

Allelopathic effects of *Zelkova schneideriana* Hand. Mazz root exudates on growth of rape, wheat, maize and tall fescue

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ABSTRACT

Intercropping is an efficient way to utilize growth resources and for economic benefits. We evaluated the possibility of *Zelkova schneideriana* Hand. Mazz intercropping with maize (*Zea mays* L), wheat (*Triticum aestivum* L), rape (*Brassica rapa* C), and tall fescue (*Festuca elata* K). A total of 39 compounds were identified in *Z. schneideriana* root exudates by GC-MS analysis; 1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester had the highest content (50.36 %), followed by phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl- (21.56 %). Growth of the test crops was inhibited by root exudates, when applied at 4 mg·ml⁻¹, however, promoted at 2 mg·ml⁻¹. The strongest inhibition was observed in maize and the lowest in tall fescue. The physiological responses of the crops to the root exudates of *Z. schneideriana* showed similar trends. Malondialdehyde (MDA) content was the most sensitive parameter followed by relative electrical conductivity (REL) and chlorophyll (Chl) content, indicating the trait-specific responses. Rape growth was the most promoted at 2 mg·ml⁻¹, followed by tall fescue. Rape was found the ideal specie for intercropping with *Z. schneideriana*, followed by tall fescue.

Key words: Allelopathic effects, crop, GC-MS, root exudates, *Zelkova schneideriana*.

INTRODUCTION

During the plant growth and development, root tissues secrete multiple allelopathic metabolites (organic acids, phenols, alcohols, esters, and flavonoids), which lead to changes in soil ecology and affects the growth of surrounding plants (1). Such interactions have been reported in many plants (2-3) and are widely used in agricultural production (4). For example, rotation or intercropping of *Solanum melongena* L and *Allium sativum* L increases the yield of eggplant (5); *Zea mays* L grew better in rotation with *Nicotiana tabacum* L than with *Vigna radiata* L (6); *Gossypium hirsutum* L intercropping with *Sorghum bicolor* L, *Glycine max* L and *Sesamum indicum* L mutually promoted the growth and yield of each other (7); intercropping of *Banana* (*Musa* spp.) with *Allium tuberosum* Rottler controls diseases and increase farmland biodiversity (8); the aqueous extracts of white fescue inhibits the root length, shoot length, germination rate and fresh weight of perennial ryegrass and hence, the compounds of aqueous extract could be potential candidate for herbicides (9). Mixed-sowing of *Festuca arundinacea* Schreb variety Millennium2 and Arid3 in 4:1 mixture ratio showed that their best physiological response and growth performance. It was concluded that in mixed-sowing compatible species and appropriate ratio in mixed-sowing is important to improve the landscape and ecological roles of the grasses (10).

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The allelopathic principle was also used in forest industry, not only to improve forest condition (11) but also to develop the underneath plants (12). Li (13) reported that the plant height increments of *Scutellaria baicalensis* Georgi seedlings treated with the aqueous extract of walnut leaves at 5, 20, 60, and 100 mg·ml⁻¹ concentrations were 5.06, 6.01, 4.18, and 4.06 cm, respectively. The percolated water solution of topsoil of *Pinus sylvestris* L. understory at ≤1:40 concentration stimulates the growth of radish seedlings (4). And the aqueous extracts of larch (*Larix gmelini*) roots, bark and branch at 12.5 mg·ml⁻¹ concentration promoted the seeds germination and seedling growth of *Juglans mandshurica*, *Brassica campestris* and *Raphanus sativus* (14).

Root-secreted signalling chemicals (15) always determine neighbouring plant allelochemical response. *Zelkova schneideriana* Hand. Mazz is an arbor tree mostly used for landscaping and silviculture. Their broad planting intervals showed a great potential for forest understory management. This research aimed to investigate the compounds composition of roots exudates and study their impacts on the growth of crops, to lay foundation for the management of *Z. schneideriana* chemical compound and provide new theoretical supports for plant interactions.

MATERIALS AND METHODS

Pot culture

This study was conducted from September 2021 to September 2022 in Beijing Forestry University, Beijing (Longitude, 116°23'17"N; latitude, 39°54'27"E; Altitude, 60 m; Annual precipitation, 600 mm; Maximum and minimum temp for study site, -11°C to 37°C).

The one-year old seedlings of *Zelkova schneideriana* Hand. Mazz were planted in pots (20 cm × 25 cm × 20 cm) with soil substrate and watered once a week during growth. The seeds of maize (*Zea mays* L), wheat (*Triticum aestivum* L), rape (*Brassica rapa* C) and tall fescue (*Festuca elata* K) were planted in pots (10 cm × 10 cm × 10 cm) with vermiculite substrate, watered every three days and irrigated with Hoagland solution weekly. All plants were grown in a growth incubator (25 °C, 16 h light/8 h dark photoperiod) (Fig. 1).



Figure 1. Photographs of one-year old *Z. schneideriana* and two-weeks old seedlings of the crops. The plants from left to right are *Z. schneideriana*, *Zea mays* L, *Triticum aestivum* L, *Brassica rapa* C, and *Festuca elata* K

Preparation of *Z. schneideriana* root exudates

The one-year old *Z. schneideriana* plants were taken out of the cultured pots carefully. After cleaning the root soil, the plant was cultured in 200 ml distilled water for two days. The roots were then collected, dried in oven (80 °C) overnight, and weighted. The root exudates solution was collected and diluted to 4 mg dry roots·ml⁻¹ concentration. Five ml was used for chromatography-mass spectrometry (GC-MS) analysis, and the left was diluted as the treatment solution at 0, 2, 4 mg·ml⁻¹ concentration, respectively, for the next allelopathic study.

GC-MS analysis

The *Z. schneideriana* root exudates solution at 4 mg dried roots·ml⁻¹ concentration was filtered with millipore filter (0.45 µm). Five ml filtrate was mixed with 2 ml of ethyl acetate, then centrifuged. Its 1 µl ethyl acetate extract was taken out from the layered solution and analysed by GCMS-Qp2010 Ultra GC-MS (manufactured by Shimadzu Corporation). The GC conditions were set as follows: inlet temperature 250 °C, helium (99.99 %) carrier gas flow at constant 1ml·ml⁻¹, injector split ratio 10:1. The temperature program started at 50 °C and increased at the rate of 15 °C·min⁻¹ to 180 °C for 5min, and then at the rate of 10 °C·min⁻¹ until it reached 250 °C for 15min. MS analysis was conducted in positive electron ionization mode, with the source temperature at 230 °C and an electron ionization potential of 70 eV. Ions were scanned over the molecular weight range of 33~ 400 atomic mass units. Each metabolite was identified via the standard mass spectrum database of NIST 11.lib and NIST11s.lib, and the relative content of each component was counted by area normalization.

Measurements of plant growth and physiological index

The 2-weeks old crop seedlings were irrigated twice (4 d interval) with 20 ml per pot of the *Z. schneideriana* root treatment solution at 0, 2, 4 mg·ml⁻¹ concentration, respectively. After 7 d treatment, the plant stems, leaves, and roots were harvested, weighed, and used for physiological index assays. The physiological indexes measured were as under:

(i). **Relative electrical conductivity (REL)** (16): 0.1 g fresh leaves were cut into 0.5 cm pieces in 30 ml deionized water, and shaken at 28 °C for 24 h. The conductivity was measured with a conductivity meter as R1. Then it was autoclaved and shaken at 28 °C for 24 h again. The conductivity was measured as R2. The REL (%) was calculated by R1/R2.

(ii). **Malondialdehyde (MDA) content** (17): 0.1 g fresh leaves were grinded in 1 ml 10 % TCA solution. After centrifugation at 4000 r·min⁻¹ for 10 min, 800 µl supernatant (water for control) was mixed with 800 µl 0.6 % TBA solution, and kept on boiling water bath for 15 min. After centrifugation, the supernatant was used for the absorbance measurements at wavelength of 532, 600 and 450 nm. The MDA content [µmol·g⁻¹ (FW)] was calculated as under:

$$\text{MDA content} = [6.45 \times (\text{OD}_{532} - \text{OD}_{600}) - 0.56 \times \text{OD}_{450}] \times V/W.$$

Where, V: Total volume of extract (ml) and W: Sample weight (g).

(iii). **Chlorophyll (Chl) content** (18): 0.1 g mature leaves were immersed in 10 ml DMSO solution at room temperature in dark until the leaves completely lost green colour. The solution was used to measure the absorbance at wavelength of 663 nm and 645 nm. The total chlorophyll content was calculated as under:

Chl content = $[(0.0127 \times A663 - 0.00269 \times A645) + (0.0229 \times A645 - 0.00468 \times A663)] \times V/W$.

Where, V: Total volume of extract (ml) and W: Sample weight (g).

(iv). **Response index (RI)** (19): The allelopathic effects of *Z. schneideriana* root exudates on crop growth were evaluated as under:

$$RI = (T1/T0-1) \times 100\%.$$

Where, T0: Control data (0 mg·ml⁻¹ treatment solution) and T1: Treatment data (2, 4 mg·ml⁻¹ treatment solution).

Statistical analysis: Three replicates for each line/treatment were conducted. The data are means \pm SD. SPSS23.0 software was used for difference comparison between the treatments (2 mg·ml⁻¹ or 4 mg·ml⁻¹) and control (0 mg·ml⁻¹) in a crop at P value < 0.05 and P value < 0.01.

RESULTS AND DISCUSSION

I. Compounds identification in *Z. schneideriana* root exudates

The *Z. schneideriana* root exudates were collected for 2-days from the water culture solution of one-year old plants. Based on GC-MS analysis, 39 compounds were identified in the *Z. schneideriana* root exudates (Table 1). They were primarily alkanes and esters. The chemical 1,2-benzenedicarboxylic acid and bis (2-methylpropyl) ester had the highest proportion (50.38 %), followed by phenol, 2,2'-methylenebis [6-(1,1-dimethylethyl)-4-methyl (21.56 %). The content of 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester in plants [*Alocasia macrorrhiza* (L.) Schott, a traditional Chinese medicinal species] was highest 32.51 %, in plant volatiles and these proved effective for pest defence (20). *Astilbe chinensis* (Maxim.) Franch. et Savat root also produced 1,2-benzenedicarboxylic acid and bis (2-methylpropyl) ester was 2.88 % contents of its root substances (21), while *Sibiraea angastata* (Rehd.) Hand-Mazz, leaves had the substance content (3.21 %) of its volatile oil (22). Phthalic acid, a precursor for synthesizing the bis (2-methylpropyl) ester of 1,2-benzenedicarboxylic acid, are effective allelopathic compounds. Treatments with phthalic acid severely reduced the root dry weight, root length, root volume, root surface area, root nodule number, root nodule fresh mass, and root vigour of *Adzuki bean* (23). The effects increased as its concentration was increased. Therefore, higher amounts of bis (2-methylpropyl) ester of 1,2-benzenedicarboxylic acid in *Z. schneideriana* has critical allelopathic effects on the surrounding plants.

II. Effects of *Z. schneideriana* root exudates on the growth of crop seedlings

The *Z. schneideriana* root exudates had various impacts on the leaf development of 4-test crops (Fig. 2). At 4 mg·ml⁻¹ solution, no significant differences were found in the fresh weight of stem and leaves than control (0 mg·ml⁻¹). However, the treatment of 2 mg·ml⁻¹, all four crops stimulated seedling growth. Rape had the highest increase (39.8 %), followed by tall fescue (27.0 %) and maize (14.1 %). Therefore, *Z. schneideriana* root exudates stimulated the test crops growth at low concentrations, but high concentrations were inhibitory. These results are in line with previous investigations, such as the high concentration of aqueous extracts of *Cunninghamia lanceolata* Hook tissues and rhizosphere soil significantly inhibited cedar seed germination. However, the inhibitory effects weaken, fade away, and change to stimulatory effects with decreasing concentration (24).

Table 1. The allelopathic compounds in the root exudates of *Z. schneideriana* by GC-MS analysis

Retention time (min)	Compounds	Area (%)
7.16	Benzene, 1,4-dimethyl-2,5-bis(1-methylethyl)-	0.16
9.46	Methyl 2,5,8,11,14,17-hexaoxonadecan-19-oate	0.28
9.56	Phenol, 2,5-bis(1,1-dimethylethyl)-	0.86
10.62	Silane, [(fluoromethylphenylsilyl)methylidyne]tris[trimethyl-	0.11
11.14	Octane, 3,5-dimethyl-	1
11.67	Eicosane	0.12
12.26	Imidazole, 4,5-dichloro-1-(2-chlorobenzyl)-	0.54
12.4	1-Ethanone, 1-[4-[(2-chlorophenyl)methoxy]phenyl]-	0.63
12.79	4-Amino-5-[.beta.,.beta.-dimethyl-.alpha.-[4-chlorostyryl]]-6-chloropyrimidine	2.68
13.72	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	50.38
16.3	Decane, 2,3,8-trimethyl-	0.2
17.86	Heneicosane	0.33
18.32	Silane, [[4-[1,2-bis[(trimethylsilyl)oxy]ethyl]-1,2-phenylene]bis(oxy)]bis[trimethyl-	0.23
19.16	Heptadecane	0.51
19.29	Tetrahydropyran-4-carboxamide, 4-(3,4-dimethoxyphenyl)-N-(3,4-dimethylphenyl)-	0.09
19.4	1-Docosene	0.46
20.07	1,2-Benzenedicarboxylic acid, bis(trimethylsilyl) ester	0.85
20.27	Heneicosane	1.56
20.43	2-Butene-1,4-diol, O-chlorodifluoroacetate-O'-trifluoroacetate-	0.54
20.67	2-Methyl-3-phenyl-benzo-1,4-dioxin	0.7
20.77	1-Hydroxymethyl-3,3-dimethyl-2-(3-methylbuta-1,3-dienyl)cyclohexanol	0.31
20.87	4-Hydroxybenzyl alcohol, bis(tert-butyl dimethylsilyl) ether	0.67
21.26	Tetracosane	1.72
21.5	1,2-Benzenedicarboxylic acid, bis(trimethylsilyl) ester	1.24
21.56	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	21.56
21.94	Silane, dimethyl(2-isopropylphenoxy)octyloxy-	0.62
22.2	Pentacosane	1.03
22.25	2-Amino-2-oxo-acetic acid, N-[3,4-dimethylphenyl]-, ethyl ester	0.75
22.81	Bis(2-ethylhexyl) phthalate	4.02
22.92	tert-Butyl 2-aminophenylcarbamate ditms	1.63
23.29	Octadecane	1.01
23.95	1,3-Dithiane, 2-butyl-2-[2-(1,3-dithian-2-yl)phenyl]-	0.36
24.2	Phenol, 2-(2H-benzotriazol-2-yl)-4,6-bis(1,1-dimethylpropyl)-	0.06
24.61	Hexadecane, 7,9-dimethyl-	0.22
24.77	Phenethylamine, N-methyl-.beta.,3,4-tris(trimethylsiloxy)-	0.77
26.28	trans-2,3-Methylenedioxy-b-methyl-b-nitrostyrene	0.35
27.3	Mercaptoacetic acid, bis(trimethylsilyl)-	0.58
29.35	Sulfurous acid, butyl nonyl ester	0.47
30.8	Silane, [[4-[1,2-bis[(trimethylsilyl)oxy]ethyl]-1,2-phenylene]bis(oxy)]bis[trimethyl-	0.43

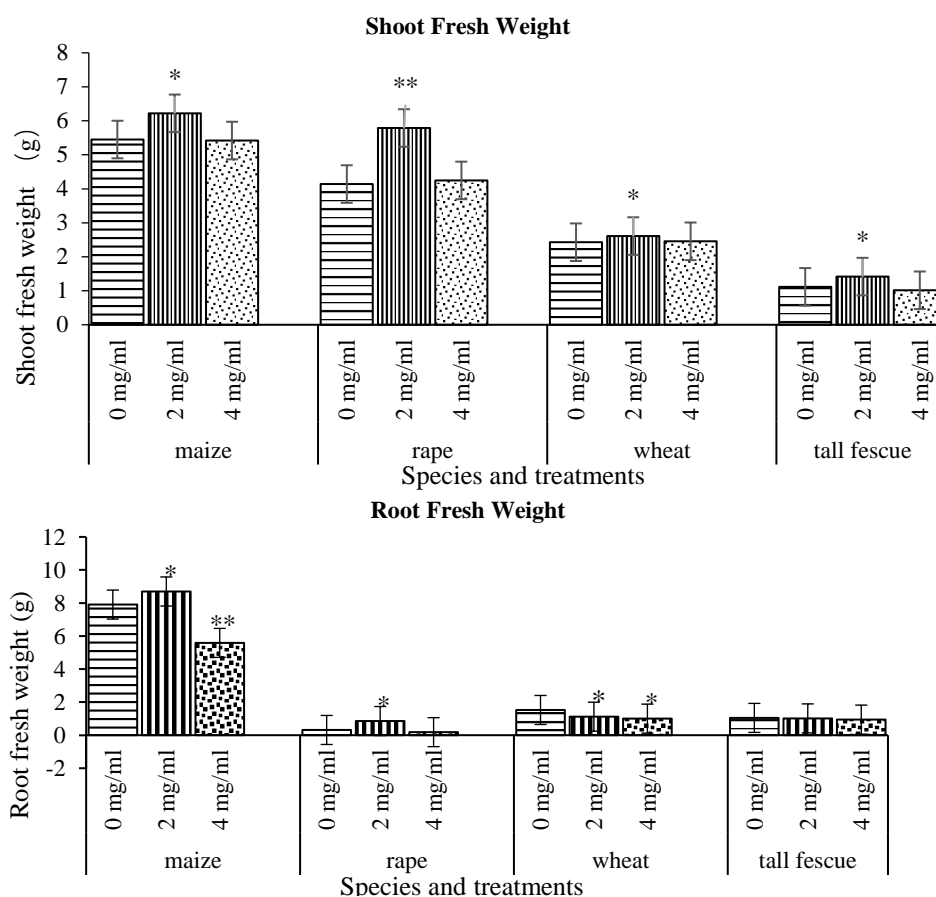


Figure 2. The effects of *Z. schneideriana* root exudates on fresh weight of shoot (Top) and roots (Bottom) of test crops. * and ** indicate significant difference at P value <0.05 and P value <0.01 between the treatments (2 mg·ml⁻¹ or 4 mg·ml⁻¹) and control (0 mg·ml⁻¹) in a crop.

Relatively, the root exudates had more severe effects on the root growth of four test crops (Fig. 2). At 4 mg·ml⁻¹ solution, the fresh root weight of all crops was inhibited than their control. Rape was the most affected 41.6 % decrease, followed by wheat (34.1 %) and maize (29.3 %). While at 2 mg·ml⁻¹ solution, the fresh root weight of rape and maize increased by 170.3 % and 9.9 %, respectively, although, weight of wheat fresh root decreased by 26.6 %. At the same time, tall fescue had no change. It was concluded that the crop roots were more responsive to *Z. schneideriana* root exudates than leaves; especially the inhibitory effects were more prominent at higher concentrations. This is also comparable to the earlier report of *Agrostis stolonifera* L root exudates impacting *Festuca arundinacea* Schreb plants, which revealed that root weight was reduced by 27 % by a high-concentration treatment, while leaf weight decreased by 8 % (25). The allelopathic response was organ specific.

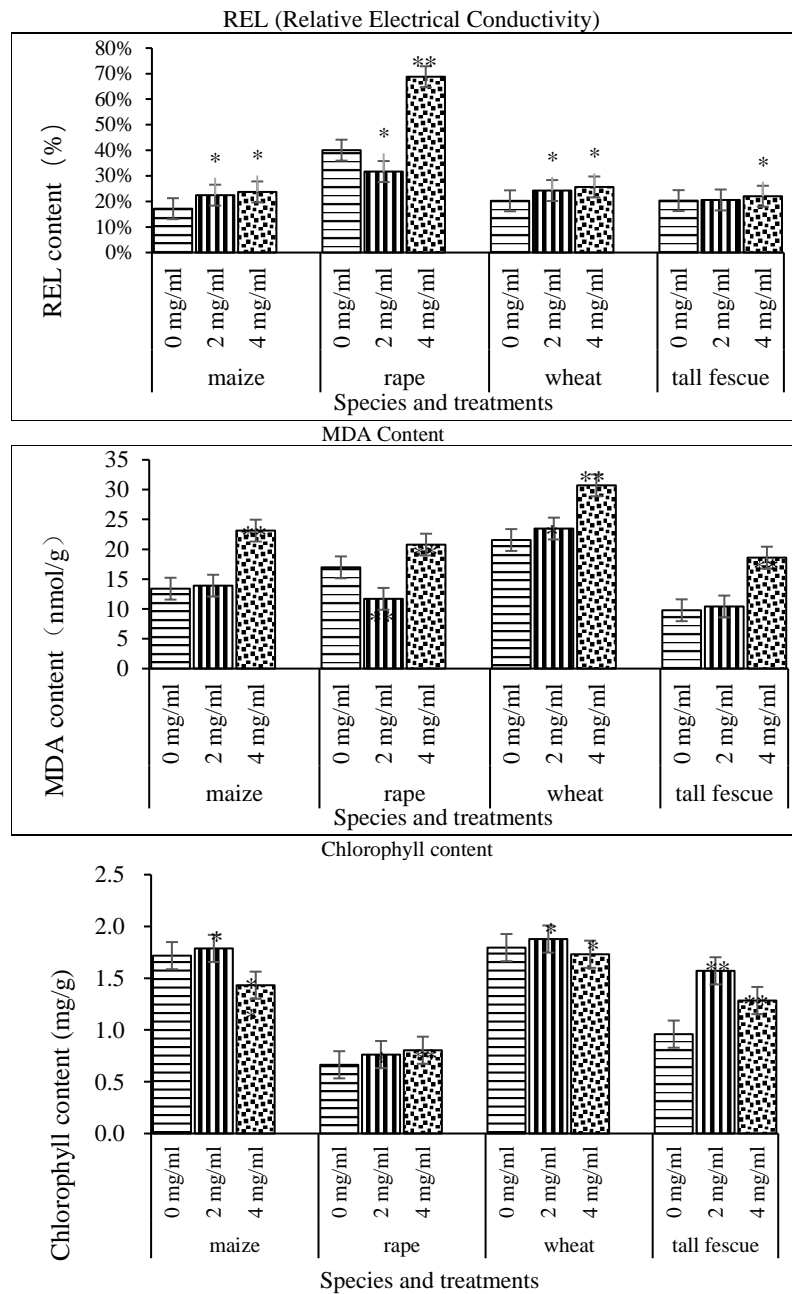


Figure 3. Effects of *Z. schneideriana* root exudates on the physiological responses (REL, MDA, Chl) of test crops. * and ** indicate significant difference at P value <0.05 and P value <0.01 between the treatments (2 mg·ml⁻¹ or 4 mg·ml⁻¹) and control (0 mg·ml⁻¹) in a crop.

III. Physiological response of crops to *Z. schneideriana* root exudates

Relative electrical conductivity (REL): With the treatment of *Z. schneideriana* root exudates, the REL value of the 4-test crops was changed (Fig. 3). At 4 mg·ml⁻¹ solution, the conductivity of all crops increased significantly, indicating that the high-concentration metabolites destroyed the permeability of the membrane. Rape REL was most increased by 71.7 % compared to control, followed by maize (38.2 %) and wheat (26.7 %). At 2 mg·ml⁻¹ solution, a significant increase was found in maize (30.8 %) and wheat (19.8 %) than control. Still, the extent was lower than the treatment of 4 mg·ml⁻¹ solution, especially in rape. It demonstrated that the allelopathic effect on crops by the *Z. schneideriana* root exudates was in response to the acceptors, physiological index, and the dosage of allelopathic chemicals.

Malondialdehyde (MDA) and chlorophyll (Chl) content: The MDA content and Chl content had similar trends (Fig. 3). At the treatment of 4 mg·ml⁻¹ solution, the MDA content of all crops significantly increased, indicating an increase in oxygen radicals and membrane lipid peroxidation in plants. Tall fescue is the most impacted species, followed by maize and wheat. Meanwhile, Chl content significantly decreased in maize and wheat and conversely increased in rape and tall fescue. Further, at 2 mg·ml⁻¹ solution, wheat showed the most significant rise in MDA content, but maize and tall fescue were less affected. In contrast, the Chl content of all crops exhibited an ascending pattern, indicating the trait-specific responses.

Overall, the physiological responses of crops to root exudates of *Z. schneideriana* were stimulatory or mildly inhibitory at low concentrations and were significantly inhibitory at high concentrations. This is consistent with the findings of other investigations, such as the root exudates of *Cynodon dactylon* L at 0.75 mg·ml⁻¹ concentration, stimulated the performance of *Festuca arundinacea* Schreb plants, but was inhibited at 1.50 mg·ml⁻¹ concentration (26). The physiological response was also displayed by specific species and physiological indexes. For instance, the varieties of *F. arundinacea*, Aries, Millennium2, and Free star are sensitive to the leaf extracts at Millennium2, while Greenlabel, Lenge master and Arid3 are least sensitive. The REL value was more sensitive than the MDA content (27). In this report, maize, rape and tall fescue were more susceptible to *Z. schneideriana* root exudates than wheat, while the MDA content was more sensitive than chlorophyll content. Plant interactions are a complicated process.

4. Comprehensive allelopathic responses of crops to *Z. schneideriana* root exudates

Four test crops responded differently to the root exudates of *Z. schneideriana*, considering their physiology and growth performance. Maize was most inhibited from the high dosage of allelopathic chemicals, with a combined RI value of -1.572, followed by rape and wheat, whereas tall fescue plants were less affected. Meanwhile, rape and tall fescue were really improved under the treatment of the low dosage of allelopathic chemicals, especially rape, with a combined RI value of 2.768. Conversely, maize exhibited less sensitivity and wheat showed specific inhibitory effects (Table 2). Therefore, rape could be an ideal specie for intercropping with *Z. schneideriana* trees. Tall fescue is another that might be intercropped more freely in distance.

Table 2. Response index (RI) of the crops to the *Z. schneideriana* root exudates

Species	Root exudates concentration	MDA content	REL	Chl content	Shoot biomass	Root biomass	Total*
Maize	2 mg/ml	0.037	0.308	0.04	0.141	0.099	-0.065
	4 mg/ml	0.726	0.382	-0.166	-0.005	-0.293	-1.572
Rape	2 mg/ml	-0.311	-0.208	0.148	0.398	1.703	2.768
	4 mg/ml	0.224	0.717	0.211	0.025	-0.416	-1.121
Wheat	2 mg/ml	0.089	0.198	0.046	0.074	-0.266	-0.433
	4 mg/ml	0.425	0.267	-0.036	0.011	-0.341	-1.058
Tall fescue	2 mg/ml	0.064	0.012	0.637	0.27	-0.03	0.801
	4 mg/ml	0.902	0.084	0.338	-0.089	-0.096	-0.833

*The data was obtained based on the RI values of a species in a concentration treatment by the formula (Chl content + shoot biomass + root biomass - REL - MDA content)

CONCLUSIONS

Z. schneideriana root exudates contains higher amounts of 2-benzenedicarboxylic acid and bis (2-methylpropyl) ester, these caused significant allelopathic effects on test crops growth depending on crop species and exudates concentrations. Rape and tall fescue can be used as intercropping species for better yield with significant economic benefits.

DECLARATION

We declare that all authors of this Ms have made substantial contributions. We have not excluded any author that substantially contributed to this Ms. We have followed our ethical norms established by our respective institutions.

CONFLICT OF INTEREST

The authors announce that they have no conflict of interest.

ETHICAL APPROVAL

The authors declare that the study was carried out following scientific ethics and conduct. However, this study did not involve any use of animals, hence no ethical approval has been obtained from the concerned committee.

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