ISSN 2449-8955

European Journal of Biological Research

Research Article

Physiological effects of allelopathic activity of *Citrullus colocynthis* on *Vicia faba* and *Hordeum vulgare*

Hediat M. H. Salama* and Hala K. A. Al Rabiah

Botany and Microbiology Department, Faculty of Science, King Saud University, Riyadh, Saudi Arabia.
*Corresponding author: Hediat M. H. Salama; Botany and Microbiology Department, Faculty of Science, King Saud University, Riyadh, Saudi Arabia; e-mail: hoda.salama@hotmail.com

TMKARPIŃSKI PUBLISHER

Received: 26 March 2015; **Revised submission:** 11 May 2015; **Accepted:** 13 May 2015

ABSTRACT

The objective of this study was to determine the impact of allelopathic potentials of different extract of *Citrullus colocynthis* shoot system on germination and metabolite accumulation in *Vicia faba* and *Hordeum vulgare*. *Citrullus colocynthis was* collected from Al-Thomamah region, Saudi Arabia and the experimental design was a complete randomized with three replicates. Water, chloroform and methanol extracts from dried shoot system of *Citrullus colocynthis* with different concentrations (25, 50, 75 and 100%) were prepared, in addition to distilled water (as Control). The results showed that great reduce in germination of *Hordeum vulgare* but *Vicia faba* was not affected. The seedling growth was more sensitive than the seed germination. The allelopathic effects of *Citrullus colocynthis* on growth showed slightly increase in shoot and root lengths. The leaf area was decreased in *Hordeum* and *Vicia* for all concentrations. Pigments (Chlorophyll A, B and caroteins) were increased in *Vicia* for all concentrations of extracts, and showed variations in *Hordeum* compared to control. Results also showed decrease of carbohydrates and increase of proteins for the both studied plants.

Keywords: *Citrullus colocynthis*, Allelopathy, Germination, Carbohydrates, Growth, Dry weight.

1. INTRODUCTION

Plants may affect other plants growing in their vicinity in a stimulatory or inhibitory manner through released biologically active compounds often termed as allelopathics, allelocompounds or allelochemicals. This phenomenon is termed as allelopathy, receiving an increased attention recently and is considered to be applied in practice for weeds and pest managements [1]. Allelopathic effects are common to many plant species and can be observed at any level of biological organization [2-6]. Plant extract that is not decomposed was thought to contain secondary compounds with allelochemical activity or phytotoxic which cause growth inhibiton [7]. However allelopathy may alter the available resources in the environment [8]. Allelochemicals are believed to be a joint action of several secondary metabolites including phenolic compounds [9], flavonoids [10], juglone [11] and terpenoids [12]. Many researchers have found that the inhibitory substances involved in allelopathy are terpenoids and phenolic substance [13, 14].

Citrullus colocynthis (L.) is an important medicinal plant belonging to the family Cucurbitaceae. It is an annual herb widely distributed in Saudi Arabia [15]. The curative properties of medicinal plants are mainly due to the presence of various complexes chemical substances of different composition which occur as secondary metabolites [16] several active chemical constituents of *C. colocynthis* plant were recorded. They are grouped as alkaloids, flanonoids, tannins, and essential oils [17]. A number of plant secondary metabolites including flavonoids and cucurbitacins have previously been reported from *C. colocynthis* [18]. The cucurbitacins are of great interest because of the wide range of biological activities exhibited in plants and animals. Plant based natural constituents can be derived from any part of the plant like stems, leaves, flowers, roots, fruits and seeds [19]. Sunil et al. [20] studied antioxidant and free radical scavenging potential of *C. colocynthis* methanolic fruit extract. *Citrullus colocynthis* is also one of the plants belonging to family Cucurbitaceae. It has a fruit commonly known as bitter apple. It has been used in herbal treatment of diabetes [21]. The aqueous pulb extract of fruit is used for kidney, liver function treatment [22]. The phenolic compounds isolated from plants are of great interest due to their antioxidative and anticarcinogenic activity. They play a very important role in absorbing and neutralizing free radicals. They contain not only minerals and primary metabolite but also a diverse array of secondary metabolite with antioxidant potential [23].

The purpose of this study was to assess the allelopathic effects of *Citrullus colocynthis* on seed germination characteristics, primary growth and biochemical changes associated with *Vicia faba* and *Hordeum vulgare*.

2. MATERIALS AND METHODS

Samples of *Citrullus colocynthis* were collected from Al-Thomamah region, Saudi Arabia during April 2011 and identified by plant taxonomist. A voucher specimen has been deposited at the Herbarium of Botany Department, Faculty of Science, King Saud University. The seeds of studied plants were also collected as follows: seeds of bean, *Vicia faba* crop 2011, seeds of barley, *Hordeum vulgare* crop 2010.

2.1. Preparation of water extract

10 gm of powder air dried *Citrullus collocythis* in a flask and added 200 ml of distilled water. Then magnetic stirrer for 15 minutes was carried out. The samples were left for 48 hours, and then filtrate. The filtrate was centrifuged for 15 min. to get a clear solution (stock solution) [24]. Prepare of 25, 50, 75 and 100% concentrations.

2.2. Preparation of methanol extract

According to Laddy et al. [25], 10 gm of air dried plant material was extracted by 200 ml methanol in soxhlet extractor for 24 hours at 40-45˚C. The residue obtained after rotary evaporator was dissolved in 3 ml methanol and completed to 100 ml by distilled water. It were prepared the 25, 50 and 100 % concentrations.

2.3. Preparation of chloroform extract

According to Laddy et al. [25], the concentrations of 25, 50 and 100% of chloroform extract were prepared.

2.4. Germination experiment

The experimental design was randomized complete block design with three replications. Seeds of *Vicia faba* and *Hordeum vulgare* were sterilized in 5% sodium hypochlorite solution for 10 minutes, rinsed through with de ionized water several times. Their germination was conducted on water porous paper support in petri dishes (25 seed per dish) at controlled temperature of 25± 1˚C and adds 7.5-15.0 ml of extract or distilled water. Then cover the dishes with paper para film to prevent evaporation and pollution and leave for 10 days. The number of germinated seeds was recorded in each dish [26].

2.5. Determination and analysis of growth parameters

Seeds of *Vicia faba* and *Hordeum vulgare* were germinated in pots. The pots of 14 cm diameter and 18 cm in height were filled with fertile

loam up to ¾ the height of the pot. Daily supply with 15 ml of *Citrullus colocynthis* extracts (25, 50, 75 and 100% water, chloroform and methanol) and control is added to the study plants. Plant growth being conducted in controlled conditions of temperature (25± 1˚C) illumination (dark/light cycle: 14/10 h) and 80% humidity into a green house of Botany Department, Faculty of Science, King Saud University.

Extracts of *Citrullus* were added daily in different concentrations (25, 50, 75 and 100%) for each test plants. Each concentration was prepared in three replicates. After 36 days of growth, the shoot and root lengths were long enough to measure using a ruler.

Fresh and dry weights were measured, leaf surface area were measured using portable area meter Model Li – 3000. Chlorophyll A, chlorophyll B and carotenoid pigments were accomplished based on method of Stirban [27], carbohydrate content was measured according to Nelson [28] and Sonogyi [29]. Protein content was measured according to Lowry et al. [30].

2.6. Statistical analysis

Each treatment was conducted with their replicates and the results were presented as mean ± SD (standard deviation). Each of the experimental values was compared to its corresponding control. The results were analyzed by one way Anova with used statistical package for social sciences (SPSS) Version 11.5.

Table 1. Germination percentage of *Hordeum vulgare and Vicia faba.*

Treatment	*Hordeum vulgare* germination %	LSD 0.05	*Vicia faba* germination %	LSD 0.05
Control	100 ± 0		100 ± 0	
Water 100%	15 ± 1	85.00 (*)	100 ± 0	.00
Water 75%	42.33 ± 2.51	57.66 (*)	100 ± 0	.00
Water 50%	65 ± 1	35.00 (*)	90.33 ± 0.57	9.6667 (*)
Water 25%	66.16 ± 1.25	33.83 (*)	100 ± 0	.00
Chloroform 100%	-	-	100 ± 0	.00
Chloroform 50%	5 ± 1	95.00 (*)	100 ± 0	.00
Chloroform 25%	70 ± 5	30.00 (*)	100 ± 0	.00
Methanol 100%	32 ± 2	68.00 (*)	94.66 ± 0.577	5.33 (*)
Methanol 50%	46.83 ± 4.53	53.16 (*)	100 ± 0	.00
Methanol 25%	85.33 ± 1.52	14.66 (*)	100 ± 0	.00

* The mean difference is significant at the .05 level. Mean of three replications in duplicates ± Standard deviation.

3. RESULTS AND DISCUSSION

3.1. Seed germination

The effect of *Citrullus colocynthis* extracts on the seed germination of *Hordeum vulgare* and *Vicia faba* is shown in (Table 1). Water extract at low concentrations (25, 50%) have slightly inhibitory effect on the seed germination of *H. vulgare* compared to control. At high concentrations (75, 100%) it showed significance inhibition. While seed germination of *V. faba* is not affected by water extract. Chloroform and methanol extracts showed significant inhibition at high concentration, the inhibition increase by increasing the concentration. While, seed germination of *V. faba* is not affected by chloroform and methanol extracts.

This indicates that the aqueous, chloroform and methanol extracts contained growth inhibiting allelochemicals and their effects were dependent on the extract of *Citrullus* concentration. These results were in agreement with Abdel Fattah et al. [31] who

observed that allelopathic effects can cause both stimulatory and suppressive effects at lower and higher concentrations respectively.

The same results were obtained by Seyed et al. [32] who showed that in different extract concentration of *Artemisia annua*, the most germination percentage is related to control and the least was related to 100% of the extract. Also, other scientists such as Mahmood *et al*. [33] and Abhinav and Kanade [34] revealed that the inhibitory effect of the extracts increased with increasing extract concentration.

Table 2. Length of shoot at start and after 36 days of treatment for *Hordeum vulgare and Vicia faba.*

Treatment	*Hordeum vulgare*				*Vicia faba*			
	Length of shoot at the start of experiment cm^2	**LSD 0.05**	**Length of shoot after 36 days of experiment cm^2**	**LSD 0.05**	**Length of shoot at the start experiment cm^2**	**LSD 0.05**	**Length of shoot after 36 days of experiment cm^2**	**LSD 0.05**
Control	12.667 ± 0.577		18.667 ± 0.577		20 ± 1		52 ± 2	
Water 100%	12.667 ± 0.577	.000	16.333 ± 1.155	2.33	17 ± 2	3.00(*)	30 ± 3	22.00(*)
Water 75%	13 ± 1	.333	14 ± 1	4.6667(*)	17 ± 2	3.00(*)	31 ± 2	21.00(*)
Water 50%	13 ± 1	.333	20 ± 1	1.33	17 ± 2	3.00(*)	41 ± 2	11.00(*)
Water 25%	13 ± 0	.333	28 ± 2	9.33(*)	20 ± 1	.00	30 ± 3	22.00(*)
Chloroform 100%	14 ± 1	1.333(*)	17 ± 2	1.6667	20 ± 1	.00	41 ± 2	11.00(*)
Chloroform 50%	12.667 ± 0.577	.00	19 ± 1	.333	20 ± 1	.00	30 ± 3	22.00(*)
Chloroform 25%	12.667 ± 0.577	.000	16 ± 1	2.6667	20 ± 1	.00	38 ± 2	14.00(*)
Methanol 100%	12.667 ± 0.577	.000	13.333 ± 0.577	5.33(*)	20 ± 1	.00	36 ± 4	16.00(*)
Methanol 50%	13 ± 1	.333	17 ± 1.732	1.6667	20 ± 1	.00	40 ± 2	12.00(*)
Methanol 25%	13.333 ± 0.577	.6667	22.667 ± 3.786	4.00(*)	20 ± 1	.00	33 ± 3	19.00(*)

* The mean difference is significant at the .05 level. Mean of three replications in duplicates ± Standard deviation.

3.2. Shoot and root lengths

Table 2 shows that the low concentrations of aqueous extract (25, 50%) had stimulation effect on shoot lengths in *Hordeum vulgare* while higher concentrations (75, 100%) induced greater inhibition after 36 days of treatment. similar results were recorded by chloroform and methanol extract. In *Vicia faba*, various solvent extracts reduced shoot length at all different concentration after 36 days of treatment.

Table 3 shows that the aqueous extract revealed inhibition of root lengths at 100, 75 and 50% and stimulated root lengths at 25%. However, chloroform extracts stimulated root lengths at 100%, 50% and 25%. Methanol extracts stimulated root lengths at 50% only for *Hordeum vulgare.* In case of *Vicia faba* all extracts of *Citrullus colocynthis* showed significance stimulation of root lengths at all concentrations. The same results were obtained by Mahmood et al. [33], who recorded that methanolic extract significanthy inhibited root and shoot growth. The allelopathic effect of *Citrullus colocynthis* has been attributed to the production of several active chemical constituents. They are grouped as alkaloids, flavonoids [17], saponins, tannins, carbohydrates, glycosides and essential oils [19].

Table 3. Length of root after 36 days of treatment for *Hordeum vulgare and Vicia faba.*

Treatment	Length of root after 36 days of experiment cm^2	LSD 0.05	Length of root after 36 days of experiment cm^2	LSD 0.05
Control	16 ± 1		7 ± 1	
Water 100%	12.33 ± 0.58	3.6667(*)	10 ± 1	3.00(*)
Water 75 %	15 ± 1	1.00	15 ± 1	8.00(*)
Water 50%	14 ± 2	2.00	10 ± 1	3.00(*)
Water 25%	17 ± 2	1.00	12 ± 2	5.00(*)
Chloroform 100%	18 ± 1	2.00	11 ± 2	4.00(*)
Chloroform 50%	16.67 ± 0.58	.6667	19 ± 2	12.00(*)
Chloroform 25%	16.67 ± 0.58	.6667	9 ± 2	2.00
Methanol 100%	15 ± 2	1.00	11 ± 2	4.00(*)
Methanol 50%	17 ± 2	1.00	11 ± 2	4.00(*)
Methanol 25%	15 ± 2	1.00	15 ± 2	8.00(*)

* The mean difference is significant at the .05 level. Mean of three replications in duplicates ± Standard deviation.

Table 4. Fresh and dry weight of shoot after 36 days of treatment for *Hordeum vulgare and Vicia faba.*

Treatment	*Hordeum vulgare*				*Vicia faba*			
	Fresh wt. g	LSD 0.05	Dry wt. g	LSD 0.05	Fresh wt. g	LSD 0.05	Dry wt. g	LSD 0.05
Control	0.45 ± 0.01		0.11 ± 0.03		7.81 ± 0.02		0.8 ± 0.02	
Water 100%	0.26 ± 0.01	.186(*)	0.02 ± 0.01	.090(*)	3.6 ± 0.05	4.210(*)	0.35 ± 0.02	.450(*)
Water 75%	0.29 ± 0.01	.160(*)	0.03 ± 0.02	.083(*)	3.71 ± 0.02	4.100(*)	0.36 ± 0.03	.440(*)
Water 50%	0.39 ± 0.02	.060(*)	0.05 ± 0.01	.063(*)	3.85 ± 0.05	3.960(*)	0.38 ± 0.02	.320(*)
Water 25%	0.46 ± 0.02	.0100	0.05 ± 0.02	.063(*)	3.82 ± 0.02	3.990(*)	0.48 ± 0.04	.420(*)
Chloroform 100%	0.21 ± 0.01	.240(*)	0.02 ± 0.01	.096(*)	2.52 ± 0.02	2.350(*)	0.24 ± 0.04	.190(*)
Chloroform 50%	0.21 ± 0.01	.240(*)	0.02 ± 0.01	.096(*)	3.67 ± 0.03	5.290(*)	0.61 ± 0.02	.560(*)
Chloroform 25%	0.36 ± 0.01	.090(*)	0.04 ± 0.01	.073(*)	5.46 ± 0.02	4.140(*)	0.41 ± 0.02	.390(*)
Methanol 100%	0.29 ± 0.02	.070(*)	0.03 ± 0.01	.073(*)	2.44 ± 0.04	3.820(*)	0.22 ± 0.02	.350(*)
Methanol 50%	0.38 ± 0.03	.160(*)	0.04 ± 0.01	.083(*)	3.91 ± 0.03	3.900(*)	0.33 ± 0.03	.470(*)
Methanol 25%	0.77 ± 0.02	.320(*)	0.08 ± 0.02	.033(*)	3.99 ± 0.04	5.370(*)	0.45 ± 0.05	.580(*)

* The mean difference is significant at the .05 level. Mean of three replications in duplicates ± Standard deviation.

3.3. Fresh and dry weights

Chloroform extract had the highest inhibitory effect on both fresh and dry weight of *H. vulgare* (0.21 and 0.02 gm respectively) while the highest fresh and dry weights were observed at low concentrations of aqueous and methanol extracts (0.46 and 0.77 gm fresh wt.), (0.05 and 0.08 gm dry wt. respectively (Table 4).

In *Vicia faba* it was found that higher concentrations had pronounced inhibitory effect on shoot fresh and dry weights (Table 4). In general at all extracts, highest concentrations induced allelopathic effects for *H. vulgare* and *V. faba*. Maximum fresh and dry weights were observed in untreated control. These results were similar to those of Malik

[35], El Khawas and Shehata [36] and Yamagushi et al. [37] that studied allelopathic effects of *E. globulus* leaf extract on germination and seedling growth of some vegetable and crop plants. Fresh and dry weight in *H. vulgare* and *V. faba* were also reduced significantly in all extracts, these results are in agreement to those obtained by Djanaguiraman et al. [38] who found that seedling dry matter of rice, sorghum and blackgram significantly reduced by leaf leachate of *E. globulus* and highest inhibition was observed in highest concentration. Fresh and dry weights of three wheat cultivars decreased in response to aqueous eucalyptus extract [39].

3.4. Leaf surface area

The effect of aqueous extract of *C. colocynthis* on the surface area showed significant inhibition at both high and low concentration (0.17 and 0.14 cm^2 respectively) for *Hordeum vulgare*. The same results were observed for chloroform extract at high and low concentration (0.05 and 0.18 cm^2 respectively). But the methanol extract of *C. colocynthis* showed significant stimulation at both high and low concentration (0.28 and 0.92 cm^2 respectively) for *H. vulgare.* The effect of all extracts of *C. colocynthis* on *Vicia faba* showed significant inhibition at all concentration. At concentration 50% chloroform showed significant stimulation for leaf surface area (7.22 cm^2) for *V. faba* (Table 5). These results are in agreement with An et al. [17]. They showed that any secondary compound with allelochemical activity can cause both stimulatory and inhibitory effects.

3.5. Chlorophyll content

Table 6 shows that the effect of different extracts of *C. colocynthis* on chlorophyll content (Ch. A, B and carotenoids) were differ greatly on *Hordeum vulgare* and *Vicia faba*. Aqueous extract (50%) stimulated chlorophyll A, B and carotenoids (0.87, 0.34 and 0.468 mg/g respectively) for *Hordeum vulgare*, also methanol extract stimulated chlorophyll A, B and carotenoids (0.98, 0.43 and 0.546 mg/g respectively). High concentrations of aqueous and chloroform extract inhibit Ch. A, B and carotenoids for *H. vulgare*. In *Vicia faba* all extracts (Aqueous, Chloroform and Methanol) with different concentrations (100, 75, 50 and 25%) simulated chlorophyll A. B and carotenoids. The highest stimulatory was effect on Chl. A were found in 25% aqueous, 50% chloroform and 50% methanol extracts being (0.837, 1.524 and 0.737 mg/g respectively). These results are supported by the findings of Corsato et al. [40] and Gliessman [41], who stated that the allelopathic effects is a natural interference in which the plant produces substances and metabolites that may benefit or harm other plants when released.

Table 5. Leaf surface area of *Hordeum vulgare and Vicia faba.*

	Hordeum vulgare		***Vicia faba***	
Treatment	**Leaf area cm^2**	**LSD 0.05**	**Leaf area cm^2**	**LSD 0.05**
Control	0.27 ± 0.02		6.12 ± 0.217	
Water 100%	0.17 ± 1.02	3.2900(*)	4.093 ± 0.084	2.0267(*)
Water 75%	1.39 ± 2.02	.5700	5.583 ± 0.635	.5367
Water 50%	0.06 ± 3.02	.7933	6.1567 ± 0.482	.0367
Water 25%	0.14 ± 4.02	.6233	4.9 ± 0.056	1.2200(*)
Chloroform 100%	0.05 ± 5.02	.6133	4.7 ± 0.329	1.4200(*)
Chloroform 50%	0.24 ± 6.02	.6167	7.22 ± 0.437	1.1000(*)
Chloroform 25%	0.18 ± 7.02	.2900	4.6167 ± 0.499	1.5033(*)
Methanol 100%	0.28 ± 8.02	2.2567(*)	5.1167 ± 1.139	1.0033(*)
Methanol 50%	0.66 ± 9.02	.6533	4.383 ± 0.802	1.7367(*)
Methanol 25%	0.92 ± 10.02	1.2567(*)	5.133 ± 0.317	.9867(*)

* The mean difference is significant at the .05 level. Mean of three replications in duplicates ± Standard deviation.

Table 6. Chl. A, B and carotenoids of *Hordeum vulgare and Vicia faba.*

	Hordeum vulgare						***Vicia faba***					
Treatment	**Chl. A mg/g**	**LSD 0.05**	**Chl. B mg/g**	**LSD 0.05**	**Carote-noid mg/g**	**LSD 0.05**	**Chl. A mg/g**	**LSD 0.05**	**Chl. B mg/g**	**LSD 0.05**	**Carote-noid mg/g**	**LSD 0.05**
Control	0.76 ± 0.01		0.301 ± 0.003		0.424 ± 0.004		0.459 ± 0.009		0.203 ± 0.003		0.256 ± 0.006	
Water 100%	0.55 ± 0.05	.20967 (*)	0.248 ± 0.002	.05333 (*)	0.332 ± 0.002	.09200 (*)	0.769 ± 0.011	.3103 (*)	0.351 ± 0.002	.1480 (*)	0.458 ± 0.008	.2020 (*)
Water 75%	0.7 ± 0.09	.05667	0.339 ± 0.003	.03733 (*)	0.436 ± 0.004	.01200 (*)	0.563 ± 0.003	.1040 (*)	0.442 ± 0.002	.2390 (*)	0.348 ± 0.004	.0920 (*)
Water 50%	0.87 ± 0.02	.10800	0.34 ± 0.005	.03867 (*)	0.468 ± 0.003	.04400 (*)	0.664 ± 0.008	.2057 (*)	0.389 ± 0.004	.1860 (*)	0.426 ± 0.005	.1700 (*)
Water 25%	0.29 ± 0.2	.46900 (*)	0.203 ± 0.007	.09833 (*)	0.241 ± 0.003	.18300 (*)	0.837 ± 0.007	.3780 (*)	0.395 ± 0.005	.1920 (*)	0.561 ± 0.004	.3050 (*)
Chloroform %100	0.35 ± 0.05	.41000 (*)	0.262 ± 0.002	.03933 (*)	0.337 ± 0.007	.08700 (*)	0.584 ± 0.004	.1250 (*)	0.334 ± 0.004	.1310 (*)	0.343 ± 0.003	.0870 (*)
Chloroform 50%	0.48 ± 0.2	.27633 (*)	0.307 ± 0.006	.00567	0.37 ± 0.005	.05400 (*)	1.524 ± 0.004	1.0650 (*)	0.828 ± 0.008	.6250 (*)	0.577 ± 0.007	.3210 (*)
Chloroform 25%	0.79 ± 0.1	.02533	0.66 ± 0.004	.35867 (*)	0.517 ± 0.007	.09300 (*)	0.567 ± 0.009	.1080 (*)	0.323 ± 0.003	.1200 (*)	0.483 ± 0.003	.2270 (*)
Methanol 100%	0.77 ± 0.03	.00633	0.359 ± 0.007	.05767 (*)	0.475 ± 0.005	.05100 (*)	0.507 ± 0.007	.0480 (*)	0.314 ± 0.004	.1110 (*)	0.314 ± 0.004	.0580 (*)
Methanol 50%	0.98 ± 0.01	.22300 (*)	0.432 ± 0.004	.13067 (*)	0.546 ± 0.004	.12200 (*)	0.737 ± 0.007	.2780 (*)	0.496 ± 0.004	.2930 (*)	0.303 ± 0.003	.0470 (*)
Methanol 25%	0.41 ± 0.01	.35200 (*)	0.334 ± 0.004	.03267 (*)	0.429 ± 0.008	.00500	0.48 ± 0.005	.0210 (*)	0.94 ± 0.005	.7370 (*)	0.611 ± 0.01	.3550 (*)

* The mean difference is significant at the .05 level. Mean of three replications in duplicates ± Standard deviation.

3.6. Carbohydrate content

As show in Table 7 all extracts of *C. colocynthis* stimulated carbohydrates in *H. vulgare* and inhibit carbohydrates in *V. faba*. The highest stimulatory effect carbohydrates of *H. vulgare* were found at low concentrations of extract (5.5, 4.88 and 5.03 mg/g) for aqueous, chloroform and methanol respectively. While the lowest was found in *V. faba* (2.5 and 3.15 mg/g) for aqueous extract. The same findings were obtained by El-Darier [42] and Pandey and Mishra [43].

3.7. Protein Content

Table 8 shows that the protein content of *H. vulgare* and *V. faba* stimulated by all extracts of *C. colocynthis* for all concentrations compared to control. This may be due to interfering of allelochemicals with physiological and biochemical processes in tested crops. Similar results were observed by El-Khatib and Hegazy [44] and El-Khawas and Shehata [36].

Table 7. Carbohydrate content in *Hordeum vulgare and Vicia faba.*

	Hordeum vulgare		***Vicia faba***	
Treatment	**Carbohydrate mg/g**	**LSD 0.05**	**Carbohydrate mg/g**	**LSD 0.05**
Control	3.32 ± 0.02		5.15 ± 0.05	
Water 100%	4.7 ± 0.05	1.380(*)	3.15 ± 0.05	2.00(*)
Water 75%	3.92 ± 0.02	.600(*)	2.5 ± 0.05	2.650(*)
Water 50%	3.61 ± 0.02	.290(*)	5.47 ± 0.07	.320(*)
Water 25%	5.5033 ± 0.006	2.1833(*)	4.3733 ± 0.031	.7767(*)
Chloroform 100%	4.03 ± 0.03	.7100(*)	5.21 ± 0.1	.060
Chloroform 50%	2.36 ± 0.03	.9600(*)	5.86 ± 0.04	.710(*)
Chloroform 25%	4.88 ± 0.03	1.560(*)	4.72 ± 0.02	.430(*)
Methanol 100%	3.4067 ± 0.006	.0867	4.32 ± 0.02	.830(*)
Methanol 50%	4.1233 ± 0.025	.8033(*)	4.4 ± 0.06	.750(*)
Methanol 25%	5.0367 ± 0.56	1.7167(*)	5.41 ± 0.03	.260(*)

* The mean difference is significant at the .05 level. Mean of three replications in duplicates ± Standard deviation.

Table 8. Protein content in *Hordeum vulgare and Vicia faba.*

	Hordeum vulgare		***Vicia faba***	
Treatment	**Protein mg/g**	**LSD 0.05**	**Protein mg/g**	**LSD 0.05**
Control	3.2433 ± 0.006		4.77± 0.06	
Water 100%	3.9733 ± 0.574	.7300(*)	5.98 ± 0.04	1.21(*)
Water 75%	4.1533 ± 0.006	.9100(*)	5.23 ± 0.03	.46(*)
Water 50%	4.4067 ± 0.006	1.1633(*)	4.98 ± 0.04	.21(*)
Water 25%	4.77 ± 0.02	1.5267(*)	4.84 ± 0.04	.07(*)
Chloroform 100%	4.97 ± 0.02	1.7267(*)	5.97± 0.03	.2(*)
Chloroform 50%	5.25 ± 0.01	2.0067(*)	5.10 ± 0.05	.33(*)
Chloroform 25%	3.47 ± 0.03	.2267	5.10± 0.04	.33(*)
Methanol 100%	4.22 ± 0.02	.9767(*)	3.75± 0.05	.02(*)
Methanol 50%	5.61 ± 0.01	2.3667(*)	4.17± 0.02	.06(*)
Methanol 25%	4.84 ± 0.04	1.5967(*)	4.30± 0.04	.47(*)

* The mean difference is significant at the .05 level. Mean of three replications in duplicates ± Standard deviation.

From the present study, it can be concluded that various solvent and water extracts of shoot system of *C. colocynthis* had allelopathic effects on germination and growth of *Hordeum vulgare* and *Vicia faba*. The extracts reduced germination in *H. vulgare* and not affect germination of *Vicia faba*. The extracts of *C. colocynthis* reduced growth of *H. vulgare* and *Vicia faba* and this inhibitory effect increased with increasing extract concentration. Inhibitory effect of various solvent extracts was not equal and highest inhibition was observed in methanolic extract while the lowest one was observed in chloroformic and aqueous extracts. This study revealed decrease of carbohydrates and increased of proteins for the two studied plants.

AUTHORS CONTRIBUTION

HMHS: Conception and design, Acquisition of data, Writing, review and revision of the manuscript,

Administrative, technical or material support HMHS and HKAAL: Development of methodology, Analysis and interpretation of data. Both authors read and approved the final of the manuscript.

ACKNOWLEDGEMENT

This research project was supported by a grant from the "Research Center of the Female Scientific and Medical Colleges", Deanship of Scientific Research, King Saud University.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

REFERENCES

1. Prasanta C, Bhowmik C, Inderjit A. Challenges and opportunities in implementing allelopathy for natural weed management. Crop Prod. 2003; 22: 661-671.
2. Gawronska H, Burza W, Bolesta E, Malepszy S. Zygote and Somatic embryos of cucumber (*Cucumis sativus* L.) substantially in their levels of abscisic acid. Plant Sci. 2000; 157: 129-137.
3. Gawronska SW. Allelopathy as a strategy for weed control in organic farming. Acta Physiol. 2003; 25: 24-30.
4. Chon SU, Kim YM, Lee JC. Herbicidal potential and quantification of causative allelochemicals from several Compositae weeds. Weeds Res. 2003; 43: 444-450.
5. Gholami BA, Faravani M, Kashki MT. Allelopathic effect of aqueous extract from *Artemisia kopetdaghensis* and *Satureja hortensison* growth and seed germination of weeds. J Appl Environ Biol Sci. 2011; 1(9): 283-290.
6. AL Rabiah HK. Allelopathic effects of *Citrullus colocynthis* extracts on the germination and growth of some plants. M.SC. Thesis, King Saud University. Faculty of Science, Botany and Microbiology Department. 2012.
7. An M, Johnson IR, Lovett IR. Mathematical modeling of allelopathy: biological response to allelochemicals and its interpretation. J Chem Ecol. 1993; 19: 2379-2388.
8. Wardle DA, Nilsson M, Gallet C, Zackrisson O. An ecosystem - level perspective allelopathy. Biol Rev. 1998; 73: 305-319.
9. Dalton BR. The occurrence and behavior of plant phenolic acids in soil environment and their potentials involvements in allelochemical interference interactions: methodological limitations in establishing conclusive proof of allelopathy. In: Inderjit KM, Dakshini M, Foys CL, eds. Principles and practices in plant ecology: Allelochemical interactions.1999: 57-74
10. Berhow MA, Voughn SF. Higher plant flavonoids: biosynthesis and chemical ecology. In: Inderjit KM, Dakshini M, Foys CL, eds. Principles and practices in plant ecology: Allelochemical interactions.1999: 423-438.
11. Jose S, Gillespie A. Allelopathy in black walnut (*Juglans nigral*) allely cropping. II. Effects of Juglone on hydoponically grown corn (*Zea mays* L.) and soyabean (*Glycine max* L. Merr.) growth and Physiology. Plant Soil. 1998; 203: 199-205.
12. Langenheim JH. Higher plant terpenoids: a phytocentric overview of their ecological roles. J Chem Ecol. 1994; 20: 1223-1280.
13. Chaves N, Escudero C. Effect of allelopathic compounds produced by *Cistus ladenifer* on germination of 20 Mediterranean taxa. Plant Ecol. 2006; 184: 259-272.
14. Khanh D, Xuan TD, Chung IM. Rice allelopathy and the possibility for weed management. Ann Appl Biol. 2007; 151: 325- 339.
15. Chaudhary SA. Flora of the Kingdom of Saudi Arabia. Ministry of Agriculture and Water. Riyadh. 1999.
16. Karthikeyan A, Shanthi V, Nagasathaya A. Preliminary phytochemical and antibacterial screening of crude extract of the leaf of *Adhatoda vasica* L. Int J Green Pharm. 2009; 3: 78-80.
17. Salama HMH. Alkaloids and flavonoids from the air dried aerial parts of *Citrullus colocynthis*. J Med Plants Res. 2012; 6(38): 5150-5155.
18. Senger C, Sturm S, Mair ME, Ellmerer EP, Stuppner H. ^{1}H and ^{13}CNMR signal assignment of cucurbitacin derivatives from *Citrullus colo-*

cynthis (L.) Schrader and *Ecballium elaterium* (L.) (Cucurbitaceae). Magn Reson Chem. 2005; 43(6): 489-491.

19. Gordon MC, David JN. Natural product drug discovery in the next millennium. Pharm Biol. 2001; 39: 8-17.
20. Sunil K, Dinesh K, Manjusha M, Kamal S, Hidhan S, Bhoodev V. Antioxidant and free radical scavenging potential of *Citrullus colocynthis* (L.) Schrad. methanolic fruit extract. Acta Pharm. 2008; 58: 215-220.
21. Karim A, Nouman M, Munir S, Sattar S. Pharmacology and phytochemistry of Pakistani herbs and herbal drugs used for treatment of diabetes. Int J Pharmacol. 2011; 7: 419-439.
22. Rahbar AR, Nabipour I. The hypolipidemic effect of *Citrullus colocynthis* on patients with hypertipidemia. Pak J Biol Sci. 2010; 13: 1202-1207.
23. Chanda S, Dave R, Kaneria M. In vitro antioxidant property of some Indian medicinal plants. Res J Med Plant. 2011; 5: 169-179.
24. Hernandez M, Lopez R, Abanas RM, Paris V, Arias A. Antimicrobial activity of *Visnea mocanera* leaf extracts. J Ethnopharmacol. 1994; 41: 115-119.
25. Ladd JL, Jacobson M, Buriff CR. Japanes beetles extracts from neem tree seeds as feeding deterents. J Econ Entomol. 1978; 71: 810-813.
26. Russo VM, Webber CL, Myers DL. Kenaf extract effects germination and post-germination development of weed; grass and vegetable seeds. Indust Crops Prod.1997; 6: 59-69.
27. Stirban M. Procese primare in fotosinteza (in Romanian), Ed. Didact. Sipedag, Bucharest, Romania, 1985.
28. Nelson N. A photometric adaption of the Somogyi method for the determination of glucose. J Biol Chem. 1944; 153: 375-380.
29. Somogyi M. Note on sugar determination. J Biol Chem. 1952; 195:19-25.
30. Lowery O, Rosebrough N, Farr A, Randall R. protin measurement with the folinphenol reagent. J Biol Chem. 1951; 193: 263-275.
31. Abd El-Fattah RI, Abou-Zeid AM, Altalhi AD. Allelopathic effects of *Artemisia princeps* and *Launae sonchoids* on rhizospheric fungi and wheat growth. Afr J Microbiol Res. 2011; 5(4): 419-424.
32. Seyed M, Moussavi N, Mohammed HBK, Ali BG. *Artemisia annua* on germination and early growth of Isabgol (*Plantago ovate*). Ann Biol Res. 2011; 2(6): 687-691.
33. Mahmood D, Sedighe S, Resa A. Allelopathic effects of *Eucalyptus globules* Labill. on seed germination and seedling growth of eggplant (*Solanum melongena* L.). Int J Farming Allied Sci. 2014; 3(1): 81-86.
34. Abhinav AM, Kanade MB. Allelopathic effect of two common weeds on seed germination, root-shoot length, biomass and protein content of jowar. Ann Biol Res. 2014; 5(3): 89-92.
35. Malik MS. Effect of aqueous leaf extract of *Eucalyptus globules* on germination and seedling of potato, maize and bean. Allelopathy J. 2004; 14: 213-220.
36. El-Khawas SA, Shehata MM. The allelopathic potentialities of *Acacia nilotica* and *Eucalyptus rostrata* on monocot (*Zea mays* L.) and dicot (*Phaseolus vulgaris* L.). Plants Biotechnol. 2005; 4(1): 23-34.
37. Yamagushi MQ, Gusman GS, Vestana S. Allelopathic effect of aqueous extract of *Eucalyptus globules* Labill on crops. Semina Ciencias Agrarias Londrina. 2011; 32(4): 13561-1374.
38. Djanaguiraman M, Vaidyanathan R, Anniesheeba J, Durgadevi D, Bangatusamy U. Physiological responses of *Eucalyptus globulus* leaf leachate on seedling physiology of rice, sorghum and blackgram. Int J Agric Biol. 2005; 7(1): 35-38.
39. Ziaebrahimi L, Khavari-Nejad RA, Fahimi H, Nejadsatari T. Effects of aqueous eucalyptus extracts on seed germination, seedling growth and activities of peroxidase and polyphenoloxidase in three wheat cultivar seedlings (*Triticum aestivum* L.). Pak J Biol Sci. 2007; 10: 3415-3419.
40. Corsato JM, Fortes AMT, Santorum M, Leszczynski R. Efedto alelopathico do extrato aquoso de folhas de girasol sobre a germinacao de soja e picao-pretio. Ciencias Agrarias Londrina, 2010; 31: 353-360.
41. Gliessman R. Agroecologia: processos ecolo-

gicos em agricultura sustentavel. Porto Alegre UFRGS, 2000.

42. El-Darier SM. Allelopathic effects of *Eucalyptus rostrata* on growth, nutrient uptake and metabolite accumulation of *Vicia faba* L. and *Zea mays* L. Pak J Biol Sci. 2002; 5(1): 6-11.
43. Pandey DK, Mishra N. Relative phytotoxicity of an allelochemicalical hydroquinone to coontail (*Ceratophyllum demersum* L.) and rice (*Oryza sativa* L.). In: Proceeding and selected papers of the Fourth World Congress on Allelopathy, Charles Sturt University. Wagga NSW, Australia, 2005.
44. El-Khatib AA, Hegazy AK. Growth and physiological responses of wild Oats allelopathic potential of wheat. Acta Agronom Hungar. 1999; 47(1): 11-18.