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Herbicidal Activity of Coumarin When Applied as a Pre-plant Incorporated into Soil

Amir Hossein NAZEMI, Ghorban Ali ASADI*, Reza GHORBANI

*Department of Agronomy and Plant Breeding, College of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran; asadi@um.ac.ir (*corresponding author)*

Abstract

Due to having a short half-life and novel site of action, the herbicidal potential of natural compounds are lionized. Coumarin is a secondary metabolite from *Lavandula* sp., family Lamiacae. The impact of eight concentrations of coumarin (0, 100, 200, 400, 800, 1600, 3200 and 6400 ppm) were separately used as a pre-plant incorporated into soil on six plant species under greenhouse conditions. Generally, coumarin had phytotoxic effect against all plant species. The phytotoxic effect was concentration-dependent. The high concentrations could inhibit the emergence of seedlings (probably by stopping germination of seeds). Based on ED_{50} parameter, the ranking of plant species for tolerance to coumarin was *S. halepense* > *Z. mays* > *C. album* > *A. retroflexus* > *E. cruss-gali* > *P. oleracea*. Based on selectivity index, coumarin at a concentration of 365.69 ppm can control *P. oleracea* without damaging *Z. mays*, whereas any concentration it cannot control other weeds without damaging *Z. mays*.

Keywords: allelopathy, maize, selectivity index, weed

Introduction

Various methods to control weeds are always introduced and among them one of the best and modern method is the use of synthetic herbicides (Rashed-Mohassel *et al.*, 2011). Synthetic herbicides offer the same advantages compared to other methods. They are selective, easy to apply, act quickly, are relatively inexpensive, and can be used where other methods do not work well (Zimdahl, 2007). Therefore, their application have increased dramatically in the agricultural world and generated a series of additional problems such as herbicide resistance in weeds, soil and water pollution and the toxicity effects on human health and non-target species (Zaeri *et al.*, 2013).

In recent years, with increasing the global awareness about these problems, the herbicidal potential of natural compound are lionized due to having very short half-life in the environment (Li *et al.*, 2003) and having novel sites of action (Duke *et al.*, 2002). Many natural compounds have been reported to have herbicidal potential such as artemisinin (a compound isolated from *Artemisia annua*), ailanthone (a compound isolated from *Ailanthus altissima*), sorgoleone (a compound isolated from *Sorghum* sp.), joglan (a compound isolated from *Juglans* sp.) and so on (Upadhyaya and Blackshaw, 2007). Research progress in this field caused that a secondary metabolite from the Australian bottlebrush (*Callistemon citrinus*), namely mesotrione, and a secondary metabolite from some lichens, namely sulcotrione, are commercialized as agrochemicals (Duke *et al.*, 2002).

Lavender (*Lavandula* sp.) is a famous medicinal plant which belongs to the family Lamiacae. The antibacterial (Karamanoli *et al.*, 2000), antifungal (Moon *et al.*, 2007), insecticidal (Papachristos *et al.*, 2004) and herbicidal activities (Goodwin and Taves, 1950) were demonstrated by the lavender extracts. The herbicidal activity of lavender extracts is due to the main secondary metabolite, so called coumarin. It is a well-known phytotoxin that has been tested in laboratory studies for its effect on germination and growth of some plant species (Chon and Kim, 2004; Pergo *et al.*, 2008; Zaeri *et al.*, 2013).

The current research aimed to determine the herbicidal potential of coumarin on six plant species when applied as a pre-plant incorporated into soil.

Materials and methods

The seeds of redroot pigweed (*Amaranthus retroflexus*), common lambsquarters (*Chenopodium album*), barnyardgrass (*Echinochloa cruss-gali*), johnsongrass (*Sorghum halepense*) and common purslane (*Portulaca oleracea*) were collected from plants from the field near Mashhad, Iran. The seeds of maize (*Zea mays* cv. Single Cross 704) were obtained from the Agricultural and Natural Resources Research Center of Mashhad, Iran.

Before the start of the experiment, the seed dormancy-breaking treatments were conducted to increase the weed species seeds' coat permeability. Hence, the seeds of *A. retroflexus*, *C. album*, *E. cruss-gali*, *S. halepense*, and *P. oleracea* were acid-scarified in concentrated sulfuric acid (98%) for 5, 1, 3, 4 and 1 min, respectively, then rinsed with distilled water (Zaeri *et al.*, 2013). The seed germination percentages were increased up to 93% for *E. cruss-gali* and 99% for *P. oleracea* by the aforementioned method.

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Bioassays were conducted between June and September 2014 in a greenhouse located on the Ferdowsi University of Mashhad, Iran. Six experiments were separately set up as a completely randomized design with eight treatments (eight concentrations). There were four replications. A mixture of sand and clay loam soil (1:1 v/v) in 1.5 L plastic pots was supplied. Then, 30 ml of a coumarin solution of 0 (control), 100, 200, 400, 800, 1600, 3200 and 6400 ppm were added to each pot, and then incorporated with upper layer of soil mixture. Then, 25 seeds of the aforementioned plant species were separately planted at 0.5 cm depth in each pot. The pots were irrigated every 5 days with tap water.

Four weeks after planting, the total number of emerged seedlings was determined; then shoots of the plants in each pot were harvested and oven-dried at 75 °C for a period of 48 h and the dry weight was determined.

The data were changed to individual plant and subjected to analyze by two variance analysis using PROC GLM in SAS and the non-linear regression analysis using open-source statistical software, $R_{2.62}$, using the *drc* statistical addition package. The response of each plant species (the biomass produced of individual plants in pot) to coumarin concentration was assumed separately using two log-logistic models (3 or 4 parameters) that were described elaborately by Tind *et al.* (2009) as follows; respectively:

$$Y = C + \{D - C / 1 + \exp[b(\log X - \log ED_{50})]\}$$

$$Y = D / 1 + \exp[b(\log X - \log ED_{50})]$$

where :

'Y' is the response (dry weight);

'C' is the lower limit;

'D' is the upper limit corresponding to the response of the untreated control;

'b' is the slope of the line;

'ED' is the required concentration of coumarin to give 50% control;

'X' is the coumarin concentration.

The coumarin concentration needed to obtain 10% and 90% reduction in dry weight (ED_{10} and ED_{90}, respectively) were also determined. The ratio between the concentration that caused 10% of damage to maize and 90% of damage to each weed was used as a selectivity index. According to Tind *et al.* (2009), a selectivity index above 2 can be safely used in a crop.

Results and discussions

Generally, the produced biomass of all plant species under study was significantly ($P \leq 0.01$) influenced when coumarin was applied as a pre-plant incorporated into soil (Table 1). There were differences between the different concentrations of coumarin over inhibition of the germination and growth of the tested plant species. The phytotoxic effect was concentration-dependent. These differences are reported in detail in the following sections. At high amount of coumarin, the seeds did not emerge (probably did not germinate) and/or the seedlings emerged with short and pale leaves. In this regard, *P. oleracea* had a small number of seedlings that were observed alive in experimental units (Table 1).

Exhaustively, the phytotoxic effect of coumarin on *E. cruss-gali* enhanced with increasing its concentration (Table 1). A concentration of 100 ppm reduced significantly the dry weight of *E. cruss-gali* from 2.62 g (control) to 1.70 g. Relative to the control, the greatest reduction in the dry weight of *E. cruss-gali* was observed with applying a concentration of 3200 ppm. There were no significant differences between 3200 and 6400 ppm. The application of a concentration of 6400 ppm coumarin inhibited completely the emergence (probably germination) of *E. cruss-gali*. Coumarin is a well-known phytotoxin that has been tested in laboratory studies for its effect on germination and growth of some plant species such as wild oats (*Avena fatua*) (Goodwin and Taves, 1950), timothy grass (*Phleum pratense*) (Avers and Goodwin, 1956), alfalfa (*Medicago sativa*), velvetleaf (*Abutilon theophrasti*) and ryegrass (*Lolium multiflorum*) (Dornbos and Spencer, 1990), barnyard grass (*Echinochloa crus-galli*) (Chon and Kim, 2004) and beggar' sticks (*Bidens pilosa*) (Pergo *et al.*, 2008). Haig *et al.* (2009) also reported that coumarin applied to post-emergence at a concentration of 100 ppm resulted in a reduction by almost 80% in annual ryegrass (*Lolium rigidum*) shoot weight.

In case of *A. retroflexus*, the lowest coumarin concentration had a significant herbicidal effect so that when coumarin was applied at a concentration of 100 ppm, it reduced significantly the dry weight of *A. retroflexus* from 2.48 g (control) to 1.02 g. Nevertheless, no significant difference was observed among the

Table 1. The effects of different concentrations of coumarin when applied as a pre-plant incorporated into soil on the produced biomass

Coumarin (ppm)	Shoot dry weight (g)					
	Z. mays	*S. halepense*	*E. cruss-gali*	*A. retroflexus*	*C. album*	*P. oleracea*
0 (control)	3.81 a	4.01 a	2.62 a	2.48 a	1.79 a	2.31 a
100	2.66 b	3.18 a	1.70 b	1.02 b	1.41 b	1.34 b
200	3.01 bc	3.26 a	1.10 c	2.08 b	1.10 b	0.23 c
400	2.59 b	1.98 b	0.51 d	0.83 b	0.62 c	0.23 c
800	2.76 b	1.69 c	0.28 de	0.33 c	0.18 d	0.21 c
1600	2.30 b	1.31 cd	0.10 e	0.12 c	0.12 d	0.00 c
3200	1.01 c	0.56 d	0.10 e	0.00 c	0.02 d	0.00 c
6400	0.52 c	0.62 d	0.00 e	0.00 c	0.00 d	0.00 c
LSD = 0.05	0.90	0.84	0.39	0.36	0.29	0.27
ANOVA	Mean squares					
Concentration (d.f.= 7)	4.82 **	6.24 **	3.53 **	3.83 **	2.24 **	2.84 **
Error (d.f.= 24)	0.22	0.36	0.08	0.11	0.15	0.04
CV (%)	24.93	17.69	15.53	14.74	21.01	25.09

30 ml of coumarin solutions were added to each pot. Means in each column followed by same letter are not significantly different at the 0.05 probability level determined by Least Significant Difference Test (LSD)

Notes: ns: not significant. * $p \leq 0.05$. ** $p \leq 0.01$

Table 2. Estimated parameters for the dose-response curves

Selectivity index	ED$_{90}$ (ppm)	ED$_{50}$ (ppm)	ED$_{10}$ (ppm)	Upper limit (g)	Lower limit (g)	Slope	Plants
-	16252.80	370.33	365.69	3.26	-	1.14	*Z. mays*
0.06	5743.58	467.78	38.09	4.01	0.24	0.87	*S. halepense*
0.44	777.08	156.22	31.40	2.62	0.27	1.36	*E. cruss-gali*
0.40	847.06	212.23	53.17	2.54	-	1.58	*A. retroflexus*
0.43	792.36	266.70	89.76	1.83	-	2.01	*C. album*
2.06	167.97	101.41	57.34	2.32	-	3.44	*P. oleracea*

ED$_{10}$ of *Z. mays* / ED$_{90}$ of each weed were used to calculate the selectivity index

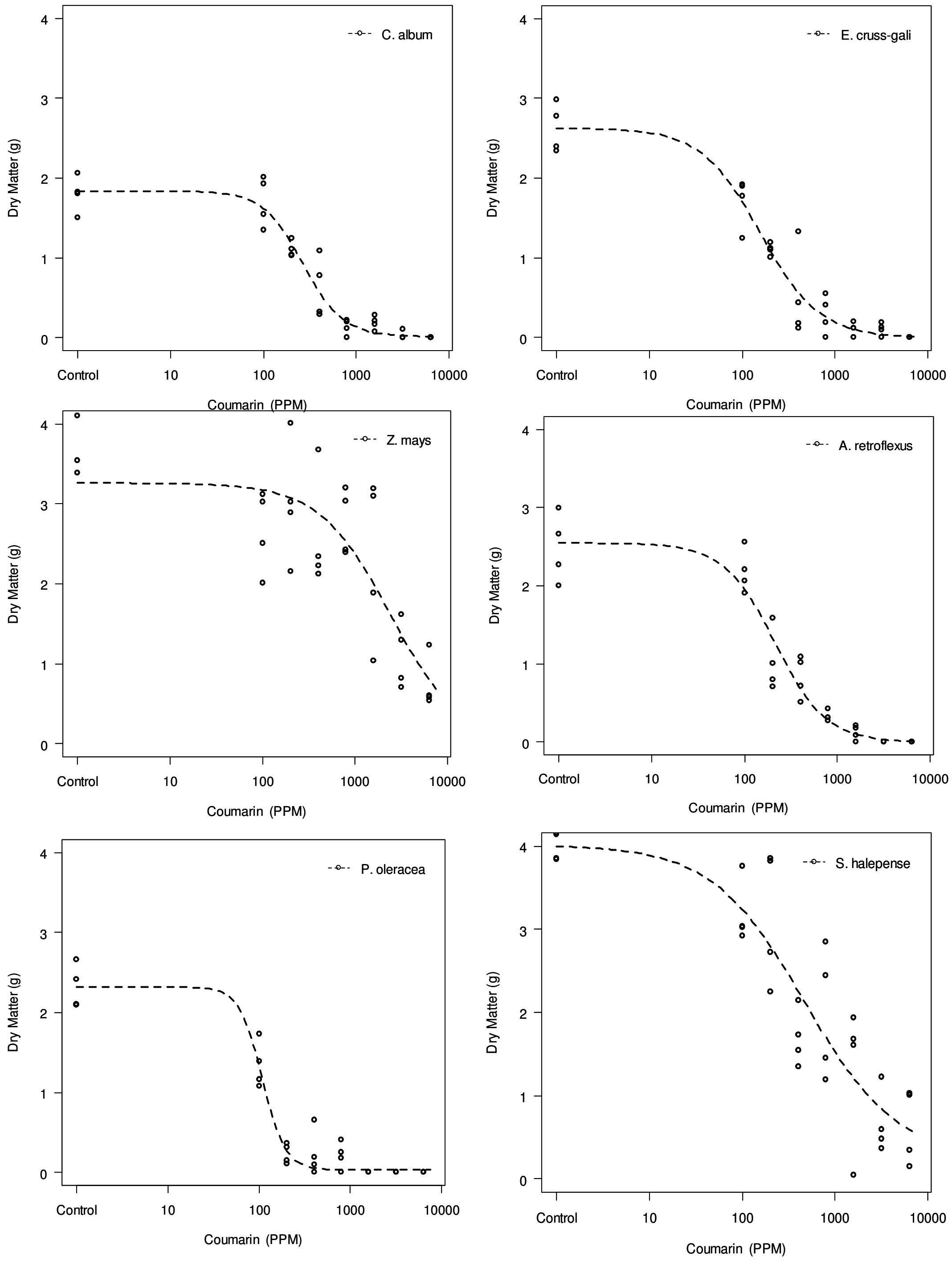

Fig. 1. Dose-response curves of the dry weight of plants on different concentrations of coumarin applied as a pre-plant incorporated into soil. The points are the replications of each treatment

treatments of 100-400 ppm. Relative to the control, the greatest reduction in the dry weight was observed at the concentrations of 3200 and 6400 ppm. The application of these two concentrations inhibited completely the emergence (probably germination) of *A. retroflexus* (Table 1).

As compared with other plant species, it seems that *P. oleracea* was very sensitive to coumarin so that the application of highest three concentrations of 1600, 3200, and 6400 ppm inhibited completely its emergence (probably germination). The lowest coumarin concentration had a significant herbicidal effect on *P. oleracea*. As with applying a concentration of 100 ppm, the produced dry weight of *P. oleracea* was reduced from 2.31 g (control) to 1.34 g (Table 1).

In case of *Z. mays*, the phytotoxic effect of coumarin rose with increasing its concentration (Table 1). When coumarin was applied at a concentration of 100 ppm, the produced dry weight of *Z. mays* reduced significantly from 3.81 g (control) to 2.66 g. No significant difference was observed among the concentrations of 100-1600 ppm. The highest reduction in dry weight of *Z. mays* was obtained with application of 6400 ppm (0.52 g). Nevertheless, no significant difference was observed between the treatments of 3200 and 6400 ppm. Unlike *E. cruss-gali*, *P. oleracea*, and *A. retroflexus*, some seeds of *Z. mays* were able to emerge in all concentrations of coumarin applied as a pre-plant. The reason for this behavior (relative tolerance) may be attributed to two factors: i) large-seeded *Z. mays* versus small-seeded weed. The 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 (Chase *et al.*, 1991; Putnam and DeFrank, 1983); ii) High rate of metabolism. The previous studies indicated that the metabolism of coumarin by maize, wheat (Zaeri *et al.*, 2013) and canola (Haig *et al.*, 2009) appears to play an important role in the relative tolerance to low concentrations of coumarin and involves mainly oxidation, reduction and/or hydrolysis.

As compared with other plant species, the lowest coumarin concentration (100 ppm) did not have any significant effect on *S. halepense*. But coumarin applied at a concentration of 400 ppm reduced significantly the produced dry weight of *S. halepense* from 4.01 g (control) to 1.98 g (Table 1). No significant difference was observed among the treatments of 0 (control)-200 ppm. The toxicity of coumarin for *S. halepense* increased with increasing its concentration. The highest reduction in dry weight of *S. halepense* was obtained with application of 3200 ppm (0.56 g). Nevertheless, no significant difference was observed among the concentrations of 1600-6400 ppm. Like maize, some seeds of *S. halepense* were able to emerge in all concentrations of coumarin applied. Given that *S. halepense* is a small-seeded weed, it seems that the plant metabolism plays a primary role in the relative tolerance to coumarin.

In the case of *C. album*, the phytotoxic effect of coumarin enhanced with increasing its concentration (Table 1). When coumarin was applied at a concentration of 100 ppm, it significantly reduced the dry weight of *C. album* from 1.79 g (control) to 1.41 g. Relative to the control, the greatest reduction in the dry weight of *C. album* was observed at the concentration of 3200 ppm. There were no significant differences between 3200 and 6400 ppm. The application of a concentration of 6400 ppm coumarin inhibited completely the emergence (probably germination) of *C. album*.

Dose-response curves for coumarin against the tested species are shown in Fig. 1. Table 2 summarized the parameters for these dose-response curves. Based on the ED_{50} parameter, the coumarin concentration needed to obtain 50% reduction in dry weight of *S. halepense*, *Z. mays*, *C. album*, *A. retroflexus*, *E. cruss-gali* and *P. oleracea* equal to 467.78, 370.33, 266.70, 212.23, 156.22 and 101.41 ppm, respectively. Therefore, the most tolerant and sensitive to the coumarin application were *S. halepense* and *P. oleracea*, respectively. The values of selectivity index (ED_{10} for *Z. mays* / ED_{90} for each weed) showed that with the exception of *P. oleracea*, the value of selectivity index of coumarin measured for *Z. mays* to control other weeds was less than 2 (Table 2). Therefore, coumarin can only be used selectively to control *P. oleracea* in *Z. mays*.

Conclusions

The results of this study confirmed not only the previous results, but also showed that coumarin has the phytotoxic effect against *Z. mays*, *S. halepense*, *E. cruss-gali*, *A. retroflexus*, *C. album* and *P. oleracea*. Generally, *S. halepense* and *P. oleracea* were the most tolerant and sensitive to coumarin when applied as a pre-plant incorporated into soil, respectively. A correlation between seed size and tolerance/sensitive to coumarin was founded, as small-seeded species appear more sensitive to coumarin. Based on the values of selectivity index, coumarin at a concentration of 365.69 ppm can only be used selectively to control *P. oleracea* in *Z. mays*. An additional approach for enhancing selectivity of coumarin is required by using a proper safener.

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