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Allelopathic effect of the two medicinal plants *Plectranthus amboinicus (Lour.)* and *Ocimum basilicum* L. on the growth of *Pisum sativum* L. and associated weeds

Kowthar G. El-Rokiek, Samia A. Saad El-Din, Mona A. El-Wakeel, Mona G. Dawood and Mohammad E. El-Awadi

Botany Department, Agricultural and Biological Research Division, National Research Centre, 33 El Buhouth St., (Former El Tahrir St.) 12622 Dokki, Giza, Egypt.

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ABSTRACT

In this study, allelopathic effects of leaf water extracts of *Plectranthus amboinicus (Lour.)* and *Ocimum basilicum* on the growth of grass weed (*Phalaris minor*) and broad leaf weed (*Anagalis arvensis*) that grow with pea (*Pisum sativum*) were investigated. The experiments were conducted in the greenhouse of the National Research Centre, Giza, Egypt. This study was carried out in the two winter seasons, 2016/2017 and 2017/2018. Plants and weeds were sprayed with different concentrations of both *P. amboinicus* and *O. basilicum* leaf water extracts (7, 12.5 and 25%). The results indicated reduction in weed growth of *P. minor* and *A. arvensis.* Increasing concentration of the two leaf extracts up to 25% maximize the inhibitions in weed growth in both seasons exceeding 80% in both *A. arvensis* and *P. minor*. On the other hand, the reduction in weed biomass was concomitant with increasing in *P. sativum* growth. The results also revealed increasing in number of pods/plant, weight of pods / plant, weight of seeds / plant and weight of 100 seeds that represented yield and yield components of *P. sativum*. Therefore, the results suggested that the leaf water extracts of *P. amboinicus* and *O. basilicum* can be used as alternative to herbicides for controlling *P. minor* and *A. arvensis* associating *P. sativum*.

***Keywords*:** Allelopathy, Weed control, *Pisum sativum*, *Anagalis arvensis*, *Phalaris minor*, *Plectranthus amboinicus (Lour.)*, *Ocimum basilicum*, water extract.

Introduction

Allelopathy is an interference mechanism, in which live or dead plant materials release chemical substances, which inhibit or stimulate the associated plant growth (Macias *et al*., 2003 and Cheng and Cheng, 2016). Allelopathic plants interfere with nearby plants by dispersing chemicals into the soil that may inhibit plant growth, nutrient uptake or germination (Singh *et al*., 2003). Medicinal plants contain large amount of various secondary metabolites (Azirak and Karaman, 2008; Mutlu and Atici, 2009; Uremis *et al*., 2009; Gitsopoulus *et al*., 2013; Soliman and Masoud, 2014; Skrzypek *et al*., 2015; Mehmet *et al*., 2016 and Shokouhian *et al*., 2016). These compounds (allelochemicals) can be used as bioherbicides in controlling weeds. Species of the family labiatae (lamiaceae) possess strong allelopathic activity against other plant species (Islam and Kato-Noguchi, 2013). *Ocimum basilicum* L. (Basil) belongs to the Labiatae family. Many workers reported allelopathic activity of *O. basilicum*. Fanaei *et al*., 2013 found that shoot and root water extract of *O. basilicum* up to 100 g/l inhibited dry weight of *Abutilon theopharasti*, *Chenopodium album* and *Centaurea depressa.* Additive similar results on allelopathic properties of *O. basilicum* extracts were obtained by several workers (Bokhari, 2009, Verma *et al*., 2012; Petrova *et al*., 2015 and Gupta, 2016).

In addition, the genus *Plectranthus* belongs to the Labiatae family, involves about 300 naturally occurring species in Africa, Asia and Australia (Harley and Reynolds, 1992). *Plectranthus amboinicus* (Lour.) (Country borage) grows naturally and distributed in tropical Africa and Asia. Aqueous extract of *P. barbatus* leaves reduced seed germination and seedling growth of *Bidens pilosa* and *Lactuca sativa*, the reduction was proportional with concentrations (Azambuja *et al*., 2008 and 2010). Pinheiro *et al*. (2015) reported that the essential oil of *P. amboinicus* and its chemotypes, carvacrol and thymol inhibited the germination and decreased root and aerial growth of *Lactuca*

Corresponding Author: Kowthar G. El-Rokiek, Botany Department, Agricultural and Biological Research Division, National Research Centre, 33 El Buhouth St., (Former El Tahrir St.) 12622 Dokki, Giza, Egypt. E-mail: kowtharelrokiek@gmail.com

sativa and *Sorghum bicolor*. Khalid and El-Gohary (2014) reported that Carvacrol and thymol represented the most abundant component of the oxygenated monoterpenes in *P. amboinicus*. Moreover, other labiatae plants were found to contain carvacrol and thymol and posses allelopathic activity, as for example, the genus *Thymus* (Yan *et al*., 2015). Regarding this concept, Safari *et al*., 2010 reported that aqueous extracts of *Thymus kotchyanus* inhibited seed germination and initial growth as well as fresh and dry weight of *Bromusto mentellus* and *Trifolium repens* at concentrations 5-100% and the growth inhibition increased with increasing concentration up to 100%. Asghar *et al*., 2012 found that *Thymus Kotchyanus* powder at concentrations (5-25g) reduced germination and seedling growth of *Sanguisorba minor* especially at 25g. Germination and growth of little seed (*Phalaris Paradoxa*), and ryegrass (*Loliummul tiflorum*) were significantly reduced by aqueous extract of *Thymus vulgaris (L.)* up to 60 mg ml^{-1} (Balah and Latif, 2013).

The aim of the study was to evaluate the effect of *O. basilicum* L. and *P. amboinicus* leaf water extract on the growth of weed species (*Phalaris minor* and *Anagalis arvensis*) and the reflection on *Pisums ativum* growth and yield.

Materials and Methods

Preparation of the *Ocimum basilicum* and *Plectranthus amboinicus* leaf water extracts

Ocimum basilicum L. and *Plectranthus amboinicus (Lour.)* fresh leaves were collected from Egyptian gardens and washed with tap water to remove dust then cut to fine particles and transferred to labeled beakers (500g of each). Two liters of distilled water were added to each beaker and shaken well and allowed to soak for 24 h at room temperature. Then the produced leaf extracts of both *O. basilicum* and *P. amboinicus* were collected and filtered through a fine mesh and compressed carefully for complete extraction. The produced extracts at 25% concentration were diluted with distilled water to concentrations of 12.5% and 7% for each extract. The method of extraction was repeated according to need so; the extracts were always fresh.

Pot experiments

Pot experiments were done in the greenhouse of the National Research Centre, Egypt during two winter seasons 2016/2017 and 2017/2018. *Pisum sativum* L. (Pea) seeds cv. Entesar 2 was obtained from Agricultural Research Centre, Egypt. The pots, 30 cm in diameter and 30 cm in height, contained equal amounts of sieved soil (2: 1 v/v clay and sand). *P. sativum* seeds were selected for uniformity by choosing those of equal size and with the same colour. Seeds were sown 2 cm deep (five seeds in each pot) and allowed to germinate. All pots (except weed free treatment) were infested with the same weight of weed (0.03 g) of both *Anagalis arvensis* and *Phalaris minor* seeds and mixed thoroughly at a depth of 2 cm in the soil. The seeds of *P. sativum* and both weeds were sown at the same time. The cultivated *P. sativum* were thinned two weeks after sowing so that three homogeneous seedlings were left per pot. Super phosphate was added to each pot before sowing while Ammonium nitrate was added during plant growth. (2:1 w/w). The experiment consisted of eight treatments including: two untreated controls, *P. sativum* only, *P. sativum* with *A. arvensis* and *P. minor* (unweeded treatment). The other six treatments were *O. basilicum* and *P. amboinicus* leaf water extract. Each treatment was represented by 6 pots. The pots were distributed in complete randomized design. The prepared leaf extracts at 7, 12.5 and 25% of *O. basilicum* and *P. amboinicus* were sprayed on the pots contained *P. sativum* plants and the two weed species at the rate of 50 ml /pot. The treatments were applied three times weekly starting from two weeks old. The data were taken at 40 days after sowing and at harvest.

Characters studied

Weeds

Three replicates were collected from each treatment at 40 days after sowing (DAS) and at the end of the season. Dry biomass of both *A. arvensis and P. minor (*g/pot) were taken.

***Pisum sativum* plants**

Plant growth

In both seasons, samples of *P. sativum* plants were collected from each treatment to determine plant height (cm), number of leaves/plant, dry biomass / plant (g) at 40 DAS.

Yield and yield components

At harvest, samples of *P. sativum* plants were taken from each treatment to determine number of pods/plant, weight of pods/ plant (g), weight of seeds / plant, and weight of 100 dry seeds (g).

Chemical analysis

Determination of total phenolic and total flavonoids contents:

Total phenolic and total flavonoids contents were determined in *O. basilicum* and *P. amboinicus* leaf water extract according to Srisawat *et al*., 2010.

Determination of total essential oils:

Total essential oils content in fresh leaves of *O. basilicum* and *P. amboinicus* were subjected to hydro-distillation (HD) for 3 h using a Clevenger-type apparatus (Clevenger, 1928). The essential oil content was calculated as a relative percentage (v/w).

Statistical analysis

All data were statistically analyzed according to Snedecor and Cochran (1980) and the treatment means were compared by using LSD at 5% probability.

Results

Weeds

The results in Table 1 indicate that *O. basilicum* and *P. amboinicus* leaf water extract at concentrations 7-25% reduced significantly dry weight of the two weeds *A. arvensis* and *P. minor* as compared to the corresponding control. The reduction in weed growth was persistent during the experimental period. Great significant inhibitions were obtained in the two weeds by using 25% of the leaf extract of both *O. basilicum* and *P. amboinicus.* The reduction in weed growth was higher with using *P. amboinicus* leaf water extract. In addition, the reduction in *A. arvensis* was higher than *P. minor.* At the end of the season, the growth of *A. arvensis* reached maximum reduction by using 25% leaf extract of *P. amboinicus,* it reduced to about 89% as compared to the control. The corresponding result in *P. minor* was about 81%.

Table 1: Effect of leaf water extract of *O. basilicum* and *P. amboinicus* on the growth of *A. arvensis* and *P. minor* (g / pot) (Average of the two seasons).

Treatments	Extract concentration (%)	Dry weight			
		At 40 days after sowing		At the end of the season	
		A. arvensis	*P. minor*	*A. arvensis*	*P. minor*
Weed free *P. sativum*	0	-	-	-	-
P. sativum +A.arvensis+ P. minor	0	3.216	4.350	38.00	88.00
P. sativum +A.arvensis+ P. minor + O. Basilicum	7	2.285	3.550	18.66	46.00
	12.5	1.838	2.995	10.50	37.00
	25	1.696	2.593	7.00	30.00
P. sativum +A.arvensis+ P. minor + P. Amboinicus	7	1.443	3.250	11.83	39.50
	12.5	1.002	2.800	9.33	33.50
	25	0.682	2.466	4.16	16.50
LSD at 5%		0.101	0.248	1.97	3.85

Growth characters of *Pisum sativum*

The results in Table 2 show significant increases in plant height, number of leaves / plant as well as dry weight of *P. sativum* at 40 DAS due to spraying with water leaf extract of *O. basilicum* and *P. amboinicus* in comparison to the unweeded control. Maximum significant increases in these characters were obtained with using 25% *P. amboinicus* leaf extract. Spraying plants with 25% leaf water extract of *P. amboinicus* induced remarkable significant increase in dry weight of *P. sativum*, it exceeded 200% over the unweeded control.

Table 2: Effect of leaf water extract of *O. basilicum* and *P. amboinicus* on different growth criteria of *P. sativum*at 40 days after sowing (Average of the two seasons).

Treatments	Extract concentration %	Plant height (cm)	Number of Leaves / plant	Dry weight (g/plant)
Weed free *P. sativum*	0	69.84	22.50	5.290
P. sativum +A.arvensis+ P. minor	0	34.00	11.00	1.750
P. sativum +A.arvensis+ P. minor + O. basilicum	7	60.00	21.33	3.500
	12.5	65.00	23.00	4.533
	25	70.00	25.00	6.333
P. sativum +A.arvensis+ P. minor + P. amboinicus	7	63.66	25.00	4.583
	12.5	67.66	27.50	5.166
	25	75.33	31.50	6.750
LSD at 5%		3.53	1.13	0.233

Yield and yield components of *Pisum sativum*

Mostly, significant increases in number of pods / plant, weight of pods/plant, weight of seeds/plant as well as weight of 100 seeds were obtained by spraying the plants with leaf water extracts of *O.basilicum* and *P. amboinicus* up to 25% in comparison to the unweeded plants (Table 3). *P. amboinicus* leaf water extract was superior in increasing yield and yield components. Weight of seeds / plant (yield /plant) exceeded 200% when spaying with 25% *P. amboinicus* leaf water extract over that of unweeded plants. In addition, the increase in weight of 100 seeds/plant exceeded 100% over that of unweeded plants.

Table 3: Effect of leaf water extract of *O. basilicum* and *P. amboinicus* on different yield of *P. sativum.*(Average of the two seasons).

Treatments	Extract concentration %	No. pods/plant	Wt. pods/plant (g)	Wt. seeds/plant (g)	Wt. of 100 seeds (g)
Weed free *P. sativum*	0	3.33	11.66	12.10	42.50
P. sativum +A.arvensis + P. minor	0	2.33	3.50	3.29	18.42
P. sativum +A.arvensis + P. minor + O. basilicum	7	2.66	5.66	5.05	21.44
	12.5	3.00	7.91	7.62	27.18
	25	3.33	10.57	9.31	32.00
P. sativum + A.arvensis + P. minor + P. amboinicus	7	3.33	7.50	6.85	22.73
	12.5	4.00	10.75	8.18	31.83
	25	5.66	15.25	10.30	39.16
LSD at 5%		0.42	0.78	0.55	2.18

Chemical analysis

The results in Table 4 reveal that the contents of total phenolics as well as total flavonoids in *O. basilicum* leaf water extract recorded high level over that in corresponding *P. amboinicus.* On the other hand, analysis of total essential oils in the fresh leaves of both plants indicated higher level of essential oils in *P. amboinicus* over their corresponding in *O. basilicum* leaves.

Table 4: Total phenolic and flavenoids contents of *O. basilicum T.* and *P. amboinicus*

Plant species	Total phenolic content as mg/100g fresh weight	Total flavonoids as mg /100g fresh weight
O. basilicum	164.00	120.50
P. amboinicus	29.50	11.18

Table 5: Total essential oils content of both *O. basilicum* and *P. amboinicus.*

Plant species	Total essential oils content as ml/ 100g fresh weight
O. basilicum	0.07
P. amboinicus	0.1

Discussion

There are many strategies in the world in ecological system to minimize the amount of chemicals used in weed control by introducing biological and ecological methods. One of the possible methods is the use of the chemical interaction between plants which means allelopathy (Cheng and Cheng, 2016).

The results of this study show that leaf water extract of *O. basilicum* and *P. amboinicus* can interfere with the growth of *A. arvensis* and *P. minor* leading to negative responses. These negative responses were indicated in dry weight reduction (Table 1). There are variation in dry weight inhibition according to the concentration and extract type in addition to variation in responses between the two weed species. Therefore, the highest concentration (25%) of the two extract overcame the other two lower concentrations (7 and 12.5%) in weed growth inhibition. Furthermore, the highest concentration of *P. amboinicus* leaf water extract was superior for all in weed growth reduction. In general, growth inhibition in *A. arvensis* was higher than *P. minor* in comparison to their corresponding untreated control. Purohit and pandya (2013) showed 80 % reduction of *Amaranthus* by *Ocimum* leaf water extract at 1% which confirmed by Baličević *et al*., 2015 who reported that 100% complete inhibition in germination and weed seedling growth of *Tripleurospermum inodorum* (L.) C.H. Schultz resulted with high concentration of *O. basilicum* extract. The reduction in plant growth by *P. amboinicus* has been documented by Pinheiro *et al*., 2015 who reported that the essential oil of *P. amboinicus* and its chemotypes, carvacrol and thymol inhibited the germination and decreased root and aerial growth of *Lactuca sativa* and *Sorghum bicolor*. Regarding other species of *Plectranthus* is as *P. barbatus*, Azambuja *et al*., 2008 and 2010 found that aqueous leaf extract reduced seed germination and seedling growth of *Bidenspilosa* and *Lactuca sativa* and the reduction was proportional with concentrations. In general, other plants belong to the family labiatae possess similar allelochemical compounds such as thymol and carvacrol cause similar effects. In this regards, *Thymus Kotchyanus* powder at concentrations (5-25g) , *Thymus vulgaris* aqueous extract up to 60 mg ml^{-1} were found to reduce germination and seedling growth of *Sanguisorba minor, Phalaris paradoxa* and *Loliummul tiflorum* (Asghar *et al*., 2012 and Balah and Latif, 2013).

Several documented works recorded similar results (Iganci *et al*., 2006; Bokhari, 2009; Safari *et al*., 2010; Verma *et al*., 2012; Fanaei *et al*., 2013; Purohit and pandya, 2013 and Petrova *et al*., 2015). In this connection, Aromatic compounds in the extracts of leaves of *O. basilicum* L. and *P. amboinicus* were identified by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) e. g. (linalool, eugenol and cineole in *O. basilicum*; thymol, carvacrol in *P. amboinicus* (Lee *et al*., 2005 and Khalid and El-Gohary, 2014) as well as different phenolic acids e.g., caffeic acid, cinnamic acid, chlorogenic acid and quinic acid (Kwee and Niemeyer, 2011 and Roby *et al*., 2013).

El-Rokiek *et al*., 2016 & 2017 interpreted the growth reduction in weed to the presence of both total polyphenols and flavonoids. Table 4 reveals that leaf water extract of both *O. basilicum* and

P. amboinicus contain flavonoids and phenolics; according to El-Rokiek (2016 & 2017) these may be the cause of weed growth inhibition. The results indicated that *P. amboinicus* leaf water extract induced more growth inhibition than *O. basilicum* although both flavonoids and phenolics in *O. basilicum* was highly elevated over that in *P. amboinicus* (Table 4). Therefore, the increase in weed growth inhibition by *P. amboinicus* may not be correlated with flavonoids and phenolics only; it may be related to other compounds in essential oils in the plant leaves. Quesequentley, total essential oils in the extracts of both plants were quantitavely determined. On the light of these results (Table 5), it could be concluded that essential oils in addition to both phenolic compounds and flavonoids may be the cause of allelopathic inhibition caused by both extracts. In addition these results explained why *P. amboinicus* extract which overcame its correspondence in *O. basilicum* extract in the inhibition of weed growth. These last results were confirmed by Viña and Murillo, (2003) and Rodrigues *et al*. (2013) who found that total identified compounds in essential oil of *P. amboinicus* leaves were 97.3% while in *O. basilicum* ranged from 14-43%. Additional confirming results were obtained by De Almeida *et al*., 2010 tested 12 essential oils of different species from Mediterranean aromatic plants; *Hyssopus officinalis, Lavandula angustifolia, Majorana hortensis, Melissa officinalis, Ocimum basilicum, Origanum vulgare, Salvia officinalis, Thymus vulgaris, Verbena officinalis, Pimpinella anisum, Foeniculum vulgare and Carum carvi* against *Lepidium sativum*, *Raphanus sativus* and *Lactuca sativa.* The authors cited that Thyme, balm, vervain and caraway essential oils were more active against both germination and radicle elongation. The authors suggested correlation between inhibition in seedling growth and monoterpenes. This fact was confirmed by Yan e*t al*., (2015).

The increasing in growth inhibition of weeds was correlated with increasing in *P. sativum* growth and yield (Tables 2&3). So, plant height, number of leaves / plant as well as dry weight / plant increased over the unweeded control. Accordingly, increases in number of pods / plant, weight of pods / plant, weight of seeds / plant and weight of 100 seeds were obtained. Controlling *A. arvensis* and *P. minor* by the leaf water extract of *O. basilicum* and *P. amboinicus* (Table 1) reduced their competition with *P. sativum* leading to increase in growth and yield of *P. sativum* (Tables 2&3). Several workers recorded increasing in crop growth and yield by controlling associated weeds (El-Masry *et al*., 2015; Ahmed *et al*., 2016 and El-Rokiek *et al*., 2013 and 2016).

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