Chemical Composition and Herbicidal Potent of Cauliflower (*Brassica oleracea* var. *botrytis*) and Cabbage Turnip (*Brassica oleracea* var. *gongylodes*)

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ABSTRACT

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This study was conducted to evaluate the phytochemical content and allelopathic potential of two cabbages botanical varieties leaves, ie. cauliflower *(Brassica oleracea* var. *botrytis)* and cabbage turnip *(B. oleracea* var. *gongylodes)*. Their aqueous and organic extracts were evaluated on lettuce (*Lactuca sativa*) and one of the most dominant weeds in Tunisia, nettle-leaf goosefoot (*Chenopodium murale*). Field experiments were conducted to evaluate the smothering potential of the two varieties. The total phenolics, flavonoïds, flavonols and flavones, alkaloïds, and proanthocyanidins contents were higher in the aqueous extracts of both varieties. For organic extracts, petroleum ether and methanol cauliflower extracts and chloroform and methanol cabbage turnip extracts were the richest ones. All aqueous and organic extracts had significantly delayed germination, reduced its rate and affected seedling growth. Reduction of germination and growth were more important using the higher concentrations and in presence of cabbage turnip extract. The organic extracts of both varieties had significantly inhibited the seedling growth of target species, especially petroleum ether, and methanol cauliflower extracts and chloroform and methanol cabbage turnip ones. Field experiment highlighted the smothering potential of the two varieties and confirmed the higher allelopathic potential of cabbage turnip as compared to cauliflower.

Keywords: Cabbage turnip, cauliflower, *Chenopodium murale*, growth, phytochemical analysis, smothering potential, speed germination

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The current trend in agriculture production is to find a biological solution to reduce the perceived hazardous impacts from herbicides and insecticides (Khanh et al. 2005). Allelopathy is a natural and an environment-friendly phenomenon which was proved to be a

promising tool for weed control, increase of crop yields, decrease of reliance on both synthetic pesticides and for the improvement of the ecological environment (Hegab and Ghareib 2010). The allelopathic properties of plants may act as a biological weed control mechanism in agro-ecosystems and are effective tools to help resolve this critical issue (Xuan et al. 2005). Allelopathic crops release chemicals into the soil that can contribute to weed management through suppression of weed seed germination, seedling emergence and establishment and seedling growth (Haramoto and Gallandt 2004).

Plants may store these chemicals in cells in bound forms, for example as water-soluble glycosides of alkaloids, flavonoids and phenols (Javed 2011) and release them into the environment by special glands on stems, leaves and roots (Sugiyama and Yazaki 2012) by a variety of mechanisms, including decomposition of residues, volatilization, and root exudation (Cruz-Ortega et al. 2007). Once released into environment, these compounds may affect the neighboring receiver plants (Javed 2011) and play an important role on the pattern of vegetation, and on crop productivity.

Brassica species, including *B. oleracea*, are frequently cited as allelopathic crops (Haramoto and Gallandt 2004) and are a significant source of glucosinolates (Iori et al. 2004; Kusznierewicz et al. 2008; Windsor et al. 2005), polyphenols, flavonoids (Jaiswal et al. 2012), proanthocyanidins (Bahorun et al. 2004), and alkaloids (Guyria et al. 2015).

Many studies have shown fungicidal (Martinez et al. 2011), nematicidal (Wu et al. 2011), and insecticidal (Schramm et al. 2012) properties of *B. oleracea* secondary metabolites, but few have evidenced the role of phenolics, flavonoids and alkaloids compounds of Brassicaceae crops in inhibiting germination and seedling growth of weeds (Batish et al. 2008; Singh and Kaur 2014). Moreover, most previous works affirmed that glucosinolates and their breakdown products are the major chemical components responsible for the herbicidal activity in *Brassica* spp. (Petersen et al. 2001).

Therefore, the first aim of this study was to confirm richness of *B. oleracea* species in polyphenols, flavonoids, flavonones/flavonols, proanthocynins and alkaloids through a phytochemical screening of aqueous and organic extracts of external leaves of two varieties of this species namely cauliflower (*B. oleracea* var. *botrytis*) and cabbage turnip (*B. oleracea* var. *gongylodes*). The second aim is to evaluate the leaf aqueous and organic extracts of both cabbages varieties effects on germination and growth of lettuce (*Lactuca sativa*) and nettle-leaf goosefoot (*Chenopodium murale*), a weed accompanying cabbage crops. A field experiment will be also conducted in order to evaluate the smothering potential of these cabbage varieties on weed biomass, especially nettle-leaf goosefoot.

MATERIALS AND METHODS

Plant material.

Cauliflower and cabbage turnip leaves were collected after eliminating fruiting heads at the harvesting stage from organically grown crops at *Institut Supérieur Agronomique* of Chott-Mariem, Sousse, Tunisia (Latitude 35°55N, Longitude 9°28E, Altitude 15 m).

Aqueous extraction.

Fresh cauliflower and cabbage turnip leaves were rinsed and then oven-

dried at 60°C for 72 h and grinded. Fifty grams of each dried material were soaked in 1 liter of distilled water at room temperature for 24 h to give a concentration of 50 g/l (Chon et al. 2005). The extracts were filtered several times and kept at 4°C in the dark until use.

Organic extraction.

Sequential extractions were carried out with organic solvents of increasing polarity: petroleum ether, chloroform, and methanol. A 100 g-sample of dried leaf powder was immersed in organic solvent for 7 days at room temperature. Organic extracts were evaporated to dryness under reduced pressure at 45-50°C, using rotavapor R-114 (Buchi, France). The residue was weighed and the yield was determined. Dry fractions were stored at 4°C until use. The extracts were tested at three concentrations (1, 3, and 6 mg/ml) in bioassays.

Phytochemical screening.

Total phenolics (TP) content. The TP content was measured using the modified Folin–Ciocalteu method (Velioglu et al. 1998). Sample extract (100 µl) was mixed with 500 µl of 1/10 diluted (in Milli-Q water) Folin–Ciocalteu phenol reagent and allowed to react for 5 min in the dark at room temperature. Then, 400 µl of sodium bicarbonate (7.5%) were added to the mixture. After 90 min of incubation in the dark at 30°C, the absorbance was read at 765 nm. TP was expressed as mg gallic acid equivalent/g dry matter (mg GAE/g dw) using gallic acid calibration curve (R^2 = 0.96).

Total flavonoïds (TFd) content. The TFd content was determined spectrophotometrically according to standard method (Quettier et al. 2000). Briefly, 0.5 ml of 2% solution of $AlCl_3$ in methanol was mixed with the same volume of extract. Absorption readings at 430 nm were taken after 30 min against a blank. TFd content was expressed as mg quercetine equivalent/g dry weight (mg QE/g dw) using quercetine calibration curve (R^2 = 0.999).

Total flavonols and flavones (TFl) content. The TFl content was determined using the method of Kumaran and Karunakaran (2007). To 2 ml of sample, 2 ml of 2% $AlCl_3$ methanol and 3 ml (50 g/l) sodium acetate solutions were added. The absorption at 440 nm was read after 2.5 h of incubation at 20°C. TFl content was expressed as mg quercetine equivalent/g dry weight (mg QE/g dw) using quercetine calibration curve (R^2 = 0.995).

Total proanthocyanidins (TPA) (condensed tannins) content. The TPA content determination was based on the procedure reported by Sun et al. (1998). A volume of 0.5 ml of extract was mixed with 3 ml of 4% vanillin–methanol (w/v) and 1.5 ml hydrochloric acid. The mixture was allowed to stand for 15 min, and then the absorbance was measured at 500 nm. TPA content was expressed as mg catechin equivalent/g dry weight (mg CE/g dw) using catechin calibration curve (R^2 = 0.998).

Total precipitable alkaloïds (TA) content. The TA content was determined by spectrophotometric method with Dragendorff reagent (Stumpf 1984). Principally, 300 µl of plant extract were mixed with 100 µl of Dragendorff reagent. After centrifugation at 7000 g for 1 min, the supernatant was removed and dissolved in 1 ml of 2.45 M NaI. An aliquot of 10 µl of each tube was added to 1 ml of 0.49 M NaI, after which the absorbance was read at 467 nm. TA

content was expressed as mg papaverine hydrochloride equivalent/g dry weight (mg PAHE/g dw) using papaverine hydrochloride calibration curve (R^2 = 0.99).

Laboratory bioassays.

Tests with aqueous extracts. Each cabbage extract was diluted appropriately with sterile distilled-water to give final concentrations of 10, 20, 30, 40 and 50 g/l. They were tested on lettuce known to be very sensitive to allelochemicals (Ervin and Wetzel 2003) and on *C. murale,* one of the most widespread weed species in Tunisia (Holmet al. 1991). Seeds were surface sterilized with 0.525 g/l sodium hypochlorite for 15 min, then rinsed four times with deionized water, imbibed in it at 22°C for 12 h and carefully blotted (Chon et al. 2005). Twenty imbibed seeds of target species were separately placed on the filter paper in 9 cm Petri plates, 5 ml of each extract were applied as per treatment. Seedlings watered with distilled water were used as control.

The Petri plates of lettuce and nettle-leaf goosefoot were then placed in a growth chamber at 21 ± 2°C and 25 ± 2°C temperature, respectively, and a 16/8 h light and dark photoperiod and relative humidity of around 75%.

Treatments were arranged in a completely randomized design with three replications. Germinated seeds were counted at 24 h intervals during 7 days. Data were transformed to percent of control for analysis.

The total germination G was determined using the formula (Anjum and Bajwa 2005): G = (Nt/N) × 100, where Nt: Proportion of germinated seeds in each treatment for the final measurement and N: Number of seeds used in bioassays. This index shows the final germination percentage.

The index of germination GI was determined using the formula (Chiapuso et al. 1997): GI = (N1) × 1 + (N2-N1) × 1/2 + (N3-N2) × 1/3 +.... + (Nn-Nn-1) × 1/n, where, N1, N2, N3,…., Nn: Proportion of germinated seeds observed afterwards 1, 2, 3,…., n-1, n days. This index shows the germination delay induced by the extract (Dorado and Lopez-Fando 2006).

For growth test, twenty pre-germinated seeds of target species were separately placed on the filter paper in 9 cm Petri plates and 5 ml of each extract were applied as per treatment. Seedlings watered with distilled water were used as control. The Petri plates were then placed in the same conditions as described above. Treatments were arranged in a completely randomized design with three replications. Shoot and root length of receiver species were measured 7 days after sowing. Data were transformed to percent of control for analysis.

The inhibitory or stimulatory percent was calculated using the equation given by Chung et al. (2001): Inhibition (-)/stimulation (+) % = [(extract – control)/control] × 100, where extract: Parameter measured in presence of leaf extract and control: Parameter measured in presence of distilled water.

Tests with organic extracts. For organic extracts, two residues concentrated from petroleum ether, chloroform and methanol were dissolved in methanol and three concentrations were prepared (1, 3 and 6 mg/ml) to estimate their effect on germination and growth of target species. Two controls were considered, distilled water and methanol, to eliminate the eventual organic solvent effect. Filter papers placed in Petri plates were soaked with distilled water, methanol or various organic extracts. Organic solvent was

evaporated for 24 h at 24°C, then 5 ml distilled water was added and 20 soaked seeds were put to germinate for 7 days. Germination index and total germination were estimated as before and expressed in percent of the control. Treatments were arranged in a completely randomized design with three replications.

For growth test, 20 pre-germinated seeds of target species were placed in Petri plates as described above for seven days, then shoot and root length of receiver species were measured 7 days after sowing. Data were transformed to percent of control for analysis. The inhibitory or stimulatory percent was calculated using the same equation as described above.

Field experiment.

Field study was conducted during 2013 and 2014 at *Institut Supérieur Agronomique* of Chott-Mariem, Sousse, Tunisia. In the study area, the climate is typically Mediterranean with hot-dry summers and mild-rainy winters. According to long term weather data (1973-2006), maximum monthly temperatures ranged between 16 and 31°C and minimum monthly temperature varied from 7 to 21°C. Mean relative humidity varied from 69 to 71%. Monthly rainfall ranged between 2 and 58 mm (Bhouri Khila et al. 2013).

The field essay was carried out to evaluate and compare the smothering effect of the two varieties of *B. oleracea* on total weed biomass and nettle-leaf goosefoot biomass. It was carried out in randomized complete block design and occupied a total area of 100 m^2 (10 m × 10 m). Blocks were replicated three times and each block contained three plots, each plot (3 m × 2 m) were occupied with one of both varieties, and one plot was left uncultivated (fallow) and considered as control. Varieties of *B. oleracea* (namely *B. oleracea* var. *botrytis* (cauliflower) and *B. oleracea* var. *gongylodes* (cabbage turnip)) were planted in four rows at a density of 0.5 m × 0.6 m and 0.5 m × 0.3 m, respectively.

Weeds were sampled at crop harvest, from 1 m^2 quadrate thrown at the middle of each plot. Aboveground weeds biomass were collected, identified and counted, then dried and weighed.

Statistical analysis.

All data were reported as mean ± standard deviation (SD) of three replicates for biological activities and of five replicates for phytochemical analysis. ANOVA and a post hoc Duncan Multiple Range tests were performed with IBM SPSS Statistics version 20, for Windows program, to analyze treatment differences. The means were separated on the basis of least significant differences (LSD) at the 0.05 probability level.

Weed biomass data were analyzed by ANOVA (IBM SPSS Statistics version 20) for randomized complete block design and performed for all treatments in the field experiment. The treatment and interaction LSD of the means were used to separate treatment means at 5% level of significance.

RESULTS

Phytochemical analysis.

Cauliflower aqueous extract was shown to contain higher amounts of total polyphenols (7.17 mgGAE/g dw) and total alkaloids (2.27 mg PAHE/g dw) whereas cabbage turnip aqueous extract was richer in condensed tannins (19.28 mg CE/g dw) and flavonones (4.26 mg QE/g dw) (Table 1). Important amounts of alkaloïds were detected in cauliflower petroleum ether (2.38 mg PAHE/gdw) and chloroform extracts (2.25 mg PAHE/gdw) and at little amounts in methanol extract. Contrarily for cabbage

turnip, higher amounts of alkaloids were recorded in methanolic extract (1.71 mg PAHE/gdw) (Table 1), whereas condensed tannins were completely absent in both methanolic cabbage extracts.

Table 1. Total phenols (TP), flavonoïds (TFd), proanthocyanidins (TPA), flavonols and flavonones (TFl) and alkaloids (TA) contents recorded in petroleum ether (PE), chloroform (CH), methanol (MET) and water (W) cauliflower and cabbage turnip extracts

Extract	TP (mg GAE/g dw)	TFd (mg QE/g dw)	TPA (mg CE/g dw)	TFl (mg QE/g dw)	TA (mg PAHE/g dw)
Cauliflower					
PE	2.97±0.07 c	1.3±0.01 b	3.07±0.61 b	1.37±0.05 c	2.38±0.35 c
CH	0.16±0.02 a	0.23±0.01 a	0.3±0.02 a	0.11±0.01 b	2.25±0.02 a
MET	2.23±0.05 b	1.92±0.11 c	0.00a	1.06±0.03 a	0.77±0.06 b
W	7.17±0.37 d	3.45±0.26 d	16.55±1.18 c	2.95±0.07 d	2.27±0.35 c
Cabbage turnip					
PE	0.67±0.01 a	0.73±0.03 a	2.02±0.08 c	0.38±0.04 b	0.54±0.03 a
CH	0.44±0.08 a	2.2±0.07 c	1.21±0.05 b	0.76±0.01 a	0.78±0.06 b
MET	1.87±0.35 b	1.13±0.12 b	0.00a	0.74±0.04 c	1.71±0.29 c
W	5.43±0.34 c	3.5±0.05 d	19.28±0.42 d	4.26±0.18 d	2.06±0.16 d

GAE: Gallic acid equivalent; QE: Quercetine equivalent; CE: Catechin equivalent; PAHE: Papaverine hydrochloride equivalent; dw: Dry weight; Means within columns sharing the same letters are not significantly different at $P \leq 0.05$.

Phytotoxicity of aqueous extracts .

Aqueous extracts effect on seed germination. Water extracts from cauliflower and cabbage turnip leaves showed inhibitory effects on germination of lettuce and nettle-leaf goosefoot seeds. For lettuce, germination was suppressed from 40g/l with cauliflower extract and at 50 g/l with cabbage turnip extract (Table 2). However for nettle-leaf goosefoot seeds, the inhibitory effect was observed rather in delay of germination speed (GI) than in the final germination (G). GI reached 50 and 17.97%, respectively with cauliflower and cabbage turnip leaves extracts at 50 g/l (Table 2).

Table 2. Germination index (GI) and total germination (G), expressed in percentage over control (C), of target species lettuce and nettle-leaf goosefoot germinating in presence of water extracts from leaves of cauliflower and cabbage turnip tested at different concentrations

Variety	Concentration (g/l)	Lettuce		Nettle-leaf goosefoot	
		G (% C)	GI (% C)	G (% C)	GI (% C)
Cauliflower	10	100.00c	93.52±12.65 d	86.66±4.71 a	78.35±6.59 c
	20	96.66±2.35 c	42.23±4.61 c	90±4.08 a	63.54±2.10 b
	30	57.54±5.36 b	19.35±4.35 b	86.66±2.35 a	57.60b±4.68a
	40	1.66± 2.35 a	0.47±0.67 a	83.33±4.71 a	52.10±2.63 a
	50	0.00a	0.00a	86.66±2.35 a	50.82±2.34 a
Cabbage turnip	10	100.00a	88.40±6.57 a	60.00b	34.27±0.80 bc
	20	96.60±2.42 b	79.80±6.12 a	66.66±6.23 b	36.61±2.40 c
	30	96.40±2.35 b	76.70±16.3 a	70±4.08 b	37.38±3.66 c
	40	25.40±0.61 c	13.20±2.70 b	61.66±4.71 b	27.56±2.15 b
	50	0.00d	0.00c	41.66±12.47 a	17.97±6.31 a

For each tested Brassicaceae, means within columns sharing the same letters are not significantly different at $P \leq 0.05$.

Aqueous extracts effect on seedling growth. Both extracts reduced the root length of lettuce and nettle-leaf goosefoot seedlings (Fig. 1). At lower concentration (till 20 g/l), cauliflower and cabbage turnip extracts stimulated shoot growth of lettuce seedlings (63 and 30%, respectively), whereas for the weed seedlings, shoot growth was slightly stimulated (30%) only by cauliflower extract applied at 10 g/l. Radical length of lettuce and nettle-leaf goosefoot was totally inhibited by both extracts from concentrations of 30 and 20 g/l, respectively. Shoot length inhibition over control of target species exceeded 60% in presence of cabbage turnip extracts from concentration of 30 g/l.

Phytotoxicity of organic extracts.

Yield of organic extracts. Cabbage turnip leaves had the highest yield compared to cauliflower leaves (2.44% vs. 0.82% with petroleum ether and 3.39% vs. 1.18% with chloroform, respectively). Concerning methanol extract, leaves of both varieties showed the same extraction yield (4.4%). Compared with petroleum ether and chloroform, methanol extract gave the highest yield for both cabbage species (Table 3).

Effect of organic extracts on germination. Cauliflower extract in all solvents did not influence the germination rate (G) of lettuce except with petroleum ether extract used at 3 mg/ml, where a slight inhibition (15% over control) was recorded. However, the extract reduced the germination index (GI) of lettuce seeds with petroleum ether (GI reached means of 45% over control at all concentrations) and with methanol (GI ranged from 32% to 77.68%) extracts. However using the chloroform extract, the germination was slightly slowed (8% over control) (Table 4).

For nettle-leaf goosefoot, cauliflower extract was found to be very inhibitory for germination as estimated by rate and speed in all cases. Germination rate inhibition reached 76, 85, and 80%, respectively, with petroleum ether (at 6 mg/ml), chloroform (at 6 mg/ml) and methanol (at 1 mg/ml). Corresponding values of GI were 13.47, 7.7, and 16.18%, respectively.

The cabbage turnip extracts of petroleum ether and chloroform did not

affect G and GI of lettuce which was around 98%. However, weed germination rate was canceled in petroleum ether and decreased from 58.7% at 1 mg/ml to 21.74% at 6 mg/ml in chloroform (Table 4).

The methanol extract slightly affected the lettuce germination rate, at 6 mg/ml, which was reduced by 10% over control, but was very toxic for germination index which ranged from 65.77 to 29.63% over control at all concentrations. In presence of methanol extract, nettle-leaf goosefoot germination rate decreased from 28.26 to 10.86% when used at the concentrations 1 mg/ml and 3 mg/ml, respectively and was suppressed at 6 mg/ml (Table 4).

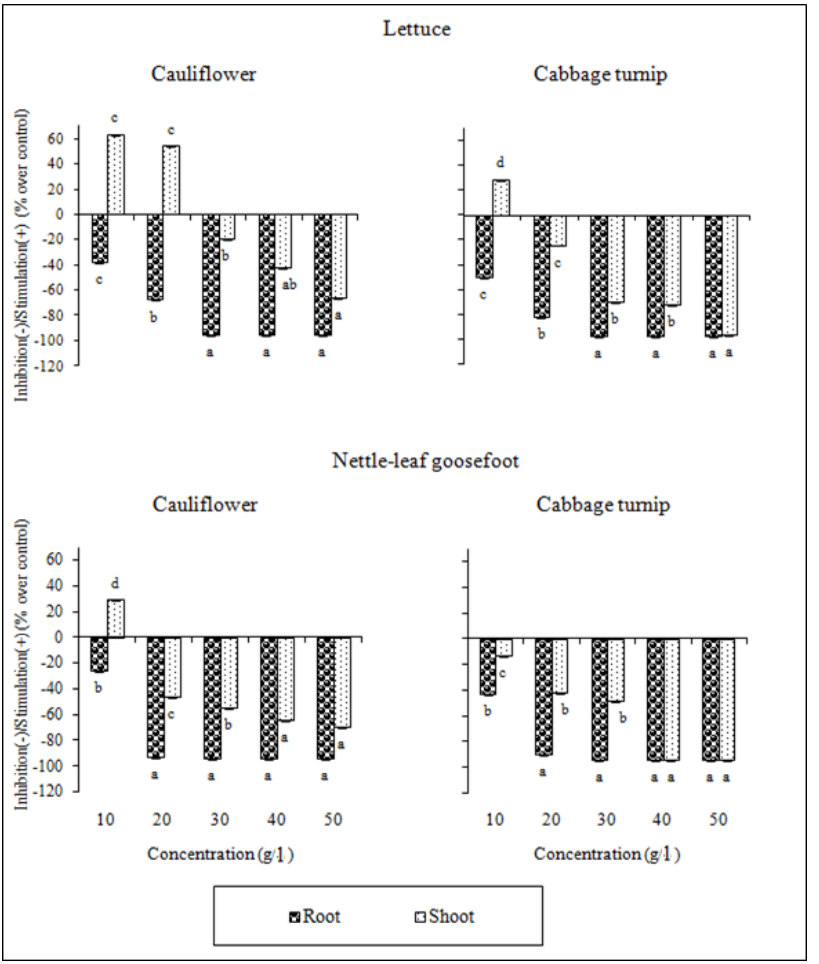

Fig. 1. Effect of aqueous extracts of leaves of cauliflower and cabbage turnip on root and shoot growth of lettuce and nettle-leaf goosefoot, 7 days after germination.
Value = Average ± SD, n=3. Different letters of bars indicate significant differences among treatments at $P \leq 0.05$ based on Duncan Multiple Range test.

Table 3. Residues yield (percentage of dry matter) after successive extraction in three organic solvents (petroleum ether, chloroform, methanol) of leaves of cauliflower (*Brassica oleracea* var. *botrytis*) and cabbage turnip (*Brassica oleracea* var. *gongylodes*)

Extract solvent	Cauliflower yield (%)	Cabbage turnip yield (%)
Petroleum ether	0.82	2.44
Chloroform	1.18	3.39
Methanol	4.43	4.4

Table 4. Germination index (GI) and total germination (G), expressed in percentage over control (C) of target species: Lettuce and nettle-leaf goosefoot germinating in presence of organic extracts from cauliflower and cabbage turnip leaves applied at different concentrations

Extract/ Concentration (mg/ml)		Lettuce		Nettle-leaf goosefoot	
		GI (%C)	G (%C)	GI (%C)	G (%C)
Cauliflower					
Petroleum ether	**1**	48.66 bc	96.66 b	32.7 bc	43.47 b
	3	37.96 ab	85 a	28.57 abc	41.30 ab
	6	49.13 bc	98.33 b	13.47 ab	23.91 ab
Chloroform	**1**	100.71 d	98.33 b	39.62 c	47.82 b
	3	92.3 d	98.33 b	33.92 bc	47.82 b
	6	100.3d	100 b	7.70 a	15.21 a
Methanol	**1**	58.77 c	100 b	16.18 ab	19.56 ab
	3	77.68 bc	98.33 b	21.7 abc	32.60 ab
	6	32.01 a	96.66 b	20.12 abc	32.60 ab
Cabbage turnip					
Petroleum ether	**1**	98.58 c	98.33 b	10.47 ab	13.04 ab
	3	94.53 c	96.67 b	0.00 a	0.00 a
	6	93.71 c	98.33 b	0.00 a	0.00 a
Chloroform	**1**	98.45 c	98.33 b	48.53 d	58.70 c
	3	97.08 c	98.33 b	36.63 c	52.18 c
	6	90.84 c	96.67 b	16.08 b	21.74 b
Methanol	**1**	65.77 b	100.00 b	18.52 b	28.26 b
	3	62.21 b	100.00 b	5.62 ab	10.86 ab
	6	29.63 a	90.00 a	0.00 a	0.00 a

In each column, values with the same letter are not significantly different at $P \leq 0.05$

Effect of organic extracts on seedling growth. Using cauliflower extracts, the lettuce seedling growth was close to control or was slightly stimulated except in presence of petroleum ether extract used at 3 and 6 mg/ml which caused an average reduction of 60 and 51%, respectively, for roots and shoots. With cabbage turnip extract, lettuce seedlings growth was slightly stimulated, except the extracts of petroleum ether (3 and 6 mg/ml) and methanol (6 mg/ml) where maximum root and shoot growth inhibitions reached respectively, 40 and 33% with petroleum ether applied at 6 mg/ml. In other cases, the percentage stimulation varied between 4.26 and 22.78% for roots and between 1.07 and 36.86% for shoots (Fig. 2).

For nettle-leaf goosefoot seedlings, root growth was reduced by all organic extracts of cauliflower and

cabbage turnip, except in presence of cabbage turnip in chloroform extract (6 mg/ml) where a stimulation of 45.54% was recorded. Maximum inhibitions in root growth (72.28%) were registered with all organic extracts used at 6 mg/ml and with petroleum ether at 3 mg/ml. Maximum inhibition of shoot growth (a means of 64%) was recorded with petroleum ether extracts of the two varieties at 6 mg/ml (Fig. 2).

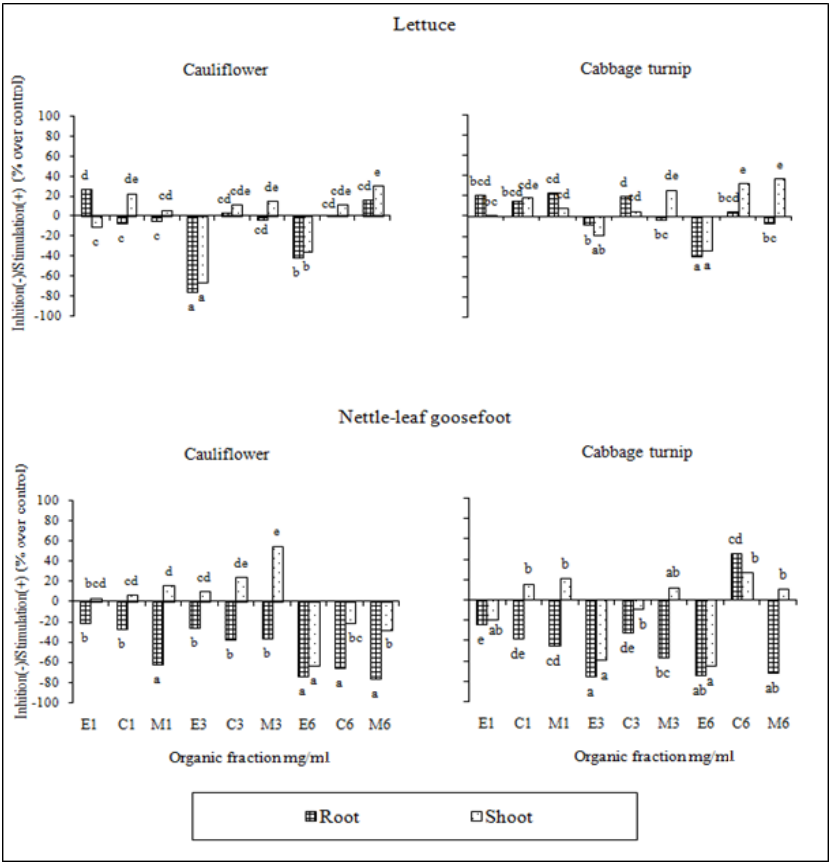

Fig. 2. Effect of organic extracts of cauliflower and cabbage turnip leaves prepared with Petroleum ether (E), Chloroform (C) and Methanol (M), applied at 1 (E1, C1, M1), 3 (E3, C3, M3) and 6 mg/ml (E6, C6, M6) on root and shoot growth of lettuce and nettle-leaf goosefoot, noted 7 days after germination. Value = Average ± SD, n=3. Different letters of bars indicate significant differences among treatments at $P \leq 0.05$ based on Duncan Multiple Range test.

Evaluation of smothering potential of cabbages varieties

There were great differences in total biomass of weed species between the plots cropped with test cabbage varieties and the fallow plots (uncultivated) in both years of experimentation (Table 5). Reduction

over fallow of total weed biomass was greater in cabbage turnip plots as compared with cauliflower plots in both years. In fact, total weed biomass was reduced over fallow plots by about 54 and 66% (means of two years), respectively in cauliflower and cabbage turnip plots. Cabbage turnip has also greater smothering potential of nettle-leaf goosefoot seedlings as compared with cauliflower.

Table 5. Dry weight of total weeds and nettle-leaf goosefoot (g/m^2), in cultivated plots by cauliflower and cabbage turnip and in fallow (not cultivated) and its reduction (% over fallow) during 2013 and 2014

Cultivated plot	Total weed dry weight (g/m^2)	Reduction (% over Fallow)	Nettle-leaf goosefoot dry weight (g/m^2)	Reduction (% over Fallow)
2013				
Fallow	571.9±177.15 a	-	58.8±30.37 a	-
Cauliflower	266.27±26.8 b	53.44	42.07±34.85 a	28.45
Cabbage turnip	211.7±18.19 b	63.00	6.11±4.06 a	89.60
2014				
Fallow	904.74 ±98.63 a	-	292.13±69.10 a	-
Cauliflower	405.7±69.66 b	55.15	48.20±24.80 b	83.50
Cabbage turnip	278.1±34.77 b	69.27	20.87±34.01 b	92.85

All values are average of three replicates ± SD. Means with the same letters in a column are not significantly different at $P \leq 0.05$.

Total biomass of nettle-leaf goosefoot was reduced over fallow by about 56% vs. 91% (means of two years), respectively, in cauliflower and cabbage turnip plots (Table 5). Total weed biomass in fallow plots was greater in the year 2014 as compared with year 2013 (904.74 g/m^2 vs. 571.9 g/m^2, respectively in 2014 and 2013). Similarly for nettle-leaf goosefoot biomass, it was greater in fallow plots in 2014 (292.13 g/m^2) than in 2013 (58.8 g/m^2). Moreover, in both cabbage varieties plots, total weed biomass decrease was most enounced in the second year of experimentation. In cauliflower plots, dry weight of nettle-leaf goosefoot decreased by 28.45% vs. 83.5% over fallow, respectively in 2013 and 2014. Similarly in cabbage turnip plots, nettle-leaf goosefoot weed dry weight was reduced over fallow by 89.6 and 92.85%, respectively in 2013 and 2014 (Table 5).

DISCUSSION

Results revealed richness of *B. oleracea* species in phenolic compounds and alkaloïds which is in accordance with previous studies (Bahorun et al. 2004; Scalzo et al. 2008; Vallejo et al. 2003; Wu and Prior 2005). Results have also shown a variation in secondary metabolites amounts with cabbage varieties. Podsedek et al. (2006) found the highest content of phenolics in red cabbage and the lowest in white cabbage in a comparison of several varieties of red cabbage, white cabbage, savoy cabbage, and Brussels sprouts. The specific phenolic compounds also varied as anthocyanins dominated in red cabbage while hydroxycinnamic acids predominated in other *Brassica* cultivars. Comparing several varieties of broccoli, Brussels sprouts, cabbage, cauliflower and Chinese cabbage showed that broccoli generally had the highest levels of phenolics, vitamin C, β-carotene, lutein

and α-tocopherol, with Brussels sprouts a close second (Singh et al. 2007).

Phytochemical analysis of organic extracts revealed more contents in phytochemicals in methanol extracts as compared with petroleum ether and chloroform. In fact, extraction yield of phenolics and flavonoïds contents depended greatly on the solvent polarity (Turkmen et al. 2006). It may be due to higher polarity of methanolic solvent (Namuli et al. 2011) which is able to extract more phenolic and flavonoïd compounds (Cowan 1999) and to draw high variety of plant constituents than the other solvents (Paulsamy and Jeeshna 2011). In particular, methanol has been generally found to be more efficient in extraction of lower molecular weight polyphenols while the higher molecular weight flavanols are better extracted with aqueous acetone (Dai and Mumper 2010). In addition, phytochemicals like, polyphenols, flavonoïds, flavonones/flavonols, were found to be present in aqueous extracts and all tested organic extracts. However, condensed tannins were present in all the extracts but completely absent in methanolic ones. This could be due to its high molecular weight and its solubility in water (Khoddami et al. 2013). Similar results were observed by Guyria et al. (2015) who revealed that the methanolic extract of broccoli (*B. oleracea* var. *italica*) contained high quantities of alkaloids, tannins, flavonoïds, phenols and proteins whereas saponins were absent. Hexane extract of broccoli contains high amount of alkaloids, phenols and proteins. Acetone and water extracts of broccoli possess little amounts of sterols, alkaloids and tannins but are rich in phenols and proteins (Guyria et al. 2015).

For germination tests, often the phytotoxic effect is not observed in the final germination percentage, but rather in the speed of germination, which can provide important indications on the allelochemicals. Ahmed and Wardle (1994) affirmed that germination index is more sensitive indicator of allelopathic effects occurring during the germination process. Plants that germinate at slower rates are often smaller (Fallah Touzi and Baki 2012) and delays in seed germination of any species can have important biological implications, because this will affect the establishment of seedlings in natural conditions (Chaves et al. 2001) and their chances of competing for resources with neighboring species (Xingxinag et al. 2009). The germination rate (G) and speed inhibition (GI) increased with the extract concentration. This finding is supported by Turk and Tawaha (2003) who registered an increase germination inhibition of wild oat (*Avena fatua*) with the increase of black mustard (*Brassica nigra*) extract.

Effects of allelochemicals on seed germination appear to be mediated through a disruption of normal cellular metabolism rather than through damage of organelles. Reserve mobilization, a process which usually takes place rapidly during early stages of seed germination seems to be delayed or decreased under allelopathy stress conditions (Gniazdowska and Bogatek 2005). Alterations in germination patterns can be caused by changes in the cell membranes permeability, RNA transcription and translation, secondary messengers integrity, respiration, conformation of enzymes and receptors, or a combination of these changes (Ferreira and Aquila 2000). For example, 6-methoxy-2-benzoxalinone (MBOA) inhibits the germination of lettuce seeds by impeding inducement of α-amylase synthesis, which mobilizes the stored reserves and maintains seed respiratory activity (Kato-

Noguchi and Macias 2005). Baleroni et al. (2000) showed that *p*-coumaric and ferulic acids increased total lipid content in the cotyledons of canola seeds and suggested that this change is due to reduced mobilization of reserves during germination in the presence of these phenolic compounds.

Target species showed different responses to aqueous and organic cabbages extracts. These results agree with earlier studies reporting that allelochemicals, which inhibited the growth of some species at certain concentrations, might stimulate the growth of same or different species at lower concentrations (Narwal 1994). In growth tests, radical length was more affected by allelochemicals than hypocotyl length, for both target species. Many studies have reported the most sensitivity to allelochemicals of roots compared to aerial parts of seedlings (Ercoli et al. 2007; Oliveira and Campos 2006; Rahman 2006). This result was attributed to the fact that roots are the first to absorb allelochemicals from the environment (Turk and Tawaha 2003) and its growth is characterized by high metabolic rates and, for this reason, they are highly susceptible to environmental stresses such as allelochemicals in soils (Hussain and Reigosa 2011). Root growth inhibition by allelochemicals can be due to changes in DNA synthesis in cells of apical root meristem, alteration of the mitochondrial metabolism (Abrahim et al. 2000) or changes in cell mitotic indices (Iganci et al. 2006) and seedling length reduction may be attributed to the reduced rate of cell division and cell elongation due to the presence of allelochemicals in the aqueous extracts (Javaid and Anjum 2006).

Toxicity of cauliflower and cabbage turnip organic was most expressed for nettle-leaf goosefoot seeds. The difference in sensitivity of both varieties shows the specificity of allelochemicals which have no, or little, affected lettuce germination in this assay. Similar results were obtained with Saad et al. (2014) where white and red cabbage extracts were most toxic for *C. murale* seeds as compared with *L. sativa* seeds and germination inhibition of target species increased with concentrations. Seigler (1996) showed that allelochemicals can be selective in their actions and plants can be selective in their responses. The selectivity in allelopathic effects may be of considerable interest for the control of weeds in crops (Seigler 1996). Fallah Touzi and Baki (2012) demonstrated that germination rate of barnyard grass (*Echinochloa crus-galli)* decreased in presence of ethanol extract of *B. juncea* and such chemicals were both species-specific and concentration-dependent and these characteristics may influence the density and the composition of individual plant communities. In fact, germination and early seedling growth assay have been regarded as a basic experiment for figuring out the effect of any plant extract upon target plant development (Madany and Saleh 2015). Results revealed that both cabbage varieties extracts caused a noticeable reduction in both germination rate and early growth of lettuce and nettle-leaf goosefoot seedlings. Such depressive effect may be imputed to the adverse impact of phytochemicals in the extracts on enzymatic processes through some interactions with organic substances of the cell (Chethan et al. 2008). Indeed, earlier studies corroborated that phytochemicals, including phenolic acids (Batish et al. 2008; Singh and Kaur 2014), phenolics, flavonoïds and alkaloïds (Rice 1979), are potent germination and growth inhibitors. These phytotoxins are known to affect the cell

structure and physiological functions of the target species resulting in impaired germination and diminished growth (Duke and Dayan 2006). Inhibition in lipid mobilization in the presence of ferulic and *p*-coumaric acids was detected during canola (*B. napus)* seed germination (Baleroni et al. 2000), as well as in sunflower (*Heliantus annus*) seeds germinating in the presence of alkaloids from thorn apple (*Datura stramonium)* (Levitt et al. 1984). Phenolic allelochemicals can also lead to increased cell membrane permeability and increase lipid peroxidation followed by slow growth or death of plant tissue (Zhao et al. 2010).

Organic extracts of cauliflower and cabbage turnip were most toxic for nettle-leaf goosefoot growth as compared with lettuce and their toxicity increased with concentration. For this species, greater inhibitions of root growth and germination speed were recorded with all organic fractions of cauliflower extract. However, in cabbage turnip extract, the highest values of growth inhibition were recorded in petroleum ether and methanol extracts and those of germination index were recorded in petroleum ether extract. The phytotoxicity variation of cabbage varieties could be attributed to the differences in secondary metabolites richness of different organic fractions. Indeed, due to the different polarities of organic solvents, the organic extracts contain different allelochemicals groups, which explain their differential toxicity (Omezzine and Haouala 2013).

The difference in toxicity between aqueous and organic extracts could be attributed to the interactions between biologically active compounds that could act in synergy or antagonism (Omezzine and Haouala 2013). Anaya (2006) suggested that the combined effect varied, and in half of the cases, it followed the pattern expected under the assumption of independence; in the other, either synergistic or antagonistic interactions were found in both germination and elongation. Lyu et al. (1990) and Rasmussen and Einhellig (1997) reported that the combined actions of phenolic compounds could be additive, antagonistic or synergistic.

Recently, Saad et al. (2014) reported the smothering potential of white and red cabbage varieties on total weed biomass and density. Such effective suppression of total weed biomass may be attributed to several reasons. Crop allelopathy may play an important role in agrosystems as it could affect the germination and growth of neighboring plants. Roots are the site of greatest activity within the soil matrix during crop growth (Bertin et al. 2003) and crop plants have the capability to produce and exude allelochemicals into their surroundings to suppress the growth of weeds in their vicinity (Huang et al. 2003).

The allelopathic potential of *Brassica* species has been well documented (Haramoto and Gallandt 2004; Matthienssen and Kirkegaard 2006). The variation in weed smothering potential with cabbages varieties revealed in field assays may be explained by the differences in their allelochemicals levels, their glucosinolates profiles and their hydrolysis products which vary between *Brassica* species and cultivars (Castro et al. 2004; Chaplin-Kramer et al. 2011; Meyer and Adam 2008). It could also be due to differences in polyphenolic and alkaloids contents among these varieties, as found above (phytochemical analysis and germination and growth tests of aqueous and organic extracts). Singh et al. (2007) demonstrated a large variation in phenolic compounds between several cabbages varieties.

Total weed biomass and nettle-leaf goosefoot biomass were greater in fallow plots in the second year of field experiment as compared with the first one. Moreover, in plots cropped with both cabbages varieties, total weed biomass reduction was most enounced in the second year of experimentation. It is possible that allelopathy of these cabbages might have contributed to the greater suppression of nettle-leaf goosefoot. This may also partially explain why comparatively less nettle-leaf goosefoot biomass was observed during 2014 than 2013 in all plots (fallow and cultivated), probably due to the residual allelopathy from those crops carried over from the previous year 2013 (Narwal 2004). Allelopathy can affect many aspects of plant ecology including occurrence, growth, plant succession, structure of plant communities, dominance, diversity and plant productivity. Plants that germinate at slower rates are often smaller. This may seriously influence their chances of competing with neighboring plants for resources (Fallah Touzi and Baki 2012).

In line with these findings and similar to the results of the germination and growth bioassay, we can confirm allelopathic potential of cauliflower and cabbage turnip and their ability to smother weeds, especially the most abundant weed in Tunisia, nettle-leaf goosefoot. We can conclude also, that variation of both cabbages varieties in phenolic compounds, flavonoids and alkaloids amounts could be the principal raison of variation of its allelopathic potential.

The results of this study indicated that the phytochemicals content may contribute to the allelopathic activity of cabbage extracts, which is strongly dependent on the cabbage variety. Cabbage turnip extracts showed most important toxicity on target species as compared with cauliflower extracts. Similarly, in field experiment, smothering potential of this cabbage variety on weeds was more important. These results confirm the utility of introducing both cabbages varieties in a rotational crop system to improve biological weed control and decreasing herbicidal products use in agriculture.

RESUME

Saad I., Rinez I., Ghezal N. et Haouala R. 2017. Composition chimique et potentialités herbicides du chou-fleur (*Brassica oleracea* var. *botrytis*) et du chou rave (*Brassica oleracea* var. *gongylodes*). Tunisian Journal of Plant Protection 12: 95-113.

Cette étude a été réalisée pour évaluer la composition chimique et le potentiel allélopathique des feuilles de deux variétés botaniques de choux, le chou-fleur (*Brassica oleracea* var. *botrytis*) et le chou rave (*B. oleracea* var. *gongylodes*). Leurs extraits aqueux et organiques ont été évalués sur la laitue (*Lactuca sativa*) et l'une des adventices les plus dominantes en Tunisie, le chénopode des murs (*Chenopodium murale*). Des expériences sur le terrain ont été menées pour évaluer le potentiel étouffant des deux variétés. Les concentrations totales de phénols, de flavonoïdes, de flavonols et de flavones, d'alcaloïdes et de proanthocyanidines ont été élevées dans les extraits aqueux des deux variétés. Pour les extraits organiques, les extraits à l'éther de pétrole et au méthanol du chou-fleur et les extraits chloroformiques et méthanoliques du chou rave ont été les plus riches. Tous les extraits aqueux et organiques ont significativement retardé la germination, ont réduit son taux et ont affecté la croissance des plantules. La réduction de la germination et de la croissance ont été plus importantes aux plus fortes concentrations et en présence de l'extrait du chou rave. Les extraits organiques des deux variétés ont inhibé de manière significative la croissance des plantules cibles, en particulier ceux à l'éther de pétrole et au méthanol du chou-fleur et au chloroforme et au methanol du chou rave. L'essai

au champ a mis en évidence le potentiel étouffant des deux variétés et a confirmé le potentiel allélopathique plus élevé du chou rave comparé au chou-fleur.

Mots clés: Analyse phytochimique, *Chenopodium murale*, chou-fleur, chou rave, croissance, potentiel étouffant, vitesse de germination

ملخص

إيناس، سعد وإيمان ريناز ونادية الغزال وربيعة حوالة. 2017. التركيبة الكيميائية وقدرة إبادة الأعشاب للقرنبيط (*Brassica oleracea* var. *botrytis*) /للبروكلو والملفوف/للكرنب السلقي (*Brassica oleracea* var. *gongylodes*). Tunisian Journal of Plant Protection 12: 95-113.

أجريت هذه الدراسة لتقييم التركيبة الكيميائية وقدرة المجاهضة لأوراق صنفين طبيعيين من الملفوف، القرنبيط (*Brassica oleracea* var. *botrytis*) والملفوف السلقي (*B. oleracea* var. *gongylodes*). تم تقييم مستخلصاتهما المائية والعضوية على نبتة الخس وعلى واحدة من أكثر الحشائش انتشارا في تونس، الخليمعة (*Chenopodium murale*). أجريت تجارب ميدانية لتقييم القدرة الخانقة للصنفين. كان المحتوى الكلي للفينول، الفلافونويد والفلافونول والفلافونون والالكالويد والاصباغ مرتفعا في المستخلصات المائية للصنفين. أما بالنسبة للمستخلصات العضوية، فقد كانت تلك المستخرجة بالايثير البترولي وبالميثانول للقرنبيط وبالكلوروفورم وبالميثانول للملفوف السلقي هي الأغنى. أدت جميع المستخلصات المائية والعضوية إلى تأخير إنبات الخس والخليمعة بشكل كبير وإلى تخفيض معدل إنبات ونمو البادرات. وسجلت أقوى التخفيضات في الإنبات والنمو مع التركيزات الأعلى لمستخلص الملفوف السلقي. وأدت المستخلصات العضوية لكل من الصنفين إلى الحد من نمو البادرات المستهدفة، وخاصة تلك المستخلصة بالايثير البترولي وبالميثانول للقرنبيط وبالكلوروفورم وبالميثانول للملفوف السلقي. أبرزت التجربة الميدانية القدرة الخانقة للصنفين والقدرة الأقوى للمجاهضة لدى الملفوف السلقي بالمقارنة مع القرنبيط.

كلمات مفتاحية: تحليل كيميائي نباتي، سرعة الإنبات، قدرة خانقة، قرنبيط، ملفوف سلقي، نمو، *Chenopodium murale*

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