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POST-EMERGENT HERBICIDAL ACTIVITY OF MONOTERPENES ON COMMON PURSLANE, *Portulaca oleracea*

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ABSTRACT

The post-emergent herbicidal activity of eight monoterpenes, namely camphor, (*R*)-carvone, 1,8-cineole, cuminaldehyde, (*S*)-fenchone, geraniol, (*S*)-limonene and (*R*)-linalool was evaluated against *Portulaca oleracea* (L.) under glasshouse conditions at 1 and 2% concentration. Results were taken after 5 days of foliar applications. The effect of monoterpenes on chlorophyll contents and total phenolic compounds was also examined at 0.5 and 1% concentrations. All the tested monoterpenes reduced fresh, dry weights and reduced shoot growth of the weed. At 1%, cuminaldehyde caused the highest reduction of 97.4, 92.0 and 74.7% on fresh and dry weights, and shoot length, respectively and geraniol caused 94.1, 90.0 and 72.4 % reductions on fresh, dry weights, and shoot length, respectively. Moreover, cuminaldehyde and geraniol caused complete burning of the plant and complete reduction 100% of fresh weight, dry weight and shoot length at 2%. In contrary, camphor caused the lowest reduction of fresh, dry weight, and shoot length at both concentrations whereas the reduction reached 32.6, 34.6 and 14.8% at 1%, and 63.1, 62.8 and 33.6% at 2%, respectively. The tested monoterpenes showed significant reduction of both chlorophyll a and b contents and phenolic compounds at concentration of 0.5 and 1%, this indicated that monoterpenes may cause adverse effect on photosynthesis and weed metabolism.

Keywords: Monoterpenes, herbicidal activity; glasshouse conditions; chlorophyll contents; phenolic compounds; *Portulaca oleracea*

INTRODUCTION

Herbicide is often used in large amounts because weeds are major problem in agriculture and this led to the evolution of many herbicide resistant weeds and to the contamination of soil and groundwater (Duke *et al*., 2000). Therefore, researchers have focused on new potential bio-herbicides, having different and selective herbicidal mechanisms in comparison to synthetic herbicides (Dudai *et al*., 1999; Duke et al., 2000; Kordali *et al*., 2007).

Plant-derived secondary metabolites including terpenes have multiple ecological functions, the most notable of these lie in the defense. Therefore, screening plant secondary metabolites could lead to discovery of new agents for pest control. Higher plants synthesize a great variety of terpenoids that play a multitude of ecological functions and emit a wide array of volatile compounds, especially the isoprene and monoterpenes, which alone may account for ~55 % of the emitted volatiles (Guenther *et al*. 1995). Monoterpenes (C_{10} unsaturated hydrocarbons) are chemically simple and the most abundant class of terpenoids. They are colorless, lipophilic volatile organic compounds and are the major constituents of floral scents, essential oils, and defensive resins. They play a prominent role in plant defense, various ecological interactions such as allelopathy, pollinator attraction, atmospheric chemistry, and plant–plant communications (Dudareva *et al*., 2006). Monoterpenes are commercially important as flavoring agents, perfumes, and they had a vast array of biological activity as insecticidal, pesticidal, and herbicidal (Isman, 1999, 2000; Kohli *et al*., 1998; Romagni *et al*., 2000). Late, studies have evaluated the use of monoterpenes or essential oils as environmentally safer pesticides and herbicides because of their non-persistence in the soil and no leaching to the ground water due to their lipophilicity, no mammalian toxicity, novel modes of action, and providing prototypes for synthesis of pesticides (Dayan *et al*., 1999; Isman, 2000; Singh *et al*. 2003; Batish *et al*. 2008; Dayan *et al*. 2009; Kaur *et al*. 2010). It has been known that monoterpenes inhibit germination and growth of other plants especially weeds and these effects may proceed through the vapor phase (Fischer 1991), and thus may possess good herbicidal potential (Singh *et al*. 2002a, b, 2006; Kordali *et al*. 2007; Dayan *et al*. 2000, 2009). However, only 1,4-cineole (Duke *et al*. 2002) and eugenol-rich clove oil (Bainard *et al*. 2006) have been used for this purpose. Monoterpene-based commercial herbicide formulations such as cinmethylin (a herbicidal analogue of 1,4-cineole) have been recommended for organic agriculture (Dayan *et al*. 2009).

Common purslane, *Portulaca oleracea*, (Family Portulacaceae), is a highly variable, fast-growing herbaceous annual plant, with a wide distribution. It is growing wildly in most parts of the world, in cold climate areas as well as warm areas. It can be found in cultivated fields, roadsides and waste places. Because of its fast growth, abundant seed production, and survival ability in all types of soils, it is considered as dangerous weed in many vegetable and fruit crops in Egypt.

The aim of this work is to study the post-emergent herbicidal activity of eight monoterpenes (camphor, (*R*)-carvone, 1, 8-cineole, cuminaldehyde, (*S*)-fenchone, geraniol, (*S*)-limonene and (*R*)-linalool) on *Portulaca oleracea* under glasshouse conditions. As far as we know there is no such study on the post-emergent herbicidal activity of monoterpenes applied as foliar application under glasshouse conditions. Also we studied the effect of monoterpenes on chlorophyll contents and total phenolic compounds after foliar application of the tested monoterpenes.

MATERIALS AND METHODS

Monoterpenes

Eight monoterpenes belong to different groups were purchased from Sigma-Aldrich Chemical Co. (Steinheim, Germany). The tested monoterpenes were camphor (98%), (*R*)-carvone (98%), 1-8-cineole (99%), cuminaldehyde (98%), (*S*)-fenchone (98%), geraniol (98%), (*S*)-limonene (96%) and (*R*)-linalool (95%) (Figure 1).

Tested weed

Common purslane, *Portulaca oleracea* (L.) seeds were obtained from Faculty of Agriculture Farm, Alexandria, Egypt. Uniform seeds were selected for the test while undersized and damaged seeds were discarded.

Glasshouse Bioassay

To study the herbicidal effects of eight monoterpenes on 3 weeks-old *Portulaca oleracea* weed, 20 seeds were sowed in plastic pots (15 cm diameter × 20 cm height) filled with clay soil. The experiment was established in glasshouse under natural conditions of sunlight 14/10 h light/dark photoperiod and 30±3˚C. After three weeks of sowing, concentrations of 1 and 2% of the tested monoterpenes were sprayed to the foliage of weed leaves. The solutions of monoterpenes were prepared in distilled water containing 0.02% (v/v) Triton X-100 as a non-ionic surfactant. Distilled water and distilled water containing 0.02% Triton X-100 served as controls. For each treatment three replicates were maintained in a completely randomized design within the glasshouse. Treated weed was kept under observation for 5 days after treatment. Injury of weed such as burning, necrosis, chlorosis, leaf distortion and stunting were visually recorded. The length and fresh weights of weed shoot were measured after 5 days of treatment. The shoots were oven-dried at 80 °C for 48 h and the dry weights were recorded.

Determination of chlorophyll a and b contents

Five monoterpenes, (*R*)-carvone, cuminaldehyde, (*S*)-fenchone, geraniol and (*R*)-linalool were applied at 0.5 and 1% of to determine the effect of these monoterpenes on chlorophyll a and b and on total phenolic compounds. After five days of application fresh leaves of *P. oleracea* (100 mg) were homogenized in 80% aqueous acetone (5 ml). The homogenate was filtered through Whatman filter paper no. 1. The final volume was adjusted at 5 ml by acetone (80%). Total chlorophyll, chlorophyll a and b contents were determined spectrophotometrically using of Unico 1200-Spectrophotometer at 663 nm for chlorophyll a and 647 nm for chlorophyll b. Concentrations were calculated using Lichtenthaler's equation (Lichtenthaler, 1987) and expressed as $\mu g\ g^{-1}$ weight.

Determination of total phenolic compounds

The total phenolic compounds were measured according to the procedure described by (Poonpaiboonpipat *et al*. 2013). In this method, 2.5 ml ethanol was added to 0.5 g of *P. oleracea* fresh leaves and kept in the freezer for 48h. The frozen samples were homogenized using a polytron homogenizer and centrifuged at 10,000 rpm for 10 min. The reaction mixture contained 1 ml of supernatant, 1 ml ethanol, 5 ml distilled water and 0.5 ml 50% Folin-Ciocalteu reagent was added. The mixture was left in the dark at room temperature for 5 min. Then, 1 ml sodium bicarbonate solution (5%) was added to the mixture. The reaction mixture was kept in the dark at room temperature for 1 h. The absorbance was measured at 765 nm by using Unico 1200-Spectrophotometer. The total phenolic was expressed as mg gallic acid equivalent/g fresh weight (mg GAE/g fw). The inhibition percentages of total phenolic were calculated from this equation: $I (\%) = [1 - T/C] \times 100$; where T is the concentration of total phenolic (mg/g fw) in treatment and C is the concentration of total phenolic in control (mg/g fw).

Statistical Analysis

Shoot length, dry, fresh weight and chlorophyll a and b contents were subjected to one-way analysis of variance followed by Student–Newman–

Keuls test (Cohort software Inc. 1985) to determine the significant differences among mean values at the probability level of 0.05.

RESULTS AND DISCUSSIONS

Herbicidal activity of monoterpenes under glasshouse conditions

Table 1 showed the effect of monoterpenes on fresh and dry weights of two-leaf stage *Portulaca oleracea* after 5 days of foliar application at rates 1 and 2%. The tested monoterpenoids reduced the fresh and dry weights of the weed in a dose dependent manner whereas, the reduction of fresh weight ranged from 32.6% to 97.4% at 1% and from 62.8% to 100% at 2%. Cuminaldehyde caused the highest reduction rates of 97.4% and 92.0% on both fresh and dry weights at 1%, followed by geraniol which caused 94.1% and 90.0%, respectively. Cuminaldehyde and geraniol caused complete reduction (100%) of fresh and dry weights at 2%. Camphor showed the lowest reduction of both fresh and dry weights with 32.6 and 34.6% at 1%, and 63.1 and 62.8% at 2%, respectively (plate 2). Some of the phytotoxicity symptoms including chlorosis, necrosis, stunting, leaves wilting and burning were observed on the treated weed. These symptoms led to plant death within 5 days of treatment. This finding implies that the tested monoterpenes like other herbicides induces severe injuries in weeds upon contact.

Previous studies showed that 1,8-cineole, linalool, limonene and camphor have phytotoxic effects against various plant species (Abrahim *et al*. 2000; Singh *et al*. 2002a; Singh *et al*. 2006). In addition, (De Martino *et al*. 2010) found that geraniol, carvone and limonene affected the germination of seeds with inhibition of 58%, 34% and 20%, respectively. But there are no reported studies on the post-emergent herbicidal activity of monoterpenes in the literature so far.

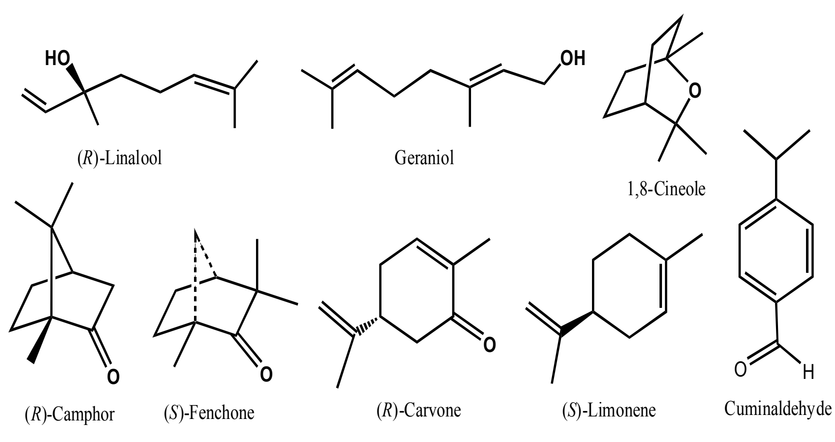

Figure 1. The chemical structures of the tested monoterpenes.

Table 1. Effect of monoterpenes on fresh and dry weights of two-leaf stage *Portulaca oleracea* weed after 5 days of foliar application

Conc (%)	Reduction (%)							
	(*R*)-Carvone		1,8-Cineole		Cuminaldehyde		(*S*)-Fenchone	
	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
0	0.00	0.00	0.00	0.00	0.0.0	0.00	0.00	0.00
1	87.0	84.6	71.4	73.0	97.4	92.00	60.5	65.4
2	90.7	88.5	83.7	80.8	100.0[a]	100.0[a]	76.7	73.0
Conc (%)	Reduction (%)							
	Geraniol		(*S*)-Limonene		(*R*)-Linalool		Camphor	
	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry Weight
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	94.1	90.0	74.4	73.0	71.2	73.0	32.6	34.6
2	100.0[a]	100.0[a]	88.6	88.5	73.3	73.0	62.8	63.1

[a] **complete burring of plant (death)**

Table 2 demonstrates the effect of monoterpenes on shoot growth of two-leaf stage *P. oleracea* weed after 5 days of foliar application. All the tested monotepenes reduced the shoot growth at both 1 and 2% concentrations in a dose dependent manner. At 1% cuminaldehyde was the most effective monoterpene causing 74.7% reduction of shoot growth, then geraniol by 72.4% growth inhibition, while camphor was the least effective one by 14.8% growth reduction. At 2% cuminaldehyde and geraniol were the most potent monoterpenes causing complete reduction (100%) of shoot growth, while camphor caused the lowest reduction of shoot growth with inhibition of 33.6% (plate 2).

Table 2. Effect of monoterpenes on shoot length (cm) of two-leaf stage *Portulaca oleracea* weed after 5 days of foliar application[a]

Conc (%)	(*R*)-Carvone		1,8-Cineole		Cuminaldehyde		(*S*)-Fenchone	
	shoot length[a]	R (%)[b]	shoot length	R (%)	shoot length	R (%)	shoot length	R (%)
0	7.98±0.99a[c]	0.00	7.98±0.99a	0.00	7.98±0.99a	0.00	7.98±0.99a	0.00
1	2.64±0.61b	66.9	4.30±0.54b	46.1	2.02±0.53b	74.7	5.04±0.78b	36.8
2	2.40±0.34b	69.9	3.40±0.48c	57.4	0.00±0.00c	100	2.80±0.41c	64.9
Conc (%)	Geraniol		(*S*)-Limonene		(*R*)-Linalool		Camphor	
	shoot length	R (%)	shoot length	R (%)	shoot length	R (%)	shoot length	R (%)
0	7.98±0.99a	0.00	7.98±0.99a	0.00	7.98±0.99a	0.00	7.98±0.99a	0.00
1	2.20±0.83b	72.4	3.80±0.21b	52.4	4.40±0.41b	44.9	6.80±0.34b	14.8
2	0.00±0.00c	100	2.90±0.19c	63.7	4.26±0.29b	46.6	5.30±0.21c	33.6

[a]**Data are expressed as means ± SE from experiments with three replicates.**
[b]**R, mean percent of reduction comparing of untreated control.**
[c]**Mean values within a column sharing the same letter are not significantly different at the 0.05 probability level.**

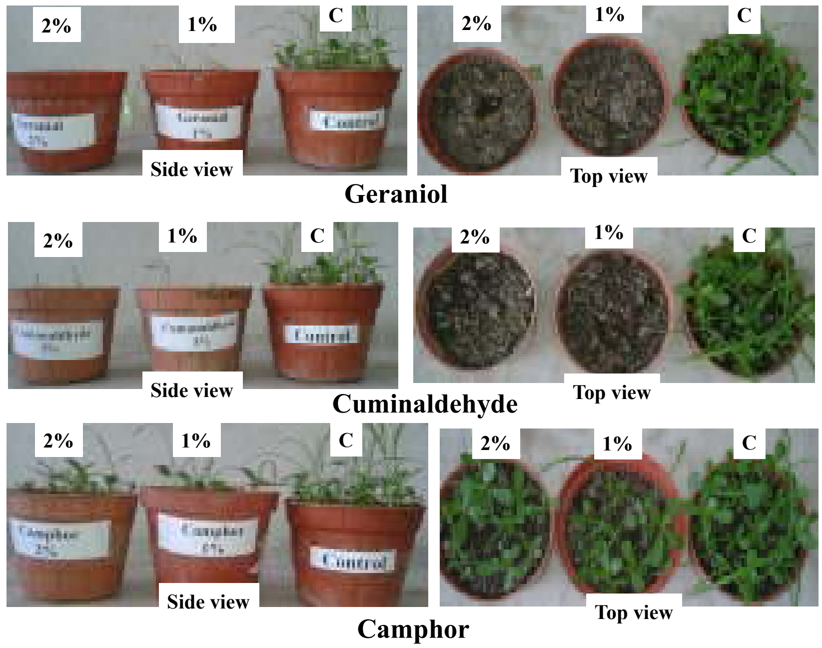

Plate 2. Herbicidal effects of geraniol, cuminaldehyde, and camphor on two-leaf stage *Portulaca oleracea* after 5 days of foliar application.

Effect of monoterpenes on chlorophyll a and b.

Table 3 shows the effect of five monoterpenes on chlorophyll a and b contents (µg g^{-1} fw) of two-leaf stage *P. oleracea* weed after 5 days of foliar application at concentration of 0.5 and 1%. The tested monoterpenes showed significant reduction of both chlorophyll a and b content. Carvone and geraniol showed the highest reduction in chlorophyll a, at concentration 0.5% causing 31.9% and 27.4% reduction, respectively. Geraniol and fenchone reduced chlorophyll b by 26.0% and 23.7%, respectively, at the same concentration. At concentration 1% geraniol caused the highest reduction in chlorophyll a and b by 44.2% and 43.5%, respectively, followed by fenchone 44.0% and 42.4% and cuminaldehyde 42.5% and 40.7%. The reduction in chlorophyll content observed in this study is in agreement with previous studies indicated that the monoterpenes had a potential to reduce chlorophyll content. For example, monoterpenes such as 1,8-cineole, citronellol, citronellal, linalool and β-pinene were reported to reduce chlorophyll content (Romagni *et al*., 2000; Singh *et al*., 2002b; Kaur *et al*., 2010; Chowhan *et al*., 2011). The mechanism of chlorophyll reduction by monoterpenes is not fully understood. However, it has been suggested that the reduction of chlorophyll content may be due to inhibition of biosynthesis of chlorophyll and/or degradation of chlorophyll.

Table 3. Effect of monoterpenes on chlorophyll a and b contents (µg g^{-1} FW) of two-leaf stage *Portulaca oleracea* weed after 5 days of foliar application

Conc (%)	(*R*)-Carvone		Cuminaldehyde		(*S*)-Fenchone	
	Chl a (µg g^{-1}) (R %)[a]	Chl b (µg g^{-1}) (R %)	Chl a (µg g^{-1}) (R %)	Chl b (µg g^{-1}) (R %)	Chl a (µg g^{-1}) (R %)	Chl b (µg g^{-1}) (R %)
0	4.96a[b] (0.00)	7.08a (0.00)	4.96a (0.00)	7.08a (0.00)	4.96a (0.00)	7.08a (0.00)
0.5	3.38b (31.9)	6.40b (9.6)	4.22b (15.0)	5.62b (20.6)	3.72b (25.0)	5.40b (23.7)
1	3.14c (36.7)	5.86 c (17.2)	2.85c (42.5)	4.20c(40.7)	2.78c (44.0)	4.08c (42.4)

Conc (%)	Geraniol		(*R*)-Linalool	
	Chl a (µg g^{-1}) (R %)	Chl b (µg g^{-1}) (R %)	Chl a (µg g^{-1}) (R %)	Chl b (µg g^{-1}) (R %)
0	4.96a (0.00)	7.08a (0.00)	4.96a (0.00)	7.08a (0.00)
0.5	3.60b (27.4)	5.24b (26.0)	4.22b (15.0)	5.86 b (17.2)
1	2.77c (44.2)	4.00c (43.5)	3.33c (33.0)	4.67c(34.03)

[a]R, mean percent of inhibition comparing of untreated control.
[b]Mean values within a column sharing the same letter are not significantly different at the 0.05 probability level.

Effect of monoterpenes on total phenolic contents

The effect of monoterpenes on total phenolic contents of two-leaf stage *P. oleracea* weed after 5 days of foliar application showed in table 4. At concentration 0.5% the tested monoterpenes reduced total phenolic contents; cuminaldehyde caused the highest reduction by 32%, then geraniol by 17.3%. However, at 1% concentration the tested monotepenes increased total phenolic compounds except for geraniol reduced phenolic contents by 43.7% and cuminaldehyde 28.3%. On the other hand linalool showed no effect on total phenolic contents. Similar findings were previously reported on the effect of α-pinene and β-pinene on phenolic compounds of maize (Areco *et al*., 2014) and some allelochemicals were shown to increase the phenolic content (Janaguiraman *et al*., 2005).

Table 4. Effect of monoterpenes on total phenolic contents of two-leaf stage *Portulaca oleracea* weed after 5 days of foliar application

Conc (%)	Reduction (%)[a]		
	(*R*)-Carvone	Cuminaldehyde	(*S*)-Fenchone
0	0.0	0.0	0.0
0.5	9.4	32	10.2
1	-10.4	28.3	-14.2

Conc (%)	Reduction (%)	
	Geraniol	(*R*)-Linalool
0	0.0	0.0
0.5	17.3	0.0
1	43.7	0.0

[a]R, mean percent of inhibition comparing of untreated control.

The present study showed that some tested monoterpenes such as cuminaldehyde, geraniol, carvone and fenchone had strong phytotoxicity against broad leave weed *P. oleracea*. It was also clear that these monoterpenes caused reduction in chlorophyll contents and phenolic compounds. These results indicating that these compounds may affect the plant photosynthesis and biosynthesis of secondary metabolites. These findings are in agreement with those reported by (Batish *et al*. 2004) who indicated that the monoterpenes may cause their phytotoxic effect by affecting the photosynthetic machinery and energy metabolism of plant. The results of this study could be useful start to develop new bio-herbicides, but further studies are needed to evaluate its herbicidal potential under field conditions, prepare formulations to enhance its activity, develop chemical derivatives to overcome its rapid volatilization and lipophilicity and study their mode of action and their effects on non-target organisms.

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نشـاط مركبـات المونوتربينـات كمبيدات حشـائش بعد الانبثـاق ضد حشيشـة الرجلـة *Portulaca oleracea*

منى منصور جبريل سعد و نعمة عبد الرازق جودة

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تم دراسة فعالية ثمانية مركبات من المونوتربينات وهى camphor, (*R*)-carvone, 1,8-cineole, cuminaldehyde, (*S*)-fenchone, geraniol, (*S*)-limonene, (*R*)-linalool رشا على المجموع الخضري على حشيشة الرجلة *Portulaca oleracea* common purslane تحت ظروف الصوبة الزجاجية. حيث تمت معاملة النباتات في مرحلة الورقتين two-le``af stage بتركيزي 1% و 2% من المركبات المختبرة. كذلك تم دراسة تأثير بعض هذه المركبات على محتوى الكلوروفيل والمركبات الفينولية بالأوراق بتركيزي 0.5% و 1%. كل المركبات أدت لتقليل الوزن الرطب والجاف وكذلك لتثبيط نمو المجموع الخضري لحشيشة الرجلة بعد 5 أيام من المعاملة السطحية بمحلول المونوتربينات. كان مركب cuminaldehyde اكثرهم تثبيطا حيث اعطى نسبة تثبيط 97.4% و 92.0% و 74.7% لكل من الوزن الرطب والجاف و طول النمو الخضري على الترتيب عند تركيز 1%. كما سبب مركب geraniol تثبيطا بنسبة 94.0% و 90.0% و 72.4% لكل من الوزن الرطب والجاف و طول النمو الخضري على الترتيب عند نفس التركيز. أما عند تركيز 2% فسبب مركبي cuminaldehyde وgeraniol احتراق وموت كامل للنبات. بينما كان مركب camphor أقل المركبات تأثيرا عند كلا التركيزين حيث سبب نسبة تثبيط 32.6% و 34.6% و 14.8% لكل من الوزن الرطب والجاف و طول النمو الخضري على الترتيب عند 1% ونسبة تثبيط 63.1% و 62.8% و 33.6% عند تركيز 2%. كما أظهرت المركبات اعراض سمية نباتية phytotoxicity وتفاوتت هذه الاعراض من اصفرار للاوراق, تبقع, ذبول و احتراق كامل للنبات. كما اظهرت المركبات تثبيطا للكلوروفيل عند معاملة النبات في مرحلة ورقتين بتركيزي 0.5% و1% وكذلك أظهرت تأثير على محتوى المركبات الفينولية بالنبات مما يعطي دلالة على ان هذه المركبات قد يكون لها تأثير على عملية البناء الضوئي والميتابوليزم في هذه الحشيشة.