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RESEARCH ARTICLE

Cuticular wax composition and anatomical features of *Cyperus rotundus* L. in relation to herbicide uptake and translocation

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Abstract

Cyperus rotundus is one of the sedges that is widely considered to be the world's worst weed and its control is troublesome due to a variety of factors. The objective of this research was to analyze the chemical composition as well as its morphology on leaves. It is important to know the barrier for absorption and translocation of foliar applied chemicals to control this weed effectively. Microscopic and SEM analysis revealed a thick epicuticular layer on the adaxial surface and thin layer on the abaxial surface, heterogenous wax coverage. While, GC-MS profiling identified major chemical constituents including pyrans, fatty acids, sesquiterpenes and nitrogenous compounds. Wax content ranged from 72.62 to 103.92 µg cm^{-2}. that contributed to the formation of a nearly impermeable membrane in leaves that aids stress tolerance and act as a transport barrier for foliar applied chemicals. Formulation of glyphosate with appropriate surfactants, particularly CTAB at higher ratios (1:2) of herbicide-to-surfactant, significantly improved translocation to primary, secondary and tertiary tubers. The findings highlight the critical role of cuticular wax composition in herbicide resistance and demonstrate the potential of adjuvant selection and usage for optimal herbicide delivery as well as development of more effective weed control measures against *C. rotundus* and similar perennial weed species.

Keywords: *Cyperus rotundus*; cuticular composition; cuticular wax; fatty acids; SEM image

Introduction

Plant surfaces are encrusted with waxes produced by the epidermal cells. The cuticle is a hydrophobic layer that covers the extracellular surface of plant leaves. Structurally, the cuticle comprises a matrix of cutin, an insoluble polyester composed of hydroxylated fatty acids and glycerol, embedded with and overlaid by a complex mixture of cuticular waxes (1, 2). The composition of these waxes varies among plant species and typically includes organic solvent-soluble lipids such very long-chain fatty acids and their derivatives (e.g., aldehydes, primary alcohols and alkanes), as well as triterpenoids in certain species (3, 4). These waxes exhibit high crystallinity, chemical inertness and strong hydrophobic properties (5).

Cuticular waxes play diverse roles in plant defense and physiology. They may protect plants from the environment by acting as a barrier against water, chemicals, UV, diseases and pests (6). Notably, plants adapted to arid environments or subjected to prolonged stress often possess thicker wax layers than species from temperate regions, indicating an adaptive response to water deficit (7-9). While the wax content partially determines the functional efficacy of the cuticle, the chemical composition of the wax layer is equally critical in influencing permeability and absorption dynamics. However, the relationship among cuticular wax content, composition and leaf surface morphology remains insufficiently understood.

Cyperus is one of the largest genera in the Cyperaceae family, comprising approximately 650-700 species, among which 220 are considered as noxious weeds (10, 11). *Cyperus rotundus* is a cosmopolitan species, thriving particularly in tropical and subtropical regions and is regarded as one of the world's most problematic weeds, causing yield losses in at least 52 crops across 92 countries (10). Despite prolific production of tubers and rhizomes, viable seed formation is minimal and sexual reproduction is largely absent. Propagation occurs asexually through rhizomes, basal bulbs and tubers, which can remain viable for 2-3 years due to dormancy mechanisms, especially when tubers are detached from the mother plant (12). The irregular emergence of this species is influenced by factors such as apical dominance (13), the presence of germination inhibitors, environmental conditions and burial depth (14).

Morphologically, *Cyperus rotundus* are dark green, linear and grooved on the upper surface, lacking ligules or auricles (15). The leaf surface is waxy and its mesophyll is made up of densely packed, thin walled cells with few vacuoles. The composition and quantity of epicuticular wax in older leaves may significantly affect herbicide absorption and translocation compared to younger tissues (16). Despite its agronomic importance, limited information exists regarding the structural features, cuticular properties and wax composition of *C. rotundus*, even though its foliar surfaces are known to support multiple pathways for chemical uptake and translocation.

A detailed understanding of leaf surface morphology and cuticular composition is therefore crucial for developing effective weed management strategies. Since the efficiency of foliar applied herbicides largely depends on their penetration through the cuticular barrier, strategies aimed at weakening or removing this barrier may enhance herbicide efficacy. In this context, the present study was undertaken to investigate the structure and composition of the cuticular wax layer in *C. rotundus* leaves under laboratory conditions.

Materials and Methods

Study location

The laboratory investigation was conducted at the Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, India. The site is situated in the western agro-climatic zone of Tamil Nadu at 11° N latitude and 77° E longitude, with an elevation of 426.8 m above mean sea level. Leaf samples were collected in bulk from healthy *C. rotundus* plants growing at the Eastern Block Farm of the Department of Agronomy.

Sample fixation and sectioning

Healthy plants and normal organs were chosen with care. Selected leaf samples were fixed in FAA solution comprising formalin (5 mL) + acetic acid (5 mL) + 70 % ethanol (90 mL). After 24 hr, samples were dehydrated through a graded series of tertiary butyl alcohol (TBA) following the protocol outlined by Sass (17). Dehydrated specimens were infiltrated with molten paraffin wax (melting point 58-60 °C) until complete saturation with TBA and then embedded into paraffin blocks for microtome sectioning.

Structural and anatomical observations

Paraffin embedded samples and sectioned at approximately 10 µm thickness using a rotary microtome (Medite M530). Sections were dewaxed and stained with Toluidine blue following O'BRIEN method, which enables differential staining of lignified tissues (18). Observations were made using a Leica DM LA light microscope and photomicrographs were captured using a Nikon lab photo 2 imaging unit. Polarized light microscopy was employed to visualize lignified cells due to their birefringent properties, which appear bright against a dark background.

Scanning Electron Microscopy (SEM)

Fresh leaf tissues were sectioned, mounted on metal stubs and coated with a thin layer of gold using a sputter coater to protect the samples from electron beam damage. The prepared samples were observed under a scanning electron microscopy (SEM, Quanta 250, FEI, Netherlands) and high-resolution images were captured digitally at various magnifications.

Extraction of cuticular waxes

Cuticular waxes were extracted from 30 fresh leaves using a Soxhlet apparatus and methanol over a 6 hr period. The entire wax mixture including epicuticular and intracuticular waxes from both leaf surfaces was collected. The solvent was evaporated and the difference in leaf weight before and after extraction (following drying at 105 °C) was used to quantify the total cuticular wax content. Wax yield was expressed as a function of leaf surface area and dry weight basis (mg/cm^2), with surface area estimated by digital imaging.

Cuticular wax composition

The *Cyperus rotundus* leaves were collected and dried under shade to make powder. The leaf powder was serially extracted in methanol by using the Soxhlet apparatus. The cycles of methanol were run till complete defatting was obtained. The solvent extracts of *Cyperus rotundus* leaf was filtered by using Whatman No. 41 filter paper. The filtrate is used for further analysis.

The leaf extract (obtained as methanol extracts from 5 g leaf powder) was analysed using gas-chromatography mass spectrometry (GC-MS). GC-MS analysis of the sample was carried out using Perkin Elmer Clarus 680 gas chromatographic instrument equipped with a mass spectrometer detector (Clarus 600 model) and an Elite-5 MS (30.0 m, 0.25 mm ID, 250 µmdf) column was used. Helium was used as the carrier gas and the temperature programming was set with the initial oven temperature at 40 °C and held for 3 min and the final temperature of the oven was 480 °C with a rate at 10 °C [min. sup^{-1}]. The sample of 2 µL was injected with a split less mode. Mass spectra were recorded over 35 - 650 AMU (atomic mass unit) range with electron impact ionization energy 70 eV. The total running time for a sample was 45 min. Quantitative determinations were made by relating respective peak areas to TIC (Total Ion Chromatogram) areas from the GC-MS spectral library (Wiley, NIST) and by comparing their fragmentation profiles with published data (19, 20). Two replicates were made per extract.

Herbicide Translocation Assay

Pot culture experiment was conducted to assess the translocation efficiency of herbicides and evaluate the role of surfactants in overcoming transport barriers. Medium-sized plastic pots (approximately 5 kg capacity) were used, each filled with a soil mixture comprising red soil, sand and vermicompost in a 2:1:1 ratio. To ensure uniformity in growth, *C. rotundus* tubers were sorted based on size and weight prior to planting. Ten tubers were sown in each pot. They were watered on a regular basis and monitored.

Various surfactants such as polyethylene glycol (PEG), polypropylene glycol (PPG), glycerine, Tween 20, Tween 80, hydrogen peroxide (H_2O_2), cetyl trimethyl ammonium bromide (CTAB) were externally mixed with a commercial glyphosate formulation at two ratios (1:1 and 1:2; glyphosate: surfactants). A 1 % solution of each mixture was prepared for foliar application. A treatment with glyphosate at 1000 ppm combined with 1 % ammonium sulphate considered as the control. The herbicide solutions were sprayed at 15 days after sowing (DAS) to examine the influence of different surfactants on translocation of glyphosate to the tubers.

Glyphosate translocation in tuber tissues

One gram of tuber tissue was homogenized with 20 mL of water and 5 mL of dichloromethane in a 50 mL tube and agitated for 60 min using a mechanical shaker. One milli litre of methanol was added and vortexed for 15 min. The mixture was centrifuged at 3000 rpm for 15 min and a 1 mL aliquot was withdrawn. To this, 0.5 mL of 5 % ninhydrin and 0.5 mL of 5 % sodium molybdate were added. The reaction mixture was sealed and incubated in a water bath at 85-95 °C for 12 min. After cooling, the mixture was transferred to a 5 mL volumetric flask and diluted with distilled water. The development of Ruhemann's purple was quantified at 570 nm using a UV-Vis spectrophotometer. Standards and blank controls were subjected to the same procedure. Glyphosate detection was based on its reaction with ninhydrin, forming a Ruhemann's purple complex via nucleophilic substitution, decarboxylation, hydrolysis and condensation mechanisms (21).

Results

The adaxial surface of the leaves from *Cyperus rotundus* was dark green with a lack of trichomes, while the abaxial surface was lighter with numerous stomata. Fig. 1 depicts an exemplary *Cyperus rotundus* leaf cross-section observed using optical microscopy, showing the thick cuticular membrane covering the adaxial leaf side deposited on the epidermal cell layer with between-cell indentations. The abaxial epidermis was made up of wide radially oblong epidermal cells with a thin cuticle (Fig. 2), while the adaxial epidermis was made up of small square cells with thick cuticles. A row of circular vascular bundles was found on the upper part of the abaxial epidermis.

The epicuticular wax coverage of the sedge leaf is dense and non-uniform. The upper layer had a thick epicuticular layer with crystalline structures and the lower surface was thin. The wax crystals varied in size and form (Fig. 3). A very thick layer of the crust was not found on the lower surface. The amorphous wax layer is attributed to the prevalence of aliphatic compounds and fatty acids. Similar films were reported (22). A report describes that alkanes form a plain layered structure while molecules with terminal polar groups such as fatty acids and alcohols formed a double layer of crystalline structure (23). Crusts have been reported from all major groups of plants including *Cynanchum sarcostemma*, *Copernicia cowellii* and *Buxbaumia viridis* (24, 25).

Cyperus rotundus leaves had a wax layer of 73 - 104 µg cm^{-2} from five replications (Table 1). Although epicuticular wax analysis has been studied extensively in dicotyledonous plants (26, 27), there are only a few studies on monocotyledonous plants like Hordeum, Wheat, *Sorghum* (28), *Alium*, *Gloriosa* and *Strelitzia* (24) and none on sedges.

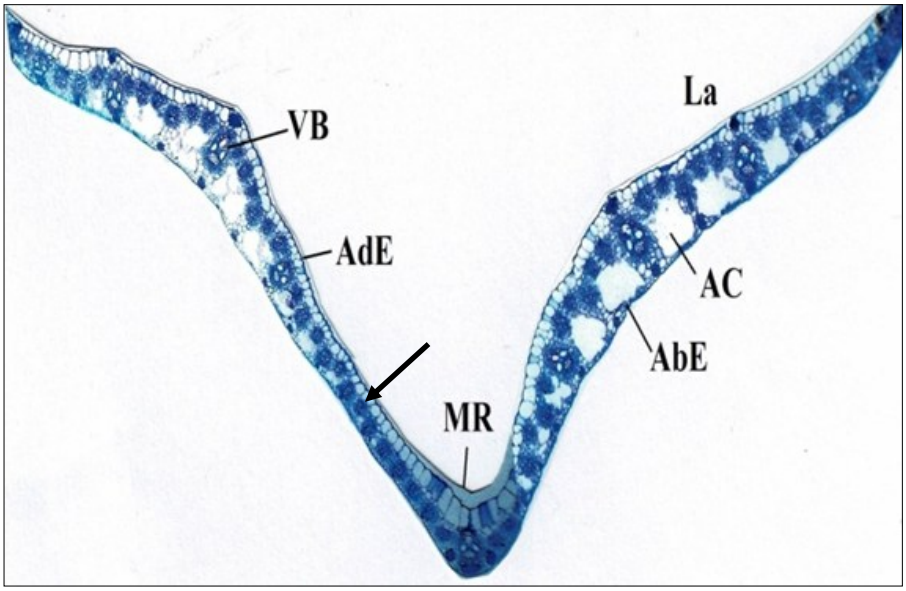


Fig. 1. Microscopic photographs of a cross section of *Cyperus rotundus* leaf (entire leaf margin under 4x).

Legends: VB - Vascular bundle, AdE - Adaxial Epidermis, MR - Midrib, AbE - Abaxial epidermis, AC - Air chamber, La - Lamina, Arrows indicate the prominent cuticular layer

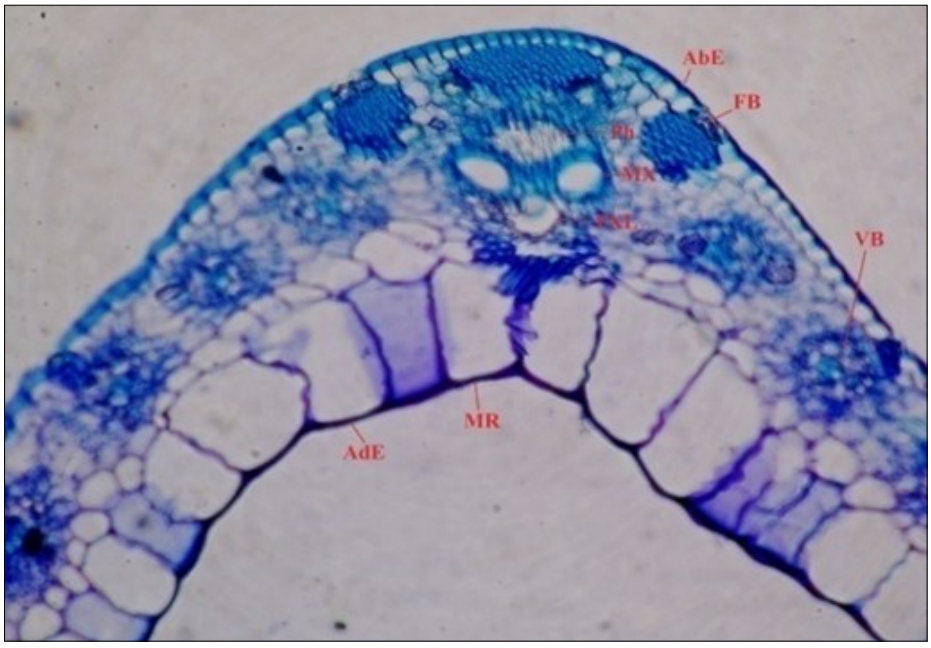
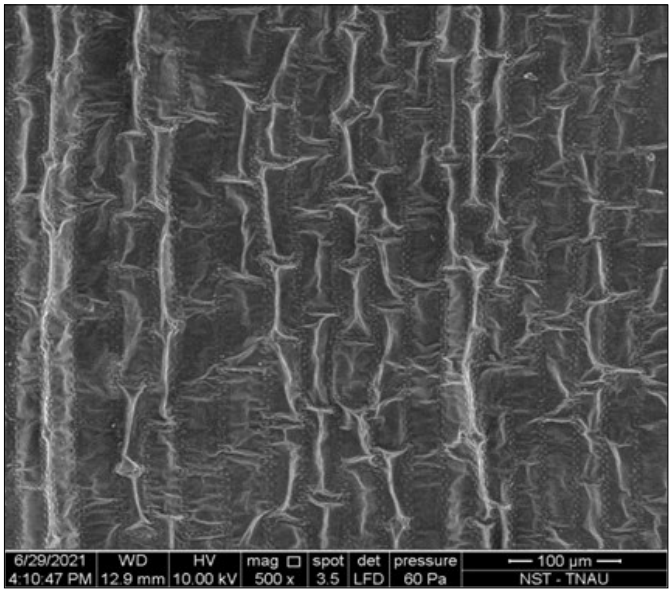
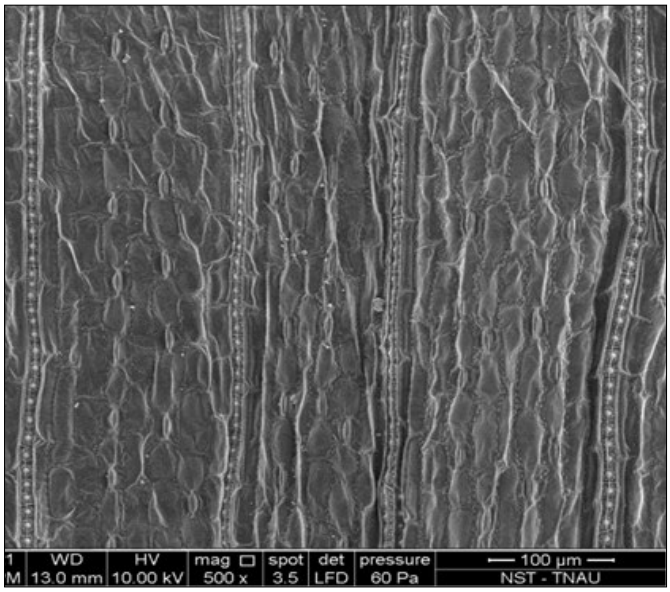


Fig. 2. Enlargement of leaf midrib under 20x.

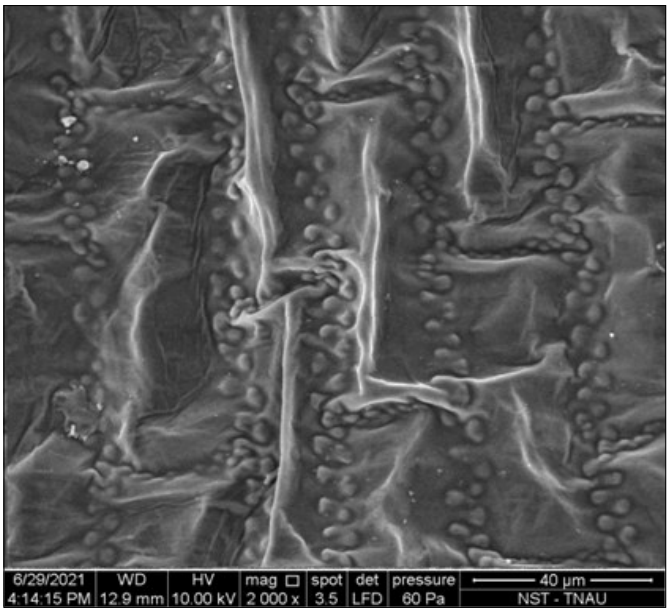
Legends: VB - Vascular bundle, AdE - Adaxial Epidermis, MR - Midrib, AbE - Abaxial epidermis *Arrows indicate the thick cuticular layer in adaxial surface



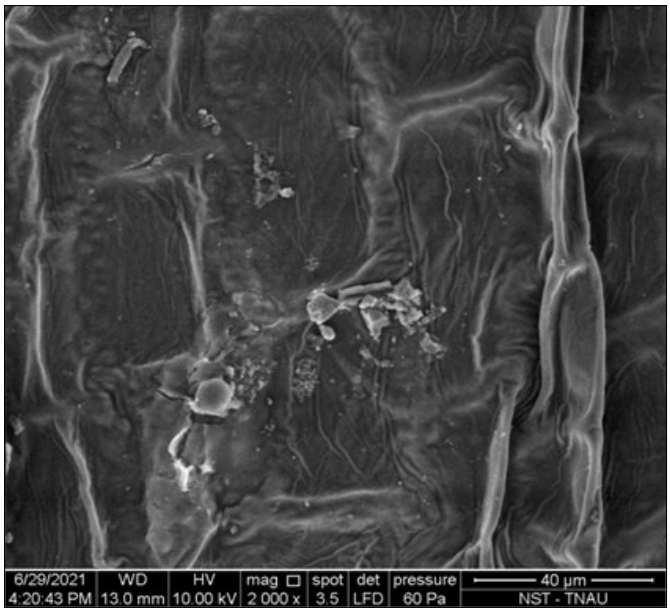
A. Thick layer of wax in upper surface



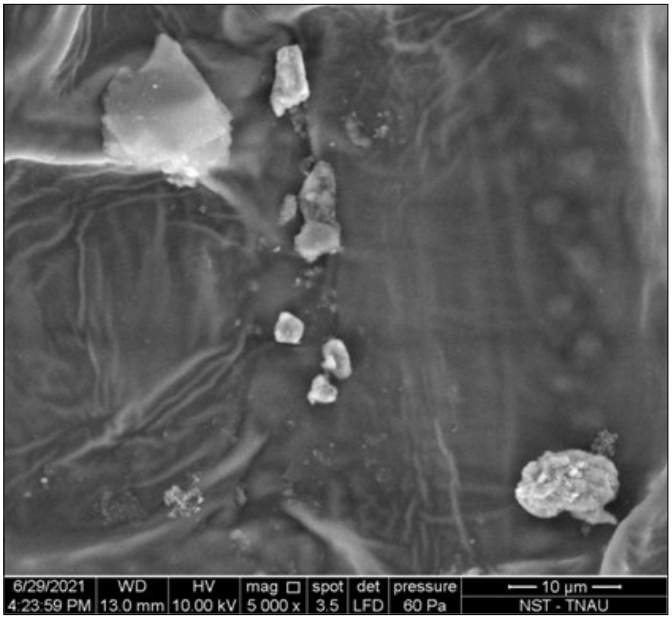
B. Thin layer of wax in lower surface with stomata



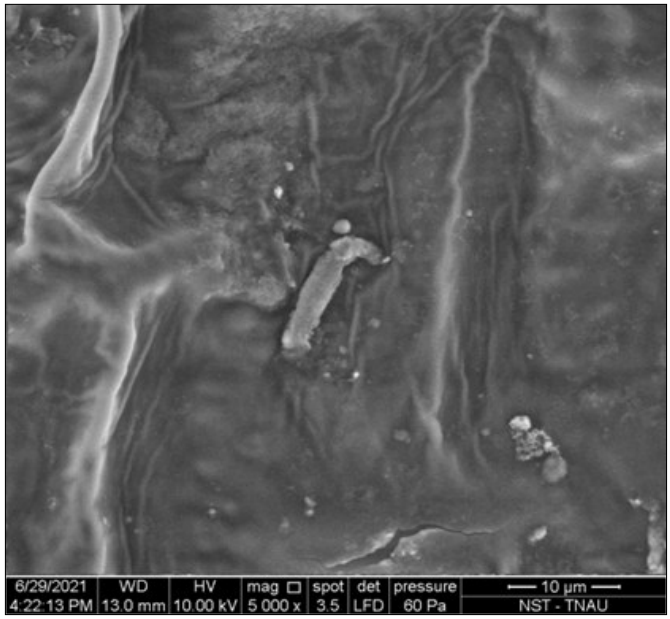
C. Wax layer in upper surface



D. Wax layer with irregular crystals



E. Wax layer with irregular crystals



F. Dense wax deposition and film on the upper surface

Fig. 3. Wax layer presence of *Cyperus rotundus* leaf under SEM.

Table 1. Cuticular wax content (µg cm^{-2}) of *Cyperus rotundus* leaves

No. of samples	Leaf area of samples (cm^2)	Weight difference (mg)	Wax content (µg cm^{-2})
1	20.4	2.12	103.92
2	22.8	2.01	88.16
3	26.3	1.91	72.62
4	24.6	2.17	88.21
5	19.5	1.78	95.90
Mean ± SE	**22.72 ± 1.26**	**1.99 ± 0.071**	**89.76 ± 5.18**

Identification of the cuticular composition

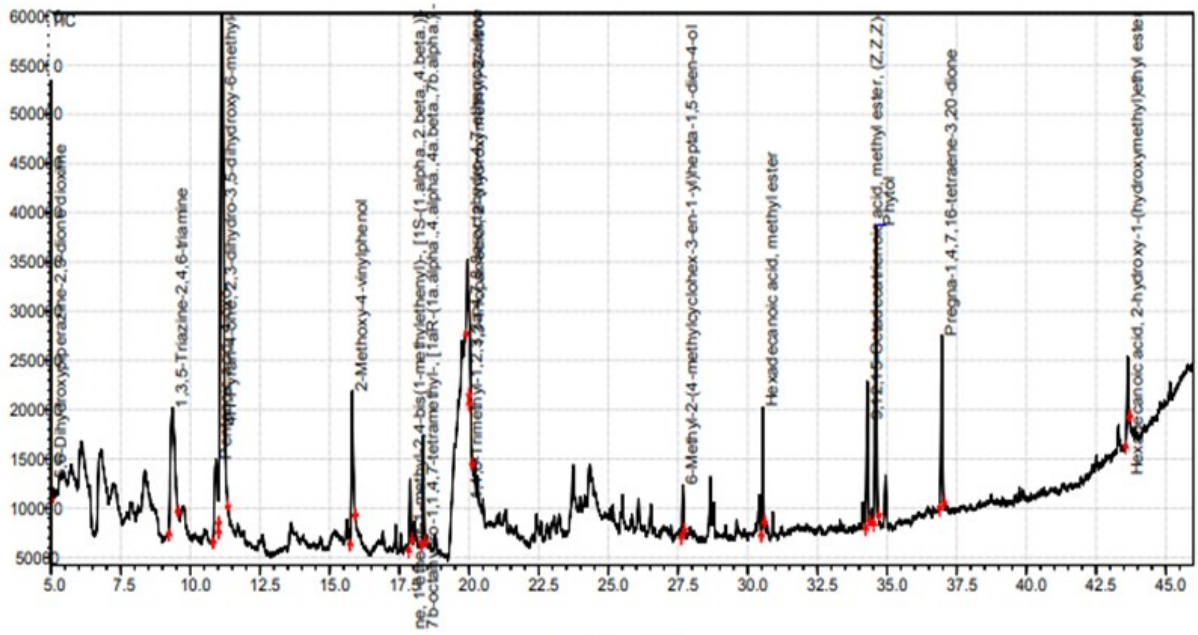
Interpretation on the mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having more than 62000 patterns. The mass spectrum of the unknown component was compared with spectrum of known components stored in the NIST library. The GC-MS was used to make quantitative determinations by relating distinct peak areas to TIC areas. The name, molecular weight, retention time and peak area percentage of the test materials was determined.

The present study was carried out on the *Cyperus rotundus* leaf to determine the presence of cuticular wax composition. In the GC-MS analysis, various compounds were identified in methanol extract. The peak area and molecular formula were used to identify the chemical components (Fig. 4). The compound 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl with RT 11.125 has the highest peak area of 40.25 %, followed by 1,3,5-Triazine-2,4,6-triamine (melamine) with RT 9.364 has a peak area of 10.48 % and Phytol (2-Hexadecen-1-ol) with RT 34.598 has a peak area of 9.94 %.

Table 2 lists the analysed major compounds of methanol extract with its retension time, molecular formula, group and peak area percentage. With regards to groups, pyrans represented 40 % of the total peak area followed by sesquiterpenes (10 %) and diterpenes (10 %). The aliphatic compounds constituted an abundant group including fatty acids (15 % of the compounds), phenols (6 %) and secondary alcohols (3 %) which together accounted for 24 % of the total compounds. Among the fatty acids, C_{19}, C_{17} and C_5 acids were found, saturated and unsaturated acids represented on average 10 % and 5 % of the total fatty acids. Nitrogen containing compounds comprised 10.48 %, steroids 6 % of all compounds and 5, 6- dihydroxy piperazine-2,3-dione (dioxime) was present in minor amounts.

Effect of surfactant-formulated glyphosate on herbicide translocation

Glyphosate translocation was quantified in primary, secondary and tertiary tubers at 15 days after herbicide application (Table 3). Among the two ratios of surfactant-herbicide formulation, 1:2



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Name
1	5.021	5.010	5.030	47236	0.40	54873	2.42	0.86	5,6-Dihydroxypiperazine-2,3-dione dioxime
2	9.364	9.230	9.560	1250947	10.48	118347	5.23	10.57	1,3,5-Triazine-2,4,6-triamine
3	10.925	10.825	11.015	549709	4.60	74331	3.28	7.40	Pentanoic acid, 4-oxo-
4	11.125	11.015	11.345	4806523	40.25	620806	27.41	7.74	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6
5	15.808	15.730	15.915	665305	5.57	142672	6.30	4.66	2-Methoxy-4-vinylphenol
6	17.891	17.835	17.955	207435	1.74	67417	2.98	3.08	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-me
7	18.358	18.300	18.435	355630	2.98	109753	4.85	3.24	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-o
8	19.947	19.890	20.005	508383	4.26	106567	4.71	4.77	1,4,6-Trimethyl-1,2,3,3a,4,7,8,8a-octahydro-4,
9	20.025	20.005	20.120	302318	2.53	101283	4.47	2.98	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-
10	27.672	27.620	27.735	151929	1.27	49806	2.20	3.05	6-Methyl-2-(4-methylcyclohex-3-en-1-yl)hept
11	30.540	30.485	30.605	372174	3.12	123983	5.47	3.00	Hexadecanoic acid, methyl ester
12	34.294	34.230	34.380	553750	4.64	145885	6.44	3.80	9,12,15-Octadecatrienoic acid, methyl ester, (Z
13	34.598	34.520	34.700	1187404	9.94	299313	13.22	3.97	Phytol
14	36.965	36.890	37.055	687688	5.76	173114	7.64	3.97	Pregna-1,4,7,16-tetraene-3,20-dione
15	43.639	43.565	43.710	294883	2.47	76686	3.39	3.85	Hexadecanoic acid, 2-hydroxy-1-(hydroxymet
				11941314	100.00	2264836	100.00		

Fig. 4. GC-MS chromatogram of methanol leaf extract.

Table 2. Chemical composition (% of all chromatograms peak areas) of leaves

Compound	Formula	R.T	Peak area (%)
Pyrans			
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	11.125	40.25
Sesquiterpenes			
Cyclohexane,1-ethenyl-1-methyl-2,4-bis(1methylethyenyl)	$C_{15}H_{24}$	17.891	1.74
1H-Cycloprop[e]azulene	$C_{15}H_{24}$	18.358	2.98
1,4,6-Trimethyl-1,2,3,3a,4,7,8,8a-octahydro-4,7ethanoazulene	C_{15} H_{24}	19.947	4.26
6-Methyl-2-(4-methylcyclohex-3-en-1-yl) hepta- 3 Cyclohexane -1-propanol	$C_{15}H_{24}O$	27.672	1.27
Diterpenes			
Phytol (2-Hexadecen-1-ol)	$C_{20}H_{40}O$	34.598	9.94
Fatty acids			
Saturated			
Pentanoic acid, 4-oxo-Levulinic acid	$C_5H_8O_3$	10.925	4.60
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	30.540	3.12
Hexadecanoic acid	$C_{19}H_{38}O_4$	43.639	2.47
Unsaturated			
9,12,15-Octadecatrienoic acid	$C_{19}H_{32}O_2$	34.294	4.64
Nitrogen containing compounds			
1,3,5-Triazine-2,4,6-triamine (melamine)	$C_3H_6N_6$	9.364	10.48
Phenols			
2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	15.808	5.56
Secondary alcohol			
1,3-Propanediol,2-(hydroxymethyl)-2-nitro-Isobutylglycerol	$C_4H_9NO_5$	20.025	2.53
Steroids			
Pregna-1,4,7,16-tetraene-3,20-dione	$C_{21}H_{24}O_2$	36.965	5.76
Others			
5,6-Dihydroxypiperazine-2,3-dione (dioxime)	$C_4H_8N_4O_4$	5.021	0.40

Table 3. Effect of glyphosate with various surfactants at two ratios (1:1, 1:2) on the translocation of glyphosate to the *C. rotundus* tubers

Treatments	Glyphosate in tubers (ppm) - 1:1 ratio			Glyphosate in tubers (ppm) - 1:2 ratio		
	1° tubers	2° tubers	3° tubers	1° tubers	2° tubers	3° tubers
T_1 - Glyphosate + PEG (1:2)	11.29	8.53	2.97	15.71	8.40	3.99
T_2 - Glyphosate + PPG (1:2)	10.95	7.83	2.05	12.28	6.76	2.06
T_3 - Glyphosate + Glycerin (1:2)	8.96	5.59	1.52	11.26	6.18	1.53
T_4 - Glyphosate + Tween 20 (1:2)	11.04	8.47	3.01	15.01	9.61	4.88
T_5 - Glyphosate + Tween 80 (1:2)	10.89	6.99	2.45	13.30	7.23	3.84
T_6 - Glyphosate + H_2O_2 (1:2)	8.58	4.97	2.02	15.26	8.14	4.23
T_7 - Glyphosate + 2 % CTAB (1:2)	15.35	9.30	3.12	17.22	11.80	6.06
T_8 - Glyphosate at 1000 ppm + 1 % Ammonium sulphate	9.54	4.37	1.23	9.37	4.12	1.06
SEd	**0.30**	**0.35**	**0.31**	**0.68**	**0.38**	**0.21**
CD(P=0.05)	**0.64**	**0.74**	**0.65**	**1.46**	**0.86**	**0.44**

ratios resulted higher translocation than 1:1 ratio formulation. Amid the treatments, T_7 (glyphosate + 2 % CTAB at 1:2 ratio) recorded a higher amount of glyphosate translocation in all the tubers, with the values of 17.22 ppm in primary tubers, 11.80 ppm secondary tubers and 6.6 ppm in tertiary tubers. In contrast, T_3 (glyphosate + glycerine at 1:2 ratios) showed the lowest translocation, followed by T_2 (glyphosate + PPG at 1: 2 ratio).

Discussion

In this study, *Cyperus rotundus* leaves had a wax layer of 73 - 104 µg cm^{-2}. The wax covering on most leaves varied between 10 and 100 µg cm^{-2} (29). Assuming a wax density of approximately 1 g cm^{-3}, this range corresponds to a wax layer thickness between 10-100 nm. Variation in wax deposition may be attributed to plant organ specificity and developmental stages (30). Cuticular wax serves as a crucial barrier against desiccation and environmental stress (31, 32). In this study, wax accumulation may explain reduced absorption and translocation of the foliar applied herbicides. Because *Cyperus rotundus* is a perennial weed and its effective management is important for obtaining more yield in crops.

Plant cuticular waxes denote a wide range of aliphatic compounds (33) that can be extracted using organic solvents (34) and analysed via GC-MS, a standard tool for profiling wax constituents (35). Previous reports on *Cyperus* species are limited, with reporting only trace amounts of triterpenoids in *Cyperus pongorei* using brief chloroform extraction (22).

In contrast, the present study identified a range of compounds including pyrans (mainly 4H-Pyran-4-one, 2, 3-dihydro -3, 5-dihydroxy-6-methyl), sesquiterpenes, diterpenes and various fatty acids (both saturated and unsaturated) and nitrogen containing compounds (Table 2 & Fig. 5). Long-chain aliphatic compounds (C_3 to C_{21}) were prevalent, corroborating findings from Jetter *et al*. (3), who observed that linear long-chain aliphatic compounds, including pentacyclic triterpenoids, can dominate wax profiles in certain plant taxa. Cuticular composition is dynamic and varies with plant species, organ, developmental stage, season, location and other environmental factors (36, 37). For example, wax esters were found to be dominant in *Musa paradisiaca* (38), *Oryza sativa*, *Hordeum vulgare* (39) and *Zea mays* (28).

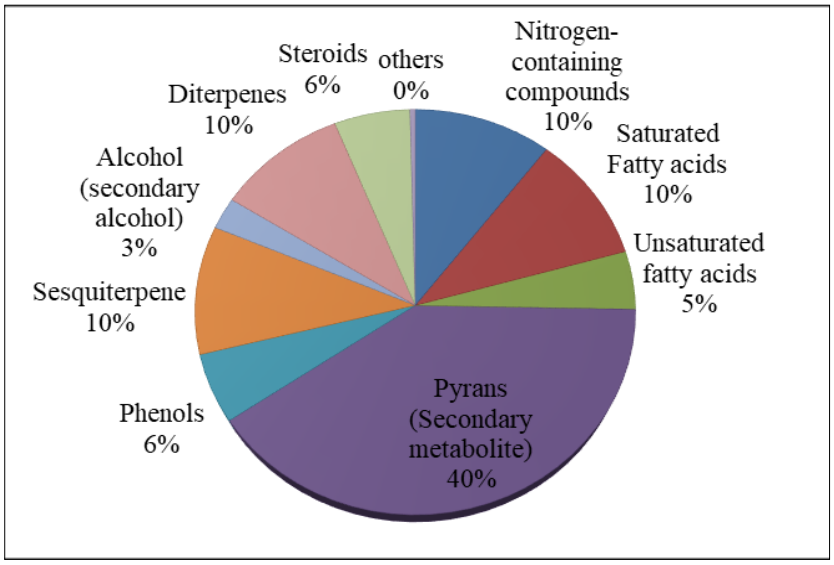


Fig. 5. Cuticular composition of *Cyperus rotundus* leaf.

The present study was revealed that the presence of various secondary metabolites and phytocompounds, in those pyrans was found to be higher. They are known for their pharmacological potential, including antimicrobial and neuroprotective properties. However, their primary function in this context may be structural, contributing to a hydrophobic cuticular matrix that resists foliar penetration of water and chemicals (40). This wax molecule is largely hydrophobic, composed of methyl and methylene groups, the cuticle acts as primary barrier to chemical infiltration. This not only impedes the entry of agrochemicals but also helps prevent pathogen ingress. Therefore, in a subsequent phase of this study, glyphosate translocation to tubers was investigated using different surfactants-assisted formulations.

Effect of glyphosate formulated with different surfactants on translocation of herbicide to the tubers

Adjuvants are critical additives in herbicide formulations, enhancing their efficacy by improving spray retention, droplet adhesion and cuticular penetration (41). Among these, surfactants have demonstrated substantial effectiveness in herbicide delivery. These compounds alter the surface properties of spray solutions, improving their spreading, wetting, emulsifying and dispersing capabilities (42). As surface-active agents, surfactants reduce surface tension, thereby enhancing the contact between spray droplets and plant leaf surfaces Curran, McGlamery (43). This improved interaction facilitates greater penetration of herbicides through the cuticle. Consequently, the inclusion of surfactants can significantly accelerate herbicide movement into plant tissues (42). The epicuticular wax layer and its composition of the target weed influences herbicide performance and efficacy (44).

Based on these principles, several surfactants were selected for formulation with glyphosate: Poly ethylene glycol (PEG), Poly propylene glycol (PPG), Glycerine, Tween 20, Tween 80, Cetyl Trimethyl Ammonium Bromide (CTAB) and Hydrogen peroxide (H_2O_2). They were combined with glyphosate in 1:1 and 1:2 ratios, and 1 % solutions were used for application. The formulation T_7 (glyphosate + 2 % CTAB at 1:2 ratio) showed superior glyphosate translocation to all the tubers (Fig. 6). This effect may be attributed to enhanced wax degradation, facilitating glyphosate absorption and systemic transport. In contrast, T_3 (glyphosate + glycerine at 1:2 ratios) showed the lowest translocation, followed by T_2(glyphosate + PPG at 1: 2 ratio). Conversely, minimal wax degradation, such as glycerine were less effective, potentially leading to suboptimal weed control due to tubers. Overall, glyphosate formulations with surfactants in 1:2 ratios demonstrated superior performance in promoting translocation to underground tubers compared to 1:1 ratios.

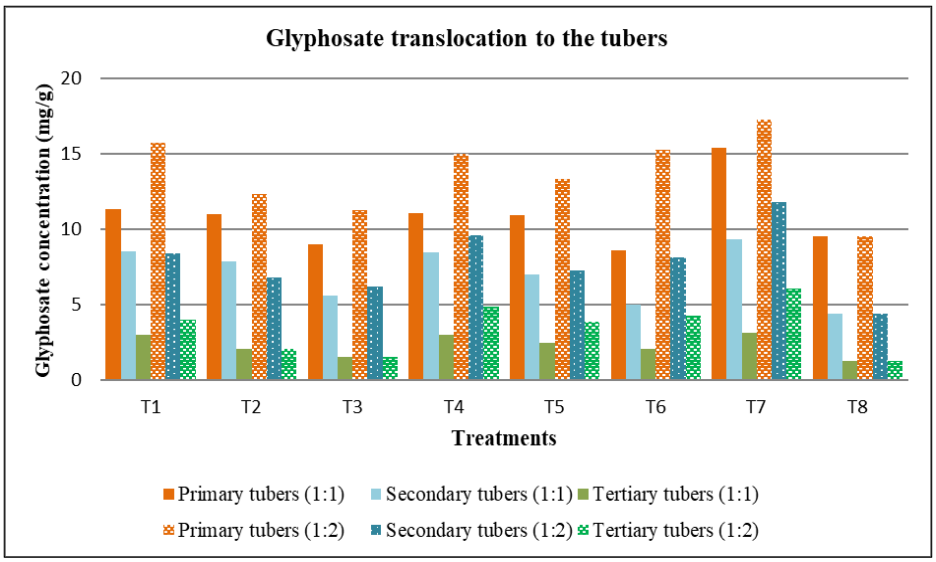


Fig. 6. Effect of surfactant-formulated glyphosate on herbicide translocation to the tubers.

These findings highlight the significance of understanding epicuticular wax content and cuticular composition for selecting appropriate surfactants to be used with herbicides, thereby facilitating cuticular disruption, enhancing herbicide uptake and improving overall weed management efficacy.

Conclusion

This study elucidated the anatomical and chemical defenses of *Cyperus rotundus*, emphasizing the role of cuticular waxes in limiting herbicide absorption and translocation. The presence of a thick, chemically complex wax layer, dominated by hydrophobic compounds such as pyrans, fatty acids and terpenoids, contributes to reduced glyphosate efficacy when applied alone. However, the use of surfactants, particularly CTAB at higher ratios, significantly enhanced glyphosate penetration and translocation to tubers. These insights can inform the development of more effective control measures against *C. rotundus* and similar perennial weed species. Future work should explore the field-scale validation of such formulations and evaluate the long-term efficacy in preventing tuber regeneration. This approach offers a promising pathway for the sustainable management of perennial weeds like *C. rotundus*.

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Authors' contributions

SK prepared the manuscript; CCR participated in the sequence alignment and drafted the manuscript. KR and GR participated in the sequence alignment. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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