trials.

Conclusion

This study demonstrates scopolamine's effectiveness against *Odontotermes obesus* through ROS activation and enzymatic inhibition. In silico screening and *in vitro* assays confirmed scopolamine's high binding affinity to key termite enzymes, leading to significant reductions in enzymatic activity vital for digestion. The dual action ROS-induced cellular damage and enzyme inhibition offers a potent, multi-faceted termite control strategy that reduces the likelihood of resistance. Scopolamine shows promise as a natural, eco-friendly alternative to synthetic pesticides. Future research should focus on optimizing delivery methods and exploring synergistic combinations for enhanced efficacy in integrated pest management.

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