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ORIGINAL ARTICLE

Management of the noxious weed; *Medicago polymorpha* L. via allelopathy of some medicinal plants from Taif region, Saudi Arabia

Mardi Mohammed Algandaby [a,*], **Salama M. El-Darier** [b]

[a] *Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia*
[b] *Department of Botany and Microbiology, Faculty of Science, Alexandria University, Alexandria, Egypt*

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Abstract Germination bioassay was carried out to test the biological activity of *Achillea santolina* L. (ASAE), *Artemisia monosperma* Del. (AMAE), *Pituranthus tortuosus* L. (PTAE) and *Thymus capitatus* L. (TCAE) aqueous extracts (collected from Taif region, KSA) on germination percentage (GP), plumule (PL) and radicle (RL) lengths (mm) besides seedling dry weight (SDW) (mg/seedlings) of *Medicago polymorpha* L. The inhibitory effect of *P. tortuosus* was insignificant compared to the other three donor species which attained the strongest allelopathic potential in the following order: *A. santolina* > *A. monosperma* > *T. capitatus*. Growth experiment using crude powder of the four donor species was conducted to examine their effects on leaf area index (LAI), photosynthetic pigments, total available carbohydrates (TAC) and total protein (TP). It is worth mentioning that each of the four donor species crude powders mixed with clay loam soil appeared to have a great inhibitory allelopathic effect on LAI, total photosynthetic pigment and chlorophyll a (Chl a) while carotenoids exhibited a slight increase with the application of the four donor species crude powders. TAC and TP were significantly decreased with increasing the crude powder concentrations while a slight decrease was recorded for carbon/nitrogen (C/N) ratio. There is possibility of using these allelochemicals directly or as structural leads for the discovery and development of environmentally friendly herbicides to control weeds. The study recommended that these species must be phytochemically examined in future for their allelochemicals in order to provide information on the possibilities of using one or more of these species as bioherbicides.

* Corresponding author. Tel.: +966 12 6400000x52291.
E-mail address: mmalgandaby@gmail.com (M.M. Algandaby).
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1. Introduction

The loss of crop yields due to weeds is enormous (Appleby et al., 2000). Potential yield reductions caused by uncontrolled weed growth throughout a crop season have an estimated range of 45–95%, depending on ecological and climatic

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conditions (Moody, 1991) whereas; it is even greater in the developing and underdeveloped countries. Lacey (1985) stated that sometimes weeds cause even 100% of crop loss. The overuse of synthetic herbicides may affect the environment, human health and food (Khanh et al., 2005). Furthermore, the increasing use of the herbicides has resulted in a dramatic increase in the herbicide resistance among weeds, and over 307 weed resistant biotype belonging to 183 species (110 dicots and 73 monocots) have been identified world over (Heap, 2006; Batish et al., 2007; Gianessi and Reigner, 2007). The use of crops having allelopathic potential can reduce the dependency on synthetic herbicides and increase crop yields (Khanh et al., 2005). The phenomenon of allelopathy has been suggested to be one of the possible alternatives for achieving sustainable weed management (Abu-Romman et al., 2010; Salhi et al., 2011, 2012). At present, there is a trend toward searching for novel natural plant products to develop bioherbicides. Numerous plants are reported to possess allelopathic potential, and efforts have been made to apply them for weed control (Salhi et al., 2014). Although most common allelopathic plants have potential for weed suppression, (Xuan et al., 2004a). The use of allelopathy for controlling weeds could be either through the direct utilization of natural allelopathic interactions or by using allelochemicals as natural herbicides (Singh et al., 2009; Batish et al., 2004). The need for finding stronger allelopathic plants for weed control is indispensable (Xuan et al., 2003).

The use of allelopathic and medicinal plants has been suggested as a viable option for alternative weed management under sustainable agriculture (Fujii et al., 2003; Hong et al., 2003; Batish et al., 2007; Zeng et al., 2009; Salhi et al., 2011, 2012; El-Kenany and El-Darier, 2013; Salhi et al., 2013, 2014). *Medicago polymorpha* (bur clover) is an annual broad leaf plant which inhabits agricultural lands especially wheat fields, road sides and other disturbed areas (Malik et al., 2012). The species can be considered as an invasive weed and can sometimes be toxic to livestock, and the seed pods can be a serious contaminant of wool (Cal-IPC, 2005). *M. polymorpha* can cause photosensitisation in horses, occasionally red gut in sheep, and bloat in cattle. Phytoestrogens/coumestrols can potentially have negative effects on the reproduction of grazing livestock (Cabi Database, 2014). The allelopathic potential of *Medicago* species has been previously reported against edible plants (El-Darier et al., 2014). The main objective of the present study was to explore the possible allelopathic effects of *Achillea santolina* L., *Artemisia monosperma* Del., *Pituranthus tortuosus* L. and *Thymus capitatus* L. (donor species) aqueous extracts and crude powder on germination efficiency, some growth parameters as well as some metabolic changes of the weedy species; *M. polymorpha* L. under laboratory conditions.

2. Materials and methods

2.1. Plant materials and soil

Samples of fresh aerial shoots of four medicinal plants as donor/allelopathic species (*A. santolina* L., *A. monosperma* Del., *P. tortuosus* L. and *T. capitatus* L.) were collected from different locations at Taif region Saudi Arabia during spring 2015. The seeds of the weedy species; *M. polymorpha* L., were collected locally from the same locations and surface cleaned then stored in paper bags at room temperature (20 ± 2 °C) until further use. Additionally, soil samples were excavated from some agricultural fields in the same locations and applied in a pot experiment.

2.2. Phytochemical screening

Test for sterols, tannins, flavonoids, coumarins and alkaloids was carried out according to the methods described by Harborne (1973, 1998, 1999) respectively. Additionally, glycosides, saponins, were detected by the procedures of Farnsworth (1966) and Lewis and Smith (1967) respectively. On the other hand, the content of total phenols and flavonoids was determined according to Meda et al. (2005) while extraction and estimation of essential oils followed the method described by Odalo et al. (2005).

2.3. Preparation of donor species aqueous extracts

The collected aerial shoot samples were air-dried, then, cut into 0.5–1 cm pieces. Stock aqueous extract was obtained by soaking 150 g of the air-dried donor plant materials in one liter of distilled water at room temperature (20 ± 2 °C) for 24 h. The mixture was filtered through four layers of cheesecloth to remove the fiber debris and kept at 5 °C in the dark until further use. Different concentrations (5%, 10%, 15% and 20%) were prepared from the stock solution, in addition to the control (distilled water).

2.4. Germination bioassay

Petri-dish experiment was carried out to investigate the potential allelopathic effects of donor species aqueous extracts on germination percentage (GP), plumule (PL) and radicle (RL) lengths, as well as seedlings dry weight (SDW) of the recipient species. Consequently, twenty-five seeds of the recipient species were arranged in 9-cm diameter Petri-dishes on two disks of Whatman No. 1 filter paper under normal laboratory conditions with day temperature ranging from 20 °C to 23 °C and night temperature from 14 °C to 16 °C. Ten cm^3 of each level of the donor species extracts were added to four replicates. Before sowing, the seeds were treated with H_2SO_4 (96%) for 2 min then washed several times with distilled water. Thereafter, the seeds were surface sterilized by soaking for two minutes in 4% sodium hypochlorite, then, rinsed four times with distilled water. Treatments were arranged in a complete randomized block design with four replications. Measurements of GP, PL and RL were recorded after 9 days at the end of the experiment. Inhibition percentage (IP) was calculated according to the following equation (Khanh et al., 2005):

$$\text{Inhibition percentage} = [1 - (\text{sample extract}/\text{control})] \times 100$$

while the reduction in Pl and RL was calculated according to the routine equation:

$$\text{Reduction percentage} = (\text{Control} - \text{allelopathic}/\text{Control}) \times 100$$

Seedlings were dried at 105 °C till constant weight for the determination of seedling dry weight (SDW).

2.5. Growth experiment

Pot experiment was performed to validate the effect of different levels of each of the four donor species (*A. santolina*, *A. monosperma*, *P. tortuosus* and *T. capitatus*) crude powder mixed (w/w) with clay loam soil (collected from control locations) on leaf area index (LAI), photosynthetic pigments in addition to carbohydrate and protein content of *M. polymorpha* (recipient species). Total plant leaf area was measured using Digital Planimeter (Placom KP-90) to the nearest cm^2. Leaf area index (LAI) was calculated by dividing the total plant leaf area by its specific ground area. The photosynthetic pigments chlorophyll a, b (Chl a, Chl b) and carotenoids (Carot) were extracted and determined using the spectrophotometric method described by Metzner et al. (1965). Total available carbohydrates (TAC) were estimated by the procedure described by Murata et al. (1968). Total proteins were extracted according to Rausch (1981) and estimated as described by Hartree (1972).

2.6. Statistical analysis

All the data of the present study were subjected, where appropriate, to standard one-way analysis of variance (ANOVA) and *t*-test (Zar, 1984) using the COSTAT 2.00 statistical analysis software manufactured by CoHort Software Company. Pairwise comparisons of means were performed using Least Significant Differences (LSD) at 0.05 probability level.

3. Results

3.1. Soil analysis

The routine analyses for soil applied in the current study are presented in Table 1. The soil texture was sandy loam with pH value of about 7.4., the $CaCO_3$ and OM percentage attained values of about 23.8 and 2.3 respectively. Additionally, cations attained considerable values.

3.2. Phytochemical screening of the donor species

The phytochemical screening of the donor species is presented in Table 2. The data showed that all the donor species were found to contain essential oils, flavonoids, glycosides, total phenols, sterols and tannins with different incidence. However, saponins were not detected in *P. tortuosus*, which is the only plant species that contains coumarins. Alkaloids were detected only in *A. santolina* and *A. monosperma*. In addition, data in Table 3 clearly demonstrated that the highest value of total flavonoids (937 mg/100 g dw), total phenols (6935 mg/100 g dw), and essential oil (2%) were detected in *A. monosperma* while the lowest values of the three phytochemical groups (246, 1057 mg/100 g dw and 0.12% respectively) were detected in *P. tortuosus* compared to the other plant species.

3.3. Germination and growth parameters

The germination percentage (GP), plumule (PL) and radicle (RL) lengths as well as SDW of *M. polymorpha* were significantly affected by applying different concentrations of *A. santolina* (ASAE), *A. monosperma* (AMAE), *P. tortuosus* (PTAE) and *T. capitatus* (TCAE) aqueous extracts (Tables 4 and 5, and Fig. 1). The inhibitory effect of the four donor species was in the following order: *A. santolina* > *A. monosperma* > *T. capitatus* > *P. tortuosus*. Noticeably, GP exhibited a gradual decrease with the increase of all donor species aqueous extracts and the germination was completely inhibited (100% inhibition) at 20% for ASAE and AMAE (Table 4).

Table 1 Determination of some physical and chemical properties ± SD of soil collected from at Taif region (KSA) during the year of 2015.

Character	Mean ± SD
pH	7.4 ± 1.5
EC (mmhos cm^{-1})	3.5 ± 0.6
OM (%)	2.3 ± 0.4
OC (%)	1.7 ± 0.1
$CaCO_3$ (%)	23.8 ± 3.2
Soluble cations and anions (mg/100 g soil)	
Total N	65 ± 5.6
P^{+++}	1.2 ± 0.2
K^{+}	67 ± 6.1
Ca^{++}	125 ± 7.2
Mg^{++}	23.4 ± 2.5
Na^{+}	90.8 ± 5.3
Cl^{-}	100.7 ± 6.3
HCO_3^{-}	9.20 ± 2.1
Soil texture (particle size distribution)	
Sand %	53 ± 5
Silt %	30 ± 3
Clay %	17 ± 2

Data are means of 5 replicates ± SD.
EC: electrical conductivity, OM: organic matter, OC: organic carbon.

Table 2 Qualitative phytochemical screening of the four donor species collected from different locations at Taif region (KSA) during the year of 2015.

Chemical group	Plant species			
	Achillea santolina	*Artemisia monosperma*	*Pituranthus tortuosus*	*Thymus capitatus*
Alkaloids	+	+	–	–
Coumarins	–	–	++	–
Essential oil	++	+++	++	+++
Flavonoids	+++	+++	+	++
Glycosides	++	++	+	++
Total phenols	++	++++	+	+++
Saponins	++	+	–	+
Sterols	++	++	++	++
Tannins	+	+	+	+

+: detected, –: not detected.
The notations, ++++, +++, ++, + and – refer to highly appreciable amounts (positive within <2 min); appreciable amounts (positive within 5 min), moderate amounts (positive after 5 min but within 10 min); trace amounts (positive after 10 min but within 15 min) and completely absent, respectively.

Table 3 Variation in the content of total flavonoids and total phenolics (mg/100 g dw) as well as essential oils (%) detected in the four donor species collected from different locations at Taif region (KSA) during the year of 2015.

Chemical group	Plant species			
	Achillea santolina	*Artemisia monosperma*	*Pituranthus tortuosus*	*Thymus capitatus*
Total flavonoids	824 ± 24	937 ± 32	246 ± 12	500 ± 18
Total phenolics	2672 ± 56	6935 ± 87	1057 ± 29	3842 ± 64
Essential oil	0.65 ± 0.01	2.00 ± 0.12	0.12 ± 0.02	1.00 ± 0.07

Data are means of three replicates ± SD.
Total flavonoids were determined as rutin and total phenolics as chlorogenic acid.
A quantitative analysis of each essential oil component was carried out by peak area normalization measurement.

Table 4 Suppression effect of aqueous extracts from different medicinal plants on germination percentage (GP) of *Medicago polymorpha*.

Treatment (%)	Plant species			
	Achillea santolina	*Artemisia monosperma*	*Pituranthus tortuosus*	*Thymus capitatus*
0	$84^{a} \pm 2$	$89^{a} \pm 2$	$82^{a} \pm 4$	$90^{a} \pm 2$
5	$49^{b} \pm 3$	$55^{b} \pm 2$	$67^{b} \pm 3$	$50^{b} \pm 1$
10	$35^{c} \pm 2$	$38^{c} \pm 3$	$64^{c} \pm 1$	$40^{c} \pm 1$
15	$13^{d} \pm 2$	$17^{d} \pm 1$	$57^{d} \pm 4$	$25^{d} \pm 3$
20	0.0^{e}	0.0^{e}	$44^{e} \pm 2$	$20^{e} \pm 2$

Data are means of 5 replicates ± SD.
Different letters within each column indicate a significant difference at $P < 0.05$ level of probability as evaluated by one-way ANOVA test.

On the other hand, PTAE and TCAE exercised germination inhibition percentages (IP) of about 46.34 and 77.78 respectively compared to control at the same concentration.

Markedly, the plumule and radicle growth followed the same trend exhibited by the GP (Table 5). The reduction exerted as a result of application of PTAE and TCAE on the recipient species for PL was 81.36% and 75.61% and for RL was 87.18% and 87.14% respectively compared to control at the maximum concentration (20%). The other two extracts attained their maximum reduction percentages of about 78.05% for PL and 85.71% for RL as well as 37.91% for PL and 76% for RL with regard to ASAE and AMAE respectively at 15% concentration level.

Consequently, the maximum reduction percentages in SDW (mg/seedling) of *M. polymorpha* (81.25% and 80.95%) were achieved respectively at the highest ASAE and AMAE concentration level (15%) compared to control (Fig. 1). The equivalent values were 66.66% and 86.36% respectively for PTAE and TCAE at the highest extract concentration level (20%).

3.4. Physiological parameters

3.4.1. Leaf area index (LAI)

Leaf area index (LAI) (cm^2/cm^2) achieved a significant reduction due to the ascending of the four donor species crude powders (ASCP, AMCP, PTCP and TCCP) (Fig. 2). The control value was about 8.31 (mm^2/mm^2) while at 8%, LAI exhibited reduced values of about 0.27, 0.31, 0.94 and 0.89 (mm^2/mm^2) for the four crude powders respectively.

3.4.2. Pigment content

A significant decrease was attained in the total photosynthetic pigment contents of the recipient species as affected by application of *A. santolina* (ASCP), *A. monosperma* (AMCP), *P. tortuosus* (PTCP) and *T. capitatus* (TCCP) donor species crude powders (Table 6). The reduction percentages were about 51.8, 45.7, 31.02 and 40.36 respectively which are compatible with 60.15%, 56.19%, 37.1% and 55.04% reduction percentages for Chl a respectively. On the other hand, carotenoids exhibited a slight increase with the application of the four donor species crude powders.

Table 5 Suppression effect of aqueous extracts from different medicinal plants on plumule (PL) and radicle (RL) lengths (mm) of *Medicago polymorpha*.

Treatment(%)	Plant species							
	Achillea santolina		*Artemisia monosperma*		*Pituranthus tortuosus*		*Thymus capitatus*	
	PL	RL	PL	RL	PL	RL	PL	RL
0	$41^{a} \pm 4$	$70^{a} \pm 6$	$46^{a} \pm 4$	$75^{a} \pm 6$	$49^{a} \pm 4$	$78^{a} \pm 6$	$41^{a} \pm 4$	$70^{a} \pm 6$
5	$26^{b} \pm 2$	$35^{b} \pm 3$	$29^{b} \pm 3$	$38^{b} \pm 3$	$29^{b} \pm 3$	$39^{b} \pm 4$	$32^{b} \pm 2$	$51^{b} \pm 4$
10	$14^{c} \pm 2$	$24^{c} \pm 3$	$18^{c} \pm 2$	$28^{c} \pm 2$	$23^{c} \pm 3$	$32^{c} \pm 3$	$26^{c} \pm 3$	$38^{c} \pm 5$
15	$9^{d} \pm 1$	$10^{d} \pm 1$	$12^{d} \pm 2$	$18^{d} \pm 2$	$16^{d} \pm 2$	$22^{d} \pm 3$	$20^{d} \pm 3$	$31^{d} \pm 4$
20	0.0^{e}	0.0^{e}	0.0^{e}	0.0^{e}	$9^{e} \pm 2$	$10^{e} \pm 2$	$10^{e} \pm 1$	$9^{e} \pm 1$

Data are means of 5 replicates ± SD.
Different letters within each column indicate a significant difference at $P < 0.05$ level of probability as evaluated by one-way ANOVA test.

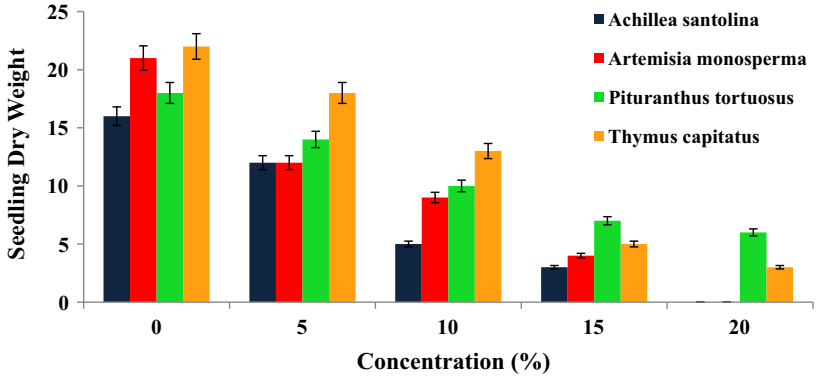

Figure 1 Suppression effect of aqueous extracts from different medicinal plants on seedling dry weight (SDW) (mg/seedling) of *Medicago polymorpha*. Vertical bar above each column represents SD.

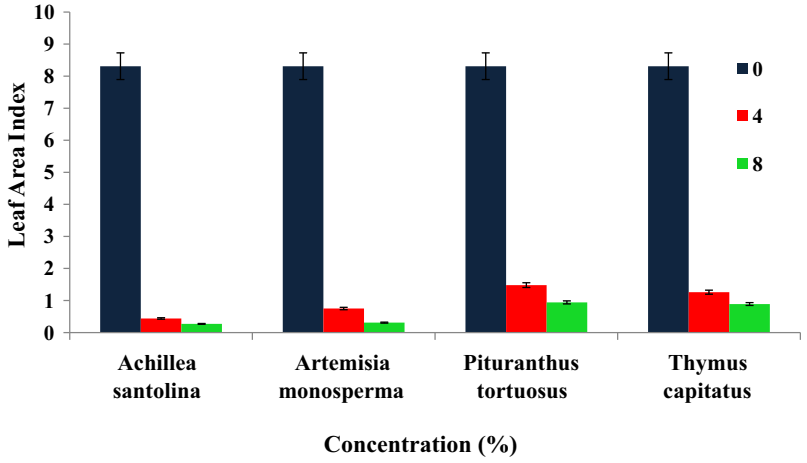

Figure 2 Suppression effect of crude powders from different medicinal plants on leaf area index (LAI) (cm^2/cm^2) of *Medicago polymorpha*. Vertical bar above each column represents SD.

Table 6 Suppression effect of crude powders from different medicinal plants on pigment content ± SD of *Medicago polymorpha*.

Donor species	Treatment (%)	Chlorophyll fractions			
		Chl a	Chl b	Carot	Total
Achillea santolina	0	15.66^{a} ± 2.1	5.89^{a} ± 0.5	0.92^{b} ± 0.01	22.47^{a} ± 1.3
	4	7.23^{b} ± 1.4	2.81^{b} ± 0.2	2.15^{a} ± 0.03	12.19^{b} ± 0.9
	8	6.24^{c} ± 1.2	1.93^{c} ± 0.1	2.66^{a} ± 0.02	10.83^{c} ± 1.3
Artemisia monosperma	0	15.66^{a} ± 2	5.89^{a} ± 0.4	0.92^{c} ± 0.01	22.47^{a} ± 1.7
	4	9.21^{b} ± 1.5	3.32^{b} ± 0.6	1.98^{b} ± 0.1	14.51^{b} ± 1.7
	8	6.86^{c} ± 1.2	2.00^{c} ± 0.2	3.34^{a} ± 0.3	12.20^{c} ± 2
Pituranthus tortuosus	0	15.66^{a} ± 1.6	6.62^{a} ± 1	0.92^{b} ± 0.04	22.47^{a} ± 2.6
	4	14.85^{b} ± 1.4	5.89^{b} ± 1.1	1.14^{ab} ± 0.01	21.88^{b} ± 1.5
	8	9.85^{c} ± 2	3.87^{c} ± 0.9	1.78^{a} ± 0.2	15.50^{c} ± 2
Thymus capitatus	0	15.66^{a} ± 2.1	5.89^{a} ± 0.6	0.92^{b} ± 0.02	22.47^{a} ± 1.3
	4	8.95^{b} ± 2	4.42^{b} ± 0.5	2.20^{a} ± 0.02	15.57^{b} ± 1.4
	8	7.01^{c} ± 1.6	3.56^{c} ± 0.8	2.83^{a} ± 0.1	13.40^{c} ± 1.2

Data are means of 5 replicates ± SD.
Chl a: chlorophyll a.
Chl b: chlorophyll b.
Carot: carotenoids.
Different letters within each column and receptor species indicate a significant difference at $P < 0.05$ level of probability as evaluated by one-way ANOVA test.

3.4.3. Total available carbohydrates, total proteins and C/N ratio

Generally, total available carbohydrates (TAC) and total protein (TP) (mg g^{-1} dw) of the recipient species were significantly decreased with increasing crude powder concentrations (Fig. 3). Application of ASCP with 8% crude powder concentration created a decrease of about 64% and 62% while the decrease due to AMCP attained values of about 71% and 68% compared to control respectively. Similarly, decrease percentages of about 51% and 45% as well as 62% and 62% were achieved for TAC and TP content as affected by PTCP and TCCP respectively. On the other hand, carbon/nitrogen ratio exhibited a slight decrease upon applying donor species crude powders.

4. Discussion

Medicinal plants have inhibitory effects on selected weeds and its allelochemicals inhibiting weed growth (Lin et al., 2003, 2004; Hemada and El-Darier, 2015). Therefore, it was easier to screen allelopathic plants from medicinal ones than other plants possibly because they have the ability to accumulate certain metabolic compounds curing many diseases of mankind (Qasem and Hassan, 2003; Salhi et al., 2014). In the present study, the aqueous extract of all the donor species (*A. santolina*, *A. monosperma*, *P. tortuosus* and *T. capitatus*) suppressed seed germination of *M. polymorpha* under different concentrations. The degree of inhibition was enhanced by increasing the concentration. The allelopathic effect of the donor species was ranked as follows: *A. monosperma* > *T. capitatus* > *A. santolina* > *P. tortuosus*. Noticeably, this alternative effect of the allelopathic substance could be related to the species specific growth regulatory effect of allelochemicals and could be concentration dependent (Einhellig, 1996; Salhi et al., 2012) expressed in the phytochemical screening of the four donor species. These results are in agreement with those previously reported by Kato-Noguchi (2001) who found that *Melissa officinalis* shoots aqueous extract inhibited the seed germination of the weed species; *Amaranthus caudatus*, *Digitaria sanguinalis*, *Phleum pratense* and *Lolium multiflorum*. Additionally, such an inhibition was supported by data provided by other studies (Batish et al., 2004; Xuan et al., 2004b; Khanh et al., 2005, 2006). Moreover, Ashrafi et al. (2008) demonstrated that aqueous extract of *Azadirachta indica* (Neem) shoots reduced the seed germination of *Amaranthus rotundus*, *Cirsium arvense*, *D. sanguinalis*, *Sinapis arvensis* and *L. multiflorum* as weed species.

Nearly, all reports on allelopathy described some types of bioassay method used to demonstrate allelopathic activity (Macias et al., 2000). The most widely used bioassay is the technique of seed germination. However, several studies had revealed that this was not the most sensitive parameter (Leather and Einhellig, 1986; Fuentes et al., 2004) and growth bioassays were often more sensitive than germination bioassays (Bhowmik and Doll, 1984) especially for the evaluation of phytotoxicity. Additionally, Hall and Henderlong (1989) and Chung and Miller (1995) had also observed that the seedling growth was more sensitive to allelochemicals than the associated seed germination.

Results of the current study suggested that aqueous extracts of all the donor species exhibited inhibitory effect on plumule (PL) and radicle (RL) lengths of the recipient species under all concentration levels, which support the previously documented GP implications. Both PL and RL of the recipient species were affected negatively due to the addition of different plant extracts and this effect was directly proportional to the concentration and more significant in the case of the radicle compared to the plumule. These results are in agreement with the declaration that water extracts of allelopathic plants have generally more pronounced effects on radicle length rather than plumule length (Turk and Tawaha, 2002; Ashrafi et al., 2007). Additionally, Ashrafi et al. (2008) stated that radicle length was more sensitive to allelochemicals than hypocotyls. Wakjira et al. (2005) reported that lettuce roots were more sensitive to the allelochemicals than shoots on applying *Parthenium hysterophorus* extracts. Similar findings were also reported by Batish et al. (2004), Modallal and Al-Charchafchi (2006), Vasilakoglou et al. (2007) and Mekky (2008). In a parallel work, aqueous extracts of *Lantana camara* reduced root growth of *Phalaris minor* and *Sorghum bicolor* (El-Kenany and El-Darier, 2013). This may be attributable to the fact that

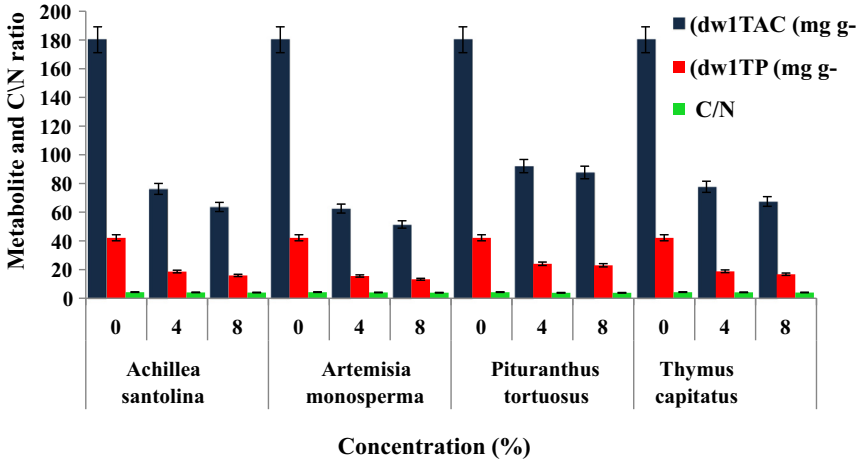

Figure 3 Suppression effect of crude powders from different medicinal plants on total available carbohydrates (TAC), total proteins (TP) as well as C/N ratio of *Medicago polymorpha*. Vertical bar above each column represents SD.

radicle had direct contact with soil or extract and can absorb many allelochemicals. The most probable explanation for the reductions in seedling root and shoot is the reduced rate of cell division and cell elongation due to the presence of allelochemicals in the aqueous extracts (Javaid and Anjum, 2006). Furthermore, allelochemicals could inhibit the elongation, expansion and division of cells which were a prerequisite for seedling growth (Einhellig, 1995). Several studies had shown that compounds of plant origin, such as allelochemicals, affect mitotic activity of growing roots (Rizvi et al., 1992; Einhellig, 1996). Such an inhibitory effect on mitotic may directly decrease plant growth, and so mitotic activity can be used to evaluate root growth resulting from cell division of meristematic cells and cell expansion in the elongation zone of roots (Dayan et al., 2000).

It was reported that measuring the seedling length might result in complicated curling and other morphological alterations such as the seedling dry weight (SDW) which may be a better selection criteria for allelopathic potential than the seedling length (Ahn and Chung, 2000). These findings agreed with that obtained by Terzi et al. (2003). The authors emphasized that the dry weights of root and the stem of cucumber seedlings were negatively influenced by the decomposed walnut leaves and juglone, depending on the concentration. Under the present study, the aqueous extract of all the donor species reduced SDW of the recipient species at different concentration levels. Reduction increased as the concentration of the donor plant increased; however the reduction degree was varied and was species dependent. *A. monosperma* extract had the greatest allelopathic potential at all concentrations, while *P. tortuosus* had the lowest ones. This reduction may be attributed to the presence of allelochemicals in the aqueous extracts. Baziramakenga et al. (1994) found that allelochemicals reduced the number of lateral roots, root and shoot dry biomass of soybean. Similar findings were also reported by Batish et al. (2004) who found that eucalypt oil could significantly reduce the seedling growth, as well as the chlorophyll content and the cellular respiration of the tested plants. Additionally, coumarin derivatives inhibited the dry matter production, the leaf area expansion, and the photosynthesis in the seedling of tobacco, sunflower and pigweed (Einhellig et al., 1970). An analogous work was conducted to determine the allelopathic effect of *Passiflora edulis* aqueous extracts on growth and seedling dry weight of two major paddy weeds *Echinochloa crusgalli* and *Monochoria vaginalis*. It was reported that the aqueous extracts strongly suppressed seedling growth.

Data also revealed that all the donor species crude powder concentrations suppressed LAI of the recipient species. This reduction may be attributed to the presence of allelochemicals in the aqueous extracts and may be related to the inhibition of cell division and/or cell expansion (El-Darier, 2002; Javaid and Anjum, 2006). Currently, total photosynthetic pigments were significantly decreased in the recipient species with the increasing of donor species crude powder levels which may be attributed to the decrease of chlorophyll a content. In general, the decrease in photosynthetic pigment may be related to the inhibitory effect of the released allelochemical substances from the donor species on the synthesis of porphyrin precursors of chlorophyll biosynthesis (Rice, 1984), inhibition of specific enzymes responsible for the synthesis of chlorophyll and/or the activation of chlorophyllase which catalyzed the catabolism of chlorophyll (Yang et al., 2004) or the modification in the integrity of chloroplast and thylakoid membranes in response to allelochemicals (Batish et al., 2004).

Throughout the present study, the total available carbohydrates (TAC) in the recipient species were significantly decreased with the increasing of donor species crude powder levels. Furthermore, the crude powder of *A. monosperma* showed the greatest allelopathic potential, while *P. tortuosus* had the lowest effect. Such decrease may be attributed to the inhibitory effect of the released allelochemical substances on the synthesis of photosynthetic pigment, and hence, on photosynthesis. Bernat et al. (2004) reported that the decrease of TAC of *Sinapis albaunder* allelochemical stress may be related to the reduction of the photosynthetic leaf area and the stomatal frequency, as well as, the inhibitory effect on the photosynthetic apparatus which may result in a decrease of CO_2 diffusion and assimilation. In addition, the reduction of photosynthetic allocation may be due to the destructive effect of allelochemical stress on plasma membrane and phloem elements (Singh and Rao, 2003). Also, Gniazdowska and Bogatek (2005) concluded that the consumption of most photosynthates in respiration for diverting energy to maintenance of the physiological processes, under allelochemicals stress, resulted in a decrease of TAC in *Zea mays* seedlings. Therefore, under prevailing experimental conditions, insufficient non-structural saccharides were converted to structural carbohydrates which were incorporated as carbon skeleton for the growth of recipient species. Thus, this suggestion, in line with the present study, may explain the decrease in dry mass and growth of the tested crop and weedy species.

It had been demonstrated that allelopathic substance stress generally induced a marked decrease in total protein content. Under the present study, total protein content in the recipient species was significantly decreased with the increasing of donor species crude powder levels. Inhibition of photosynthesis and other impaired metabolic activities, under this study, may have resulted in a decrease of protein synthesis and/or the stimulation of the protein degradation. Mersie and Singh (1993) and Hoque et al. (2007) reported that the degradation of the protein to amino acids, such as proline, might be an adaptation mechanism against the allelochemical stress and/or a means of osmolytes to prevent water loss. In addition, allelochemicals are known to generate reactive oxygen species (ROS) which caused oxidative modification/degradation of proteins (Lara-Nunez et al., 2006; Venkateshwarlu et al., 2001). Therefore, the decline in total protein content, under this study, may be related to the enhancement of protein degradation, and/or alteration in the incorporation of amino acids into protein, hence, reduce the growth of target species.

5. Conclusion

The present study evaluated the allelopathic potential of ASAE, AMAE, PTAE and TCAE on GP, PL, RL, SDW, LAI, pigments, TAC and TP of *M. polymorpha*. All these parameters were significantly decreased at different concentration levels. Based on these results, the recipient species showed distinct poor growth and metabolism under the effect of the different donor species. The species with the strongest allelopathic potential (*A. monosperma*, *T. capitatus* and *A. santolina*) must be examined for their selective action on other specific

plants including crops and weeds under field conditions, and their allelopathic activity should be further studied in detail.

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