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Article

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ALLELOPATHIC POTENTIAL OF LAVENDER'S EXTRACT AND COUMARIN APPLIED AS PRE-PLANT INCORPORATED INTO SOIL UNDER AGRONOMIC CONDITIONS

Potencial Alelopático do Extrato da Lavanda e da Cumarina Aplicado na Pré-Emergência, Incorporado ao Solo em Condições Agronômicas

ABSTRACT - This study aimed to further explore that if coumarin and lavender's extract, similar to greenhouse conditions, are phytotoxic towards some plant species under agronomic conditions. Before planting of maize, coumarin at 0, 250, 500, 1,000, 2,000, and 4,000 g h^{-1} and lavender's aqueous extract at 0, 1,000, 2,000, 4,000, 8,000, and 16,000 mL h^{-1} were applied and incorporated into soil at a 3-5 cm depth. The density and biomass of weeds was significantly reduced by applying both compounds. The inhibition ability was also rate-dependent. Although the density and biomass of maize was also decreased at high rates, this crop showed higher tolerant to both compounds that all weeds.

Keywords: allelopathy, natural herbicides, maize, weeds.

RESUMO - O objetivo deste estudo foi investigar se a cumarina e o extrato de lavanda, semelhantemente às condições de casa de vegetação, são fitotóxicos para algumas espécies de plantas sob condições agronômicas. Antes do plantio de milho, a cumarina foi aplicada a 0, 250, 500, 1.000, 2.000 e 4.000 g h^{-1}, e o extrato aquoso de lavanda, a 0, 1.000, 2.000, 4.000, 8.000 e 16.000 mL h^{-1}, e ambos foram incorporados no solo a uma profundidade de 3-5 cm. A densidade e a biomassa de plantas daninhas tiveram redução significativa com a aplicação de ambos os compostos. A capacidade de inibição também foi dependente da taxa de aplicação. Embora a densidade e a biomassa do milho também tenham diminuído em altas taxas de aplicação, essa cultura mostrou maior tolerância a ambos os compostos que todas as plantas daninhas.

Palavras-chave: alelopatia, herbicidas naturais, milho, plantas daninhas.

INTRODUCTION

In the past years, the approach to use agrochemicals for weed control has changed in a way that a shift has occurred from synthetic chemical compounds to natural ones. This change in approach is due to the fact that natural compounds have relatively short half-life in the environment (Li et al., 2003) and may provide novel sites of action (Duke et al., 2002). Many natural compounds taken from many plant species, especially aromatic ones, have been studied for their herbicidal potential. For example, sorgoleone, isolated from *Sorghum* sp.; ailanthone, isolated from *Ailanthus* sp.; artemisinin, isolated

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from *Artemisia* sp.; coumarin, isolated from *Ruta* sp. and so on (Vyvyan, 2002). Given the great interest in this field, the molecule of leptospermone isolated from *Callistemon citrinus* was optimized and then commercialized as mesotrione and sulcotrione herbicides (group F_2/27) which inhibit *p*-hydroxyphenyl pyruvate dioxygenase (HPPD), a key enzyme in the biosynthesis of carotenoid and plastoquinone (Duke et al., 2002)

Lavender (*Lavandula* spp.) is a medicinal plant with potent allelopathic properties which belongs to the Lamiacae family. The substantial components of lavender are made up of coumarin (Tiliacos et al., 2008). However, of 18 structural analogues of coumarin extracted from lavender, coumarin itself had the most phytotoxic on *Lolium regedum* (Haig et al., 2009). This compound is also active against other pest species such as bacteria (Karamanoli et al., 2000), fungi (Moon et al., 2007), and insects (Papachristos et al., 2004).

Although laboratory bioassays are often used to investigate the allelopathic potential of a natural compound or plant extract, field bioassays have been infrequently pursued to investigate the allelopathic potential of a natural compound or plant extract. To establish the allelopathic potential of natural compound or plant extract, the observations should be the same in both bioassay conditions. In our previous study, coumarin applied as a pre-plant incorporated into soil under greenhouse conditions could inhibit the emergence of seedlings (probably germination of seeds) of some weeds and a crop (Nazemi et al., 2015). This study aimed to further explore if these observations will be repeatable under agronomic conditions.

MATERIALS AND METHODS

Seedbed preparation included a moldboard plowing in December 2014 followed by two passes with a field cultivator in May 2015. According to the standard agronomic practices of Research Fields of Ferdowsi University of Mashhad, Iran (36°153 N, 59°283 E, 985 m a.s.l.), a starter fertilizer was broadcast at a rate of 240 kg ha^{-1} of diammonium phosphate (46% P and 21% N) and was incorporated by a shallow-disk and harrower. The experiment was set up as a factorial based on a randomized complete block design using two factors (compound and rate) with three replications. Coumarin (99% purity, Merck Co., Germany) was used at 0 (control), 250, 500, 1,000, 2,000, and 4,000 g h^{-1} and aqueous extract taken from lavender (*Lavandula officinalis*) was used at 0 (control), 1,000, 2,000, 4,000, 8,000, and 16,000 mL h^{-1}. The aqueous extract taken from lavender was exactly obtained by the method of Haig et al. (2009). The obtained extract was considered as basic extract (100%). The predominant weeds of site consisted of *Amaranthus retroflexus*, *Amaranthus blitoides*, *Cyperus rotundus*, *Echinochloa crus-galli*, *Chenopodium album*, *Portulaca oleracea* and *Datura stramurioum*. The treatments were applied as pre-plant with a calibrated lance sprayer (Matabi Super Agro 20 L sprayer, UK), equipped with an 8002 flat fan nozzle tip delivering 208 L ha^{-1} at a pressure of 200 kPa on soil surface and then incorporated at a 3-5 cm upper layer. Then, maize *(Zea mays* cv. Single Cross 704) was planted in rows spaced 50 cm apart at a 3-4 cm depth. Each plot was 2 m long by 2 m wide. All the plots were irrigated once a week.

The sampling of both weeds and crop was performed at 15 and 30 days after planting (DAP). At both samplings, the weeds based on plant species were separately counted and removed at the soil surface from a 0.25 m^2 within each plot, and oven-dried to weight at 75 °C. Data were multiplied by four. All data were subjected to analysis of variance (ANOVA) by using PROC GLM in SAS software (version 9.1, SAS Institute Inc., USA). Treatment means were separated, using LSD at a probability level of P = 0.05.

RESULTS AND DISCUSSION

Generally, the application of both compounds significantly resulted in a reduction in the density and biomass of all plants established in the experimental units, depending on their rates. However, the interaction effect between compound and rate was not significant (Tables 1 and 2). Since the total density and dry weight of weeds appeared to be more important than that of individual weeds, only the former are discussed. For both samplings, all experimental units that received coumarin and lavender's extract had lower weed density than the untreated

Table 1 - Effect of Lavender aqueous extract and coumarin applied as pre-plant incorporated into soil on density and dry biomass weight of plant species at 15 DAP

Compound	Rate (g or mL h^{-1})	*A. retroflexus*	*A. blitoides*	*C. album*	*D. stramurioum*	*C. rotundus*	*E. crus-galli*	*P. oleracea*	*Z. mays*	Total weeds
		Density (no. m^{-2})								
Coumarin	0 (control)	9.0	7.6	9.6	2.0	5.6	3.0	7.0	10.0	44.0
	250	4.0	5.3	5.6	0.0	3.3	3.0	0.6	9.6	21.6
	500	2.0	1.6	4.6	1.0	3.0	1.6	0.3	10.0	14.3
	1000	1.0	2.0	3.0	1.0	2.3	0.3	2.0	9.0	11.6
	2000	0.3	1.3	2.3	2.6	1.3	0.0	0.3	6.6	8.3
	4000	0.3	1.6	0.3	0.3	1.0	0.0	0.0	4.6	3.3
Extract	1000	1.3	1.3	2.6	0.0	1.3	1.3	0.6	10.0	8.4
	2000	0.3	1.0	0.0	0.0	0.0	1.0	0.0	8.3	3.6
	4000	1.0	1.3	0.6	0.3	1.3	0.6	0.3	7.6	5.6
	8000	0.3	1.0	2.3	0.0	0.3	0.0	0.3	4.0	4.2
	16000	0.3	0.0	0.0	0.0	0.6	0.0	0.0	3.6	0.9
LSD 0.05%		4.5	3.3	4.0	1.3	3.0	1.7	2.3	2.1	7.9
		Dry biomass weight (g m^{-2})								
Coumarin	0 (control)	49.9	45.8	24.6	26.8	14.9	9.6	24.7	8.9	185.9
	250	14.7	35.8	13.5	0.0	5.3	1.4	1.5	7.9	59.6
	500	8.8	11.1	7.9	3.6	2.2	2.3	2.0	8.1	38.2
	1000	9.0	9.1	3.6	4.3	4.5	1.3	8.7	8.2	40.6
	2000	0.7	8.2	5.8	8.7	4.9	0.0	1.7	5.8	30.1
	4000	0.3	9.3	1.3	0.2	1.8	0.0	0.0	2.6	12.9
Extract	1000	6.4	19.3	8.9	0.0	6.3	5.7	1.5	10.8	48.4
	2000	0.6	3.3	0.0	0.0	0.0	3.4	0.0	8.7	7.3
	4000	3.1	3.1	1.9	10.8	4.6	2.6	1.6	7.4	27.8
	8000	1.5	0.6	4.9	0.0	1.3	0.0	1.0	5.4	9.5
	16000	1.3	0.0	0.0	0.0	3.4	0.0	0.0	2.3	4.6
LSD 0.05%		20.7	20.6	10.4	20.2	8.3	5.5	12.2	1.6	36.8

The treatments of 0 g coumarin ha^{-1} and 0 mL extract ha^{-1} are the same. The latter was abbreviated.

Table 2 - Effect of Lavender aqueous extract and coumarin applied as pre-plant incorporated into soil on density and dry biomass weight of plant species at 30 DAP

Compound	Rate (g or mL h^{-1})	*A. retroflexus*	*A.blitoides*	*C.album*	*D. stramurioum*	*C. rotundus*	*E. crus-galli*	*P. oleracea*	*Z.mays*	Total weeds
		Density (no. m^{-2})								
Coumarin	0 (control)	6.6	10.3	15.0	2.3	1.0	7.6	3.0	10.0	39.2
	250	4.3	2.6	3.0	0.0	2.3	0.0	1.3	10.0	13.6
	500	4.3	3.3	0.6	1.0	0.6	0.6	0.3	8.6	11.0
	1000	0.0	2.3	1.0	1.6	0.0	0.3	0.3	9.6	5.6
	2000	2.0	0.6	0.6	0.3	1.3	0.3	0.3	5.6	5.6
	4000	2.3	3.0	0.3	0.3	0.6	0.3	0.0	3.6	7.0
Extract	1000	0.6	0.6	3.0	0.0	1.0	0.0	0.6	10.3	6.0
	2000	0.6	1.0	0.6	0.3	1.3	0.6	0.3	8.3	5.0
	4000	0.0	0.6	1.0	0.6	0.0	0.3	0.3	7.0	3.0 c
	8000	1.0	0.3	0.6	0.0	0.6	0.0	0.3	5.0	2.5 c
	16000	1.0	1.6	0.0	0.3	0.6	0.3	0.0	5.3	4.3
LSD 0.05%		5.8	4.5	4.3	1.4	2.3	4.4	1.0	2.2	8.6
		Dry biomass weight (g m^{-2})								
Coumarin	0 (control)	175.0	88.5	49.1	19.9	2.6	30.9	43.7	19.6	409.1
	250	30.7	23.5	22.3	0.0	0.3	0.0	23.3	20.3	90.2
	500	19.7	26.5	2.7	17.7	0.6	10.1	6.5	18.5	84.0
	1000	0.0	23.8	5.2	10.9	0.0	6.4	2.6	17.2	49.1
	2000	68.3	6.1	5.8	3.4	0.6	1.9	5.2	13.9	91.6
	4000	49.6	25.1	2.0	1.4	0.8	0.0	0.0	8.3	79.1
Extract	1000	11.4	5.5	20.1	0.0	3.1	0.0	15.6	22.2	48.2
	2000	15.0	7.6	2.7	3.5	4.8	3.4	5.0	19.8	41.8
	4000	0.0	4.1	4.1	17.3	0.0	1.6	5.2	8.8	32.7
	8000	17.0	2.6	2.0	0.0	1.9	0.0	6.5	6.8	30.6
	16000	10.5	10.5	0.0	3.7	1.7	2.1	0.0	5.7	28.7
LSD 0.05%		87.7	27.1	20.0	19.3	3.6	14.9	18.4	5.2	109.8

The treatments of 0 g coumarin ha^{-1} and 0 mL extract ha^{-1} are the same. The latter was abbreviated.

control. Weed density was influenced with increasing rates of coumarin and lavender's extract. By increasing their application rate, weed density decreased. Among the treatments, the application of 16,000 mL extract ha^{-1} had the highest impact by reducing total weed density (0.9 plant m^{-2}) at 15 DAP. By contrast, the application of 8,000 mL extract ha^{-1} had the highest impact on weeds at 30 DAP (2.5 plant m^{-2}). Similar results were found for total weed biomass. The untreated control had the highest weed biomass for both samplings (185.9 and 409.1 g m^{-2}, respectively). The greatest reduction in total weed biomass was found when 16,000 mL lavender's extract ha^{-1} was applied compared to the untreated control. With this treatment, total weed biomass was 4.6 g m^{-2} at 15 DAP and 28.7 g m^{-2} at 30 DAP.

Unlike all of the weeds observed in the field, the compounds used at low rates cannot influence the density and biomass of maize. However, these traits in maize were decreased when high rates of both compounds were used. Number of seedlings at both samplings did not differ among the rates of 0 (control), 250, 500, 1,000 g coumarin ha^{-1}, 1,000, and 2,000 mL lavender's extract ha^{-1}. The greatest reduction in maize density was found by applying 16,000 mL lavender's extract ha^{-1} at 15 DAP compared to the untreated control. By contrast, the greatest reduction was found by applying 4,000 g coumarin ha^{-1} at 30 DAP. Similar results were found for dry weight of maize.

It seems that all weeds were very sensitive to coumarin and lavender's extract. The results showed that weed density was significantly reduced by pre-plant application of both compounds at all tested rates. Therefore, there is potential to inhibit weed germination using coumarin and lavender's extract. Inhibition ability was also concentration-dependent. High application rates almost completely inhibited seedling emergence of weeds (Tables 1 and 2). It seems that the inhibitory effect of lavender's extract is higher than that of coumarin. Therefore, it can be concluded that other compounds within lavender's extract also have inhibitory effects. This observation and discussion confirms the results of Haig et al. (2009), who reported that among 18 coumarin structural derivatives extracted from lavender, coumarin had the most phytotoxic effect, followed by 7-hydroxycoumarin and 7 Diethylamino-4-methylcoumarin. Studies on germination inhibition ability of coumarin or lavender's extract in the laboratory (Goodwin and Taves, 1950; Li et al., 1993; Pergo et al., 2008; Zaeri et al., 2013) and in a greenhouse (Campiglia et al., 2007; Haig et al., 2009; Nazemi et al., 2015) have been reported. The mechanism of action of coumarin is not known. However, coumarin can reduce the amount and activity of Golgi body in lettuce (Li et al., 1993), block mitosis in alfalfa (Vyvyan, 2002) and modify mitochondria structure in onion (Kupidlowska et al., 1994). It was shown that maize had higher tolerance to both compounds as compared to weeds. This observation is probably due to higher and/or faster metabolism; lower absorption was due to low surface to volume ratio of its seed; and/or higher depth germination. Previous studies indicated that small-seeded species appear especially susceptible to allelochemicals because the surface-to-volume ratio of a small-seeded species is usually greater, and therefore its exposure per unit mass to allelopathic substances in the soil is also greater (Nazemi et al., 2015)

Dias et al. (2004) reported that the application of lavender extract as a pre-emergence treatment has been ineffective to inhibit the germination of *Phalaris minor* because of photo-degradation and/or volatilization. According to the recommendations of Campiglia et al. (2007) and Haig et al. (2009), the lavender's extract and coumarin were applied as pre-plant incorporated into soil in this study to minimize photo-degradation and/or volatilization. In experimental site, no crop was cultivated and no pesticide was used for two years and the soil had less than 1% organic matter. These features can presumably reduce the potential microbial degradation of the compounds used in this study because the microbial enrichment phenomenon did not occur. For this reason, a relatively high concentration range was preferably applied.

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