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Allelochemicals Analysis of *Rumex vesicarius* L. and *Zygophyllum coccineum* L., and Their Effect on Seed Germination and Seedling Growth of Wheat, *Triticum aestivum* L.

S. A. Alazzam[1], M. M. Sharqi[1] and A. F. Almehemdi[2*]
[1]Department of Biology, College of Science, University of Anbar, Iraq
[2]Center of Desert Studies - University of Anbar, Iraq
[*]Corresponding author's e-mail: ds.dr.ali.fadaam@uoanbar.edu.iq

Abstract. Plants samples were gathered from Ramadi, University of Anbar. Extraction processes performed from foliage in labs belonging to Center for Desert Studies using methanolic and aqueous extracts to assess their Allelopathy. Allelopathic bioassay applied with three concentrations 0.5, 1 and 2% On Germination%, Germination Rate, Germination Speed, Average Germination Speed, Response Index, Radicle and Plumule Length of wheat seed. Phytochemicals detection of the R. vesicarius presented highest percentage of alkaloids, flavonoids, phenols and Glycosides. GC/MS analysis proofed that R. vesicarius and Z. coccineum methanolic extracts contained highest 2-pyrrolidone-5-carboxylic acid (alkaloid) of 35.98% and 1,2-benzendicarboxyylic acid bis(-methylhexyl) ester (hydrocarbonate chain) of 35.06%, in each species extract, respectively. Methanolic and aqueous extract (%2) of R. vesicarius possessed lowest rate of germination (%13.3), (%50.7), respectively. On the other hand, methanolic and aqueous extract (%2) of Z. coccineum gave lowest germination rate of 30.6 and 81.3%, respectively. so, this extracts of R. vesicarius and Z. coccineum could be used as eco-friendly bioherbicide, and may be integrated into harmful weed control strategies.

1. Introduction

Allelopathic effects took big attention by agronomist, biologist and weed killers. Most plant types, such as wild plants, trees and crops are able of release secondary compounds into given environment lead to inhibit the development of adjoining plants [**1**] these compounds called allelochemicals. Allelochemicals are synthesized in plants, or bacteria, fungi, microorganisms, and viruses which possessed positive or negative effects on the evolution and growth of ecological and agricultural systems [**2**]. These plants- produced Allelochemicals are released to the environment through different routes via roots exudation, leaves and stems volatilization or leaching disintegration decomposition of plant residues [**3, 4**]. Allelopathic effects could be inhibitory or stimulatory, depending on type of the allelochemicals that affected the target species [**5**]. Furthermore, allelopathy is highly accepted as an interesting circumstantial factor that limits the plant growth, succession, predominance, distribution, species diversity, structure and composition of plant Societies [**6**]. *Rumex vesicarius* L. belongs to Polygonaceae, this plant is widely grown in in India; it is consumed as medicine to relief coughing, digestive problems, toothache, nausea, pain, anti-inflammatory, antitumor and antimicrobial activities and muscle tensions and used in the treatment of liver diseases [**7**]. *R. vesicarius* is considered as a dietary supplementary plant because it is a source of carotenes. Foliage is rich in ascorbic acid, citric acid and tartaric acid, glycoside, alkaloid, flavonoids, tannins and phenolic compounds [8]. Additionally, the plant is a good source of minerals such as K, Na, Ca, Mg, Fe, Mn and Cu [**9**]. Many species of Zygophyllaceae such as Zygophyllum coccineum L. are widely dole out in desert areas [**10**]. Species of this genus is belonging to a group of succulent plants that resist drought or tolerate salt, living under arid, dry environments, growth, evolution and distribution of Zygophyllum species are relied on the chemical properties of the soil of their habitats and their high tolerance to environmental stresses [**11, 12**]. Additionally, the leaves, fruits and stems of Z. coccineum are used in folk medicine as a drug active against gout, rheumatism, asthma, and hypertension. Plant is used as a diuretic antihistaminic local anesthetic and antidiabetic agent [**13**].

Based on the above, the objectives of the research were to evaluate the allelopathic effect of R. vesicarius and Z. coccineum on germination indices and seedling growth of Triticum aestivum L. Find also out the qualitative and quantitative analysis of leaves.

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2. Materials and Methods]

2.1. Plant materials

Leaves of *R. vesicarius* and *Z. coccineum* were collected from Ramadi, University of Anbar, Iraq (Fig.1and Fig.2). Collected leaves were washed thoroughly under tap water to remove chemicals materials and dust. the leaves of plants were dried by incubation in oven at 37°C for one day. Extraction processes were performed on leaves of plant in the laboratories of Desert Studies Center after drying the leaves of plants by using methanol solvent and Aqueous Extract.

Figure1. Target plant *Rumex vesicarius* L**.**

Figure2. Target plant *Zygophyllum coccineum* L.

2.2. Preparation of plant extracts

2.2.1. Methanolic and aqueous extraction

Dried powder (100g) leaves of plants were treated with 500 mL Methanolic and aqueous extraction sequentially. The mixture was homogenized with a magnetic stirrer for 4 h at room temperature and then was filtrated using Whatman filter paper No.1. The filtrate solvent was cooled and centrifuged with 1500 rpm at 15°C for 30 min. The crude fraction was evaporated at 45°C using a rotary evaporator (IKA, Germany) under vacuum to obtain the final dried crude extract. Subsequently, dilutions were made to attain required final concentrations (0.5%, 1% and 2%) [**14**].

2.3. Qualitative Phytochemical Analysis

Qualitative phytochemical screening for the crude extracts of *R. vesicarius and Z. coccineum* was carried out by following the method described by Cannell [**15**] The presence of glycosides, tannins, saponins, alkaloids, flavonoids and Phenols was analyzed.

2.3.1. Determination of Total Phenolic Content

The total amount of phenolic compounds was determined in the Methanolic extract with a standard Folin - Ciocalteu reagent. The reaction mixture contained 100 µl of the extract, and 500 µl of the Folin-Ciocalteu reagent and 1.5 ml of (20%) Na_2CO_3. The samples was then mixed on a vortex mixer and diluted with distilled water to the final volume of 10 ml. After 2 hours reaction, and the absorbance at (765 nm) was determined and used to estimate the phenolic content using the calibration curve made with gallic acid. The total amount of phenolic compounds was expressed in milligram of gallic acid equivalent (GAE) per gram dry weight [**16**].

2.3.2. Determination of total flavonoids content

The flavonoid content was determined by the aluminum chloride colorimetric method. 50 µL of crude extract of plants (1 mg/mL methanol) were made up to 1 mL with methanol, and mixed with (4 mL) of distilled water and then 0.3 mL of (5%) sodium nitrite solution; 0.3 mL of (10%) Aluminum trichloride solution was added after 5minute of incubation, and the mixture was leaved for 6 minutes. Then, 2 mL of 1 mol/L Sodium hydroxide were added, and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand for 15 minute. The absorbance was measured at (510 nm). The total flavonoid content was

calculated from a calibration curve, and the result was expressed as milligram rutin equivalent per gram dry weight [**17**].

2.3.3. Determination of Tannins content

Tannins quantitative determination was carried out using the method reported by [**18**]. Added 75 ml of distilled water to 25 g of powder leaves and put in a water bath for 30 min. Then performed centrifugal for mixture at (200* rpm) for 30 min at room temperature. take leachates and place in beaker 100 ml and complete the volume with the distilled water. add to the mixture 20 ml from solution lead acetate (4%) with continuous shaking for 6h, the solution was filtered and the precipitate was placed in evaporating dish and dried in oven 105 °c for 6 h. then the evaporating dish c was weighed . The samples were burned in the burning oven at a temperature of 550°c. and the evaporating dish was weighed once other and the percentage of tannins was calculated as:

% Tannins = Weight before burning- Weight after burning / Weight of sample × 100.

2.3.4. Determination of Alkaloids content

Alkaloids quantitative determination was carried out using the method reported by [**19**]. 200 of (10%) acetic acid in ethanol was added to each powder leaves 2.50 g in a 250 beaker and allowed to stand for 4 h. The extract was concentrated on a water bath to one-quarter of the main volume subsequently addition of 15 drops of concentrated (NH_4OH) drop wise to the extract until the precipitation was complete immediately after filtration. After 3 hours of mixture sedimentation, the supernatant was discarded and the precipitates were washed with 20 of (0.1 M) of NH_4OH and then filtered using Gem filter paper (12.5 cm). Using electronic weighing balance, the residue was dried in an oven and the percentage of alkaloid is expressed as

% Alkaloid = Weight of alkaloid/ Weight of sample × 100.

2.3.5. Determination of Glycoside content

Quantitative determination of Glycoside was according to the methodology by [**20**]. Glycosides were extracted and percentages were estimated by adding 100 ml of ethyl alcohol80% to (10) g of powder leaves and left for 24 h .Then filter the solution to obtain the ethanolic extract ,using a rotary evaporator (temperature 50 °C) to obtain a concentrated extract. 50 ml ether and 5ml Lead acetate of 0.3 M was added with shaking, ether was added three times, then the aqueous layer was taken and dried at 50 ° C to get Glycosides. the percentage of tannins was calculated as:

% Glycoside = Weight of Glycoside /Weight of sample × 100.

2.4. GS/MS Analysis

R. vesicarius and Z. coccineum dried methanolic extracts was subjected to chromatography technique of GC-2010 Plus (Shimadzu, Japan) which are equipped with column of DB-5MS (30 m length, 0.25 mm i.d. and 0.25 µm thickness, Agilent Technologies, J & W Scientific Products, USA). Temperatures of injector and detector were run at 250 and 230°C, respectively. While oven temperature was started at 100°C and raised at a rate of 5°C min^{-1} then held at 260°C for 1 min. injected Aliquot was 1 µL of sample and helium was used as the carrier gas. mass range was scanned from 50-550 amu. Identification for possible active constituents was carried out using NIST Library [**21**]. These analyses were accomplished at Ministry of Science and Technology, Department of Water and Environmental Research.

2.5. Allelopathic bioassay

A series of laboratory experiments were conducted with the Methanol Extracts and aqueous extracts of *R. vesicarius* and *Z. coccineum* separately at Center for Desert Studies Lab, University of Anbar 20/1/2019. Modified method of [22] was used. Where, the germination test was carried out in sterile Petri dishes (9 cm) lined with two filter papers Whatman No. 3. The different plant extracts were added to every Petri dish of the respective treatments daily in amounts just to keep the seeds moist enough to get favorable conditions for germination and growth. The control treatment was treated with distilled water only. Before setting for germination test, the weed seeds were surface sterilized with 1% sodium hypochlorite (NaOCl) for 3 min and rinsed thoroughly with distilled water. The experiment was arranged in the completely randomized design with three replications. 25 seeds were placed in each Petri dish. The Petri dishes were set in the Desert Studies Center Labs, University of Anbar at the room temperature ranging from 28 -30C°. A seed was considered germinated when its radicle emerged.

2.5.1. Germination study

Triticum aestivum L. was used as indicator plant in the allelopathic bioassay, as this plant is very sensitive to allelochemicals at low concentrations (0.5, 1 and 2%), aqueous and methanol extract was prepared according to the method of [**22, 23**].

2.5.2. Germination and Growth Records

Further dilutions (2, 1 and 0.5%) were made using distilled water. The seeds of Triticum aestivum L. were surface sterilized with 2% NaClO for 2 minutes and were rinsed with distilled water to remove traces of NaClO. The germination test was carried out in sterile Petri dishes of 9 cm in size placing a Whatman filter paper on Petri dishes. 25 seeds each of T. aestivum were grown in Petri dishes containing double folded Whatman filter paper. Each Petri dish was provided with 5 ml of respective extracts. Distilled water (0% extract) was used as a control. Each treatment was replicated three times and the experiment was arranged in a completely randomized design (CRD). The petri dishes were sealed and placed in a growing room at 25 ° C for 10 days. The germinated seeds were counted daily and time to start germination was noted. Data for Germination Percentage, radicle and hypocotyl length was recorded after 10 day. Seed germination percentage was calculated using the following formula

$G\% = \frac{Sg}{St} x 10^2$ …….[**24**]. G%= Seed germination percentage, sg= germinated seed and st= time to seed be germinated.

Germination Rate (GR) was calculated using the following formula

GRI (%d -1) = Σ (Ni /Ti) …….[**25**]. Ni= seed germinated percentage, Ti= time to seed be germinated.

Speed of germination was calculated by the following formula

CVG (%d -1) = 100 * ΣNi / Σ (NiTi)) given by [**26**].

Mean germination time was calculated by the formula

MGT (d) = Σ (NiTi) / (ΣNi) given by [**27**].

Response Index (RI) was calculated as per the formula for the magnitude of inhibition versus stimulation by imposed stress on seed germination and seedling growth using following formula: When germination of treatments (T) is lower than the control (C): RI = (T/C) – 1 When germination of treatments (T) is higher than the control (C): RI = 1 – (C/T). If RI > 0 Treatment stimulated germination If RI = 0 No effect If RI < 0 Treatment inhibited germination [**28**]. After 10 days after germination, shoot (SL) and root (RL) length was measured per treatment. Take ten samples were individually.

Germination % =Number of germinated seeds / Total number of seeds × 100.

2.6. Statistical analysis

All data were analyzed by using software (SAS version 9.3, 2013), As for means comparison of all data was performed using least significant difference test (LSD) at 5% probability level.

3. Results and Discussion

3.1. Qualitative Phytochemical Analysis and detection

The preliminary qualitative phytochemical screening of *R. vesicarius* and *Z. coccineum* revealed that the presence of bioactive components alkaloids, flavonoids, phenols, Glycosides, saponins and tannins in methanolic extract. However glycosides were absent in aqueous extract extracts for both plants and alkaloids absent in aqueous extract for *R. vesicarius*. The results shown in (Table.1) Showed that the leaf extract of *R. vesicarius* contained alkaloids, flavonoids, phenols, glycosides, saponins and tannins. Some results were identical to previous studies that dealt with the chemical composition of the plant *R. vesicarius*, which showed that it contains many active compounds such as alkaloids, carbohydrates, protein, cardiac glycosides, saponins, glycosides, flavonoids, tannins steroids, ascorbic acid, tocopherols, anthraquinones, cysteine, glutamic acid, proline terpenoids, phenylalanine and histidine [28]. In addition these results in Table (1) for *Z. coccineum* were in agreement with results of [**10**]. These studies showed the importance of the *Z. coccineum* plant because it contains phenols, total flavonoids, alkaloids, saponins tannins, glucosinolates, proline, ascorbate, glutathione, protein and carbohydrate in various extracts *Z. coccineum*.

Table 1. Qualitative phytochemical analysis of extracts of *Rumex vesicarius* L. and *Zygophyllum coccineum* L.

constituents	Reagents	Detection indicator	Methanolic Extract		Aqueous Extract	
			R vsicarius	*Z coccineum*	*R vsicarius*	*Z coccineum*
Saponins	Extract Mixing	Thick foaming	++	++	+	+
Alkaloids	Dragendroff	Orange brown	++	++	_	++
Flavonoids	Sodium Hydroxide Solution	Yellow	++	++	++	+
Phenolic	$Fecl_3$	Brown pcpt	+++	++	++	++
Tannins	Lead Acetate	Yellowish pcpt	++	++	+++	+++
Glycosides	Benedict	Reddish Brown pcpt	++	+	_	_

* Key = (-): absent; (+): weak; (++): strong:(+++), pcpt= precipitation

The quantitative estimation of primary metabolites (tannins, glycosides, alkaloids, Flavonoid and phenolic) revealed that the various phytochemical constituents present in the plant extract (Table.2). Show the results of the chemical analysis of the *R. vesicarius* extract that the phenols content were 68.9 mg g^{-1}, following by the Flavonoid content 48.7 mg g^{-1}, Tannin content 59.8 (%), Alkaloid content 58.7 (%) and Glycoside content 16.3 (%). Additionally, these results in Table (1) For *R. vesicarius*, Where the study [**14**] confirmed that the total phenolic content of *R. vesicariu*s have been reported 56.55 [mg GAE·g^{-1} DW], 33.87 mg CEg^{-1} DW and Condensed tannins 27.95 mg CE g^{-1} DW. Similarly, results were observed in the [**29**]. Furthermore, show the results (Table .1) of the chemical analysis of the *Z. coccineum* extract that the phenols content were 55.3 mg g^{-1}, following by the Flavonoid content 35.7 mg g^{-1}, Tannin content 63.8 (%), alkaloid content was 44.6 (%) and Glycoside content was estimated 32.4(%). Where studies confirmed such [**30**] *Z. coccineum* extract contain appreciable content of total phenols and total flavonoids, alkaloids, saponins and tannins. These results match many studies such as [**10**].

Table 2. Total Phenolic content, Alkaloid, Glycoside, Flavonoid, and Tannins of *R. vesicarius* and *Z. coccineum* leaves

Phytochemical Of Leaves	*R. vesicarius*	*Z. coccineum*
Phenolic (mg/g.)	68.9	55.3
Flavonoid (mg/g.)	48.7	35.7
Tannins (%)	59.8	63.8
Alkaloid (%)	58.7	44.6
Glycoside (%)	16.3	32.4

3.2. GC/MS Analysis

GC/MS analysis of *R. vesicarius* methanol extract was listed in Table3and, it was found that the extract contained 11 identified constituents which represent 100.00%. The major component was 2-pyrrolidone-5-carboxylic acid 35.98% followed by aspidofractinine-3-methanol 12.06%, Vitamin E (α-Tocopherol) 12.05%, 7,10,13-hexadecatrienoic acid 10.40%, 7-Tetradecanal 9.00%, n-nonadecanoic acid 5.54%, imidazo (1,2-α) pyrridine 4.75%, 2-ethoxypropane 3.59%, methyldecanoate methyl ester 2.87%, Δ-Tocopherol 2.67%. whereas, 3,4,5-trimethyl-1h-pyrano 2,3-cpyrazol-6-one was lowest component of 1.09%.

Table 3. active constituents of *R. vesicarius* and *Z. coccineum* methanolic extract via GC/MS.

P#	compounds	R. vesicarius		MW	compounds	Z. coccineum		MW
		RT	Area %			RT	Area %	
1	2-ethoxypropane	2.414	3.59	88	2-isopropoxyethanamine	2.038	2.81	103
2	3,4,5-trimethyl-1H-pyrano2,3-cpyrazol-6-one	11.457	1.09		n-nanodecanoic acid	17.160	2.04	298
3	2-pyrrolidone-5-carboxylic acid	12.482	35.98	143	1,1-bicyclopentyl	18.028	4.91	362
4	methyldodecanoate	18.923	2.87	214	E-2-tetradecen-1-ol	18.293	1.05	212
5	n-nonadeconic acid	19.341	5.54	298	1-Eicosyne	18.496	2.95	278
6	7,10,13-hexadecatrienoic acid	20.732	10.40	264	methylmyristate	19.921	1.90	242

7	7-tetradecanal	21.159	9.00	210	n-hexadecanoic acid	19.363	27.44	256
8	Aspidofractinine-3-methanol	24.700	12.06	310	6Z-6-pentadecen-1-ol	20.652	1.46	226
9	Δ-tocopherol	28.125	2.67	402	2,6,10,14-tetramethyl-2-hexadecene	20.883	4.98	280
10	Imidazo(1,2-α)pyrimidine	29.106	4.75	151	(6Z,9Z-)-6,9-pentadecadien-1-ol	21.167	13.79	224
11	α-tocopherol	30.301	12.05	430	Not match	24.158	1.61	
12					1,2-benzendicarboxyylic acid bis(-mthylhexyl) ester	24.707	35.06	390

P#= peak number, RT= retention time, MW= molecular weight.

Furthermore, Table. 3 illustrated that GC/MS analysis of Z. coccineum methanol extract splitted 12 active constituents, represent 100%. So, The major component was 1,2-benzendicarboxylic acid bis(-methylhexyl)ester of 35.06% followed by n-hexadecanoic acid of 27.44%, (6Z,9Z-)-6,9-pentadecadien-1-ol 13.79%, 2,6,10,14-tetramethyl-2-hexadecene 4.98%, 1,1-bicyclopentyl 4.91%, 1-Eicosyne 2.95%, 2-isopropoxyethanamine 2.81%, n-nanodecanoic acid 2.04%. Additionally, 4 components were possessed amounts less than 2%, represent 6.02% of total area% as methylmyristate 1.90%.

3.3. Allelopathic bioassay

Current results revealed that methanol and aqueous extract of *R. vesicarius* and *Z. coccineum* of had allelopathic activity, and it proved that the three concentrations of phytoextracts had an inhibitory effect on seed germination *T. aestivum* and inhibitory effect of Methanol and aqueous extract was enhanced by increasing extract concentration (Table.4 and Table.5). from previous results of other investigators [**31**]. it was derived that allelopathy influences on seed germination were occurred by two ways. Firstly, the chemical constituents hinder cell division. Secondly, these constituents inhibit the elongation of cells [**32**] who found that several allelopathic compounds could reduce the stimulating effect of some known growth hormones including indole acetic acid and gibberellins.

Methanol and aqueous leaf extracts effect on *T. aestivum* seeds were caused a reduction of radical length compared to control, as shown in (Table.4 and Table.5). Many investigators observed reduction in radical growth during their allelopathic studies [**33, 34**]. Plumule and radicle are the first plant organs emerge from the seeds during germination since their growth decreases if they are exposed to allelopathic compounds.

The decrease in radicle length could be due to the presence of allelochemicals including flavonoids, tannins, and phenolic acids. Furthermore, the toxicity might be due to synergistic effect rather than single one, phenolic acids in plant extracts have been investigated to exhibit a toxic effect on seed germination and various plant growth processes, phenolic acids inhibited protein synthesis in roots of *Cicer arietinum* [**35, 36, 37**].

Table 4: Allelopathic activity of *R. vesicarius* leaves extracts (Methanol, Aqueous) on Growth indices of *Triticum aestivum* seed.

Type extract	Con	G (%)	GR (%.d^{-1})	GS (%.d^{-1})	AGS (%.d^{-1})	RI (%.d^{-1})	ALR (cm)	ALP (cm)
Control))	0%	97.3	6.028	14.79	0.16589	0.000	2.983	4.217
Methanol	0.005	93.3	6.103	13.42	0.16386	-0.041	2.587	3.320
Extract	0.01	49.3	6.442	5.29	0.15525	-0.492	1.323	0.687
	0.02	13.3	6.844	1.25	0.14611	-0.863	0.242	0.100
Aqueous	0.005	90.7	6.165	12.21	0.16226	-0.068	3.207	2.210
Extract	0.01	69.3	6.318	8.38	0.15829	-0.286	2.103	0.273
	0.02	50.7	6.521	5.25	0.15338	-0.478	1.037	0.160
LSD 5%		10.02	0.1472	1.245	0.003605	0.1166	0.483	0.688

***G: Germination Percentage, GR: Germination Rate , GS: Germination Speed, AGS: Average Germination Speed, RI: Response Index, ALR: Average Length Radicle, ALP: Average Length Plumule.

Table 5. Allelopathic activity of *Z. coccineum* leaves extracts (Methanol, Aqueous) on Growth indices of *Triticum aestivum* seed.

Type extract	Con	G (%)	GR (%.d^{-1})	GS (%.d^{-1})	AGS (%.d^{-1})	RI (%.d^{-1})	ALR (cm)	ALP (cm)
Control))	0%	97.33	6.028	14.79	0.165	0.000	2.983	4.217
Methanol	0.005	93.33	6.119	13.00	0.163	-0.041	2.920	3.273
Extract	0.01	85.33	6.474	9.00	0.154	-0.122	2.030	1.860
	0.02	30.67	6.947	2.71	0.144	-0.684	0.515	0.171
Aqueous	0.005	94.67	6.099	13.54	0.163	-0.027	3.390	4.363
Extract	0.01	89.33	6.227	11.25	0.160	-0.082	2.910	2.727
	0.02	81.33	6.415	9.25	0.155	-0.164	1.680	1.677
LSD 5%		7.170	0.118	1.060	0.0026	0.080	0.3012	0.552

***G: Germination Percentage ,GR: Germination Rate , GS: Germination Speed, AGS: Average Germination Speed, RI: Response Index, ALR: Average Length Radicle, ALP: Average Length Plumule.

4. Conclusion

In the present study conclude that the *R. vesicarius* and *Z. coccineum* leaves have the potential to act as a source of useful allelochemicals because of being present of various phytochemical constituents such as alkaloids, flavonoids, phenol, Glycosides, saponin and tannins. Moreover, *R. vesicarius* and *Z. coccineum* extracts possess allelopathic potential on germination indices of wheat. GC/MS analyses was informative to fortify the phytochemicals detection. Therefore, these two species can be used as antimicrobial agents and as bioherbicidal precursors on weeds.

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