

## Effects of essential oils of cinnamon, lavender and peppermint on germination of Mediterranean weeds

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### ABSTRACT

In laboratory and green house investigations, we studied the allelopathic effects of essential oils extracted from aromatic plants cinnamon (*Cinnamomum zeylanicum* L.), lavender (*Lavandula* spp.) and peppermint (*Mentha x piperita* L.) on seed germination of 7 Mediterranean weed species [*Amaranthus retroflexus* L., *Solanum nigrum* L., *Portulaca oleracea* L., *Chenopodium album* L., *Sinapis arvensis* L., *Lolium* spp. and *Vicia sativa* L.]. Each essential oil was tested at 4 concentrations in controlled conditions (Germination chamber: 0.2, 0.6, 1.8 and 5.4 mg l<sup>-1</sup>) and in semi-controlled condition (Green house: 5.4, 21.6, 86.4 and 345.6 mg l<sup>-1</sup>) and distilled water was used as control. In controlled conditions, the 1.8 and 5.4 mg l<sup>-1</sup> concentrations totally inhibited the seed germination and also showed selectivity. While in semi-controlled condition the 345.6 mg l<sup>-1</sup> concentration of cinnamon essential oil, totally inhibited the seed germination of *Amaranthus retroflexus* L. The concentration of essential oils, greater effects on weed susceptibility than the type of oil used. However, cinnamon oil caused drastic inhibitory effects followed by lavender and peppermint. The possible use of essential oils as natural herbicides to control different weeds for sustainable cropping system is discussed.

**Keywords:** Allelochemicals, *Amaranthus retroflexus*, bioherbicide, *Cinnamomum zeylanicum*, *Chenopodium album*, essential oil, *Lavandula* spp., *Lolium* spp., *Mentha x piperita*, *Portulaca oleracea*, *Sinapis arvensis*, *Solanum nigrum*, sustainable weed control, *Vicia sativa*,

### INTRODUCTION

Weeds are major problem in agriculture and cause huge yield losses in crops (1,25,45). For weed control, chemical herbicides are used, which are harmful to both environment and human health (15,36,44), hence, the interest in environmentally friendly alternatives for weed control has increased in recent years (20,36,42). Research has mainly focused on strategies of Integrated Weed Management System (IWMS), new strategies and agronomic solutions based on the use of natural compounds have been suggested (2,5,6,11,13,19,26,28,31,38,40,42,43). This approach would mainly rely on the exploitation of allelopathic effects. Allelopathy is 'Any process that involves secondary metabolites produced by plants, algae, bacteria, and fungi that influence the growth and development of biological systems' (22). These secondary metabolites are allelochemical

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compounds that exerts their phytotoxic effects directly or indirectly as they limit the growth of competing plants in the surrounding environment (1). Allelochemicals interactions between the plants are recognized as a key factor in patterning of crop vegetation and weed growth and in influencing crop yield (32). They also have the potential to become natural pesticides in sustainable agriculture (19,38). The advantage of utilizing essential oil in sustainable organic agriculture is based on their rapid decomposition in environment (11,18,19,38,46). Essential oils are among natural compounds identified as allelochemicals. They are a mixture of secondary metabolites harmful to plants and microorganisms which are released in response to many factors (26,30). These secondary metabolites are able to inhibit weed seeds germination and may be used for weed control (18,19). The advantage in utilizing essential oils is given by the joint action of several compounds present in them and not only by a single compound, whose quantity and persistence in the environment may be not sufficient to obtain a toxic response on its own. Moreover, essential oils are classified as "Generally Recognized as Safe" (GRAS) by the US Food and Drug Administration (FDA) (38). Some studies have shown their possible application as bioherbicides (3,4,13,19,26,31,37,38,39). Essential oils extracted from the aromatic plants may be very inhibitory to seeds germination of various crops and weeds (19). However, their selectivity on weed species has yet to be investigated. Selectivity is the ability to kill or retard the growth of an unwanted plant or "weed", while causing little or no injury to desirable species (15). Not all oils exert the same effect on weeds and their inhibitory effects differ with concentrations and weed species (11,13,19,26,38). This research aimed to evaluate the effects of essential oils of cinnamon (*Cinnamomum zeylanicum* L.), lavender (*Lavandula* spp.) and peppermint (*Mentha x piperita* L.) on seed germination of some weed species common in Mediterranean environment.

## MATERIALS AND METHODS

The effects of cinnamon (*Cinnamomum zeylanicum* L.), lavender (*Lavandula* spp.) and peppermint (*Mentha x piperita* L.) essential oils were evaluated on the weed seed germination rates. The test compounds were provided by Muller & Kostner and were certified IFRA-RIFM-CEE 91/155 GMO's free and for herbalist use. The essential oils used were 100% pure. We selected major winter and summer crops weeds of Mediterranean environments. The test weeds spp. were: (i). **Spring-summer cycle:** Redroot pigweed (*Amaranthus retroflexus* L.), black nightshade (*Solanum nigrum* L.), common purslane (*Portulaca oleracea* L.) and common lambsquarter (*Chenopodium album* L.) for (ii). **Autumn-winter cycle:** Wild mustard (*Sinapis arvensis* L.), ryegrass (*Lolium* spp.), and common vetch (*Vicia sativa* L.). Before starting the test, all weeds seeds were placed in germination chamber at 5°C in dark for two days, to break dormancy and synchronize the germination.

**Germination Chamber:** The germination tests were done in Petri dishes (10 cm dia) in a germination chamber in dark at 27°C (day) and 15°C (night) for spring-summer weeds and at 20°C and 10°C for autumn-winter weeds, respectively. For each essential oil, an oil-in-water emulsion was prepared at 0.2, 0.6, 1.8 and 5.4 mg l<sup>-1</sup> concentrations (C1, C2, C3 and

C4, respectively). Each Petri dish contained 25 weed seeds placed on two layers of filter paper (Whatman<sup>®</sup> No.5) wetted with 5 ml oil-in-water emulsion. The control dishes (C0) were moistured with distilled water. The Petri dishes were sealed with laboratory film (Parafilm<sup>®</sup> M) to permit the exchange of oxygen and CO<sub>2</sub> but not the volatiles of essential oil (16). All treatments were replicated four times. Seedling emergence was recorded daily until the control reached the maximum germination. Radicle elongation is measure of inhibitory activity (19), however, coleoptile growth is more reliable (24). Thus, we used coleoptile elongation as parameter of germination inhibition. Relative germination was calculated as germination (%) of the treatment compared to control. For statistical evaluation, data were transformed to  $\arcsin [x (\%)/100]^{1/2}$  (21). ANOVA was used to test for significant differences between the means of each seed weed species and each essential oil concentration. For each weed seed species a simple linear regression analysis was used to study the correlation between seed germination (%) and essential oil concentrations. For each essential oil the regression lines of all weed species were compared using the analysis of variance as suggested in (12). For all statistical analysis, the SAS program was used (34).

**Green House:** The research was done in plastic pots of 400 ml (10 cm dia, 8 cm height) in greenhouse (controlled temperature 25°C/15°C day/night) to test the effects of inhibitory effects of essential oils. Before the trial, the soil was dried in oven at 104 °C until constant weight. Each pot was filled with 0.5 kg loamy soil. Twenty-five seeds of each weed were sown at 4 mm depth. For each essential oil, the oil-in-water emulsion was prepared at 5.4, 21.6, 86.4 and 345.6 mg l<sup>-1</sup> concentrations named as C4, C5, C6 and C7, respectively. Five ml emulsion was sprayed on the soil surface of each pot. In control only water was used. All treatments were replicated four times. The essential oil-in-water emulsion was sprayed on the soil surface just after the sowing; for daily irrigation, water gently sprayed on the soil until it got moist preventing its leaching from the pot. After sowing, the seedling emergence was observed and recorded daily till 30 days. The coleoptile length was used as parameter of germination inhibition. Relative germination was calculated as germination (%) of control.

**Statistical Analysis:** For statistical evaluation, data were transformed to  $\arcsin [x (\%)/100]^{1/2}$  (21). ANOVA was used to test for significant differences between the means of each seed weed species and each essential oil concentration. For each weed seed species a simple linear regression analysis was used to study the correlation between seed germination (%) and essential oil concentrations. For all statistical analysis, the SAS program was used (34).

## RESULTS

**Laboratory Bioassay:** The essential oils inhibited the germination of weeds. The lethal dose to completely inhibit the weeds seed germination differed for each essential oil studied and test weed species (Table 1). Cinnamon oil exerted the drastic inhibitory effect than lavender and peppermint. The germination rate decreased with increasing

Table 1. Effects of application of essential oils concentrations on seeds germination of weeds in Lab. bioassay

Weeds	Cinnamon essential oil (%)					Lavender essential oil (%)					Peppermint essential oil (%)				
	0.2	0.6	1.8	5.4	LSD (P<0.05)	0.2	0.6	1.8	5.4	LSD (P<0.05)	0.2	0.6	1.8	5.4	LSD (P<0.05)
<i>A. retroflexus</i>	11	60	100	100	23	7	26	100	100	10	6	16	100	100	9
<i>C. album</i>	71	54	100	100	8	80	77	99	100	7	61	70	97	100	8
<i>Lolium</i> spp.	3	19	68	100	11	2	7	47	100	6	2	8	35	100	5
<i>P. oleracea</i>	6	82	100	100	16	2	17	100	100	6	6	17	100	100	7
<i>S. arvensis</i>	80	76	87	100	7	74	79	92	100	7	66	67	89	100	7
<i>S. nigram</i>	63	38	100	100	14	51	39	94	100	13	40	31	94	100	14
<i>V. sativa</i>	62	41	100	100	19	17	11	100	100	19	21	47	100	100	20
LSD (P<0.05)	18	21	7	-	-	15	12	8	-	-	14	14	8	-	-

Table 2. Effects of application of essential oils on root elongation of weeds over the control (%) in Lab. Bioassay

Weeds	Cinnamon essential oil (%)			Lavender essential oil (%)			Peppermint essential oil (%)		
	0.2	0.6	1.8	0.2	0.6	1.8	0.2	0.6	1.8
<i>A. retroflexus</i>	63ab	50c	-	64a	55bc	-	65a	57bc	-
<i>C. album</i>	67c	83ab	-	88a	81b	68c	83ab	67c	60d
<i>Lolium</i> spp.	91a	81c	72d	89ab	88b	83c	88b	88b	90bc
<i>P. oleracea</i>	65a	53c	-	62ab	57bc	-	67a	61ab	-
<i>S. arvensis</i>	70ba	72a	57b	71a	70a	57b	76a	73a	57b
<i>S. nigram</i>	74ab	77a	-	76ab	77a	68c	78a	76ab	72b
<i>V. sativa</i>	67b	52c	-	76a	68b	-	75a	50c	-

Within species, values without common letters are significantly different according to LSD (0.05).

Table 3. Effects of application of essential oils concentrations on seeds germination of weeds in Pot culture

Weeds	Cinnamon essential oil (mg)					Lavender essential oil (mg)					Peppermint essential oil (mg)				
	5.4	21.6	86.4	345.6	LSD (P<0.05)	5.4	21.6	86.4	345.6	LSD (P<0.05)	5.4	21.6	86.4	345.6	LSD (P<0.05)
<i>A. retroflexus</i>	51	49	91	100	5	71	64	78	84	4	78	64	73	82	6
<i>Lolium</i> spp.	40	35	47	57	8	23	20	45	48	7	21	40	50	53	8
<i>S. arvensis</i>	51	61	69	78	8	49	37	59	61	4	40	35	47	57	6
LSD (P<0.05)	10	8	11	6	-	5	5	10	5	-	9	9	7	7	-

concentrations of essential oils, even if a dose-dependent relation between oil concentrations and inhibitory ability was not always observed. The weed species most sensitive to essential oils were redroot pigweed (*Amaranthus retroflexus* L.), common purslane (*Portulaca oleracea* L.) and common vetch (*Vicia sativa* L.), their germination was completely inhibited by all oils at concentration  $1.8 \text{ mg l}^{-1}$ . Common lambsquarter (*Chenopodium album* L.) and black nightshade (*Solanum nigrum* L.) were inhibited at  $1.8 \text{ mg l}^{-1}$  by cinnamon oil and at  $5.4 \text{ mg l}^{-1}$  by lavender and peppermint essential oils. Wild mustard (*Sinapis arvensis* L.) and ryegrass (*Lolium* spp.) were the least susceptible species, only being completely inhibited at the highest oil concentration ( $5.4 \text{ mg l}^{-1}$ ) (Table 1). Although a lethal concentration for all the weeds was identified, but the sensitivity of various species to the oils was quite different. For instance at lower concentration ( $0.2 \text{ mg l}^{-1}$ ) wild mustard was very sensitive to cinnamon (80%) and lavender oil (74%), while it was less susceptible to peppermint (66%). Similar tendency was shown by common lambsquarter, which was more sensitive to cinnamon and lavender (71 and 80% respectively) than peppermint (61%). While black nightshade also demonstrates the sensitivity to cinnamon, lavender and peppermint (63%, 51% and 40% respectively). The effects of oils at concentration  $0.6 \text{ mg l}^{-1}$  was erratic. For instance, common lambsquarter was inhibited more by cinnamon and lavender oils at concentrations  $0.2 \text{ mg l}^{-1}$  than at  $0.6 \text{ mg l}^{-1}$ , while, peppermint essential oils inhibited lambsquarter germination more at  $0.6 \text{ mg l}^{-1}$  than at  $0.2 \text{ mg l}^{-1}$ . The regression lines between seed germination and essential oil concentrations confirm the different susceptibility of weed species (Fig. 1). While the inhibition is similar among the oils, weed species differ in their response to the toxic effect of each oil. It appears that the concentration of essential oils has a greater effect on weed susceptibility than the type of oil used. This aspect is highlighted in Table 2, where root elongation was more sensitive to weed species oil concentration and oil type than control (%). At  $0.2$ ,  $0.6$  and  $1.8 \text{ mg l}^{-1}$  concentrations, redroot pigweed and common purslane were found more sensitive to essential oils. The reduction in root of redroot pigweed was 50, 45 and 47% with cinnamon, lavender and peppermint essential oils, respectively, compared with control. The cinnamon, lavender and peppermint essential oils caused 47, 43 and 39% reduction in common purslane root growth. While the root length reduction in common vetch was 48 and 50% with cinnamon and peppermint essential oil respectively and 32% with lavender. Ryegrass was less sensitive to test essential oils for root length reduction cinnamon essential oil caused maximum reduction (28%), while in general reduction was 12%. The essential oils were found harmful to root growth depending on the oil type and its concentration.

**Pot culture:** Similar to laboratory bioassay, the essential oils also reduced the seed emergence also in green house (semi-controlled condition) compared to control (C0). Cinnamon oil caused stronger inhibition as already recorded in laboratory trials, compared to lavender and peppermint. Ryegrass was less susceptible to all oils. Cinnamon oil inhibited the redroot pigweed, ryegrass and wild mustard germination by 51, 40 and 56% respectively (Table 3). Even the lower concentrations of lavender ( $5.4 \text{ mg l}^{-1}$ ) and peppermint ( $5.4 \text{ mg l}^{-1}$ ) also inhibited the germination (Table 3). The inhibitory effects increased with increasing concentrations ( $86.4 \text{ mg l}^{-1}$ ). The cinnamon oil totally inhibited the redroot pigweed (91%) germination, while ryegrass and wild mustard were inhibited

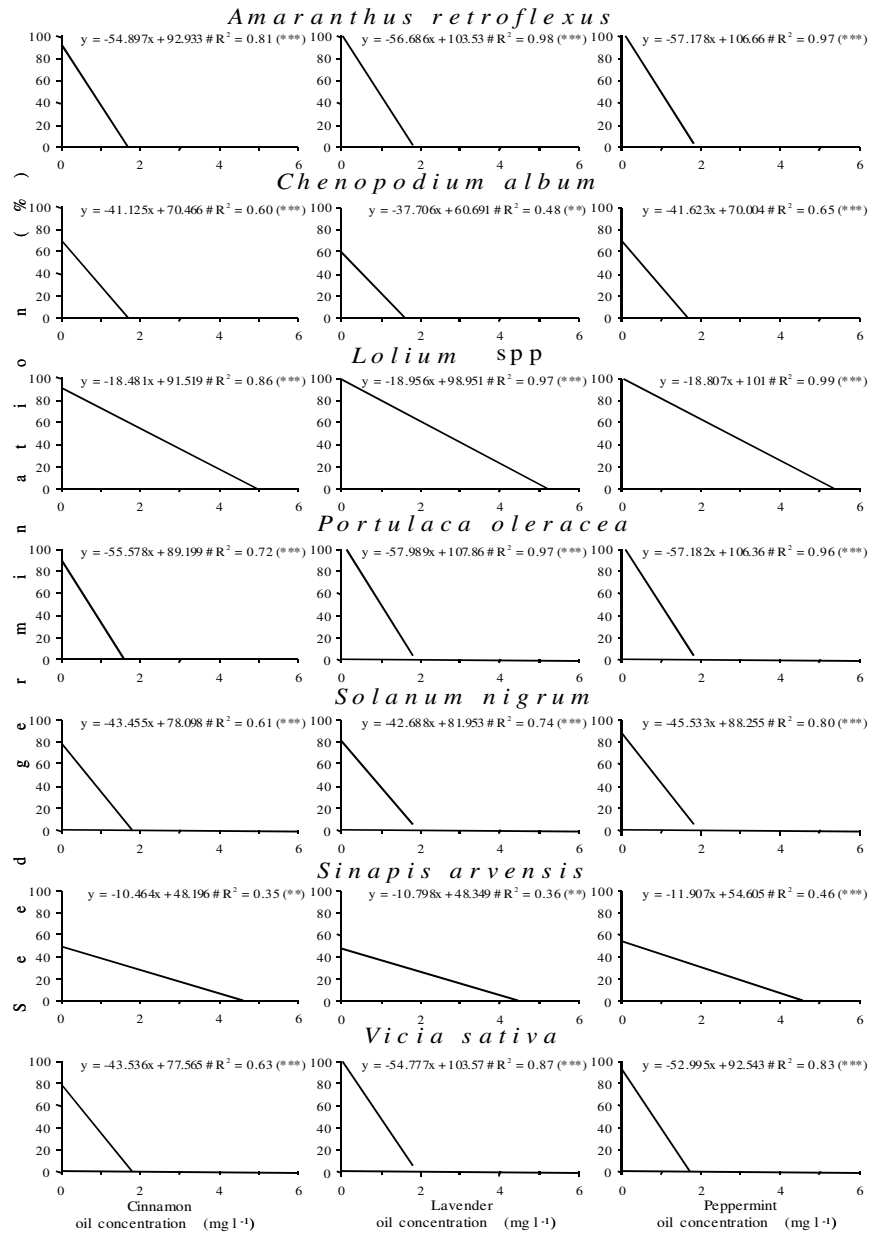


Figure 1. Regression lines of seed germination and oil concentration in seven weed species treated in controlled condition with cinnamon, lavender, and peppermint essential oils.

47% and 69%, respectively over the control (C0). Lavender oil inhibited the seed germination of redroot pigweed (78%) followed by wild mustard (59%) and ryegrass (45%). Peppermint oil also inhibited the redroot pigweed (73%), ryegrass (50%) and wild mustard (47%) respectively. At highest concentration (345.6 mg l<sup>-1</sup>) cinnamon essential oil totally inhibited the germination of redroot pigweed, followed by lavender (84%) and peppermint (82%). While wild mustard seed germination was inhibited by 78, 61 and 57% with cinnamon, lavender and peppermint essential oils, respectively. Ryegrass was inhibited 57% by the cinnamon, 53% by peppermint and 48% by lavender.

The regression analysis between oil concentrations and seed germination showed that increasing concentration of essential oil increased the inhibitory effects on weed seed germination till a lethal dose (Fig. 2).

## DISCUSSION

The cinnamon, lavender and peppermint essential oils had strong phytotoxic activity to weed seed germination, therefore, may be regarded as potential source of bioherbicides. Other studies have also shown that essential oils extracted from aromatic plants are phytotoxic (2,6,7,15,19,23,28,31,35,38,47). The regression analysis between seed germination and oil concentration (Fig. 1) showed that the phytotoxic effect of essential oils in laboratory while weeds seeds differed greatly in their susceptibility to oil concentrations. The similar results were recorded in semi-controlled conditions (Fig. 2). Thus the inhibition ability does not differ among the oil types but it depends on their concentration. The mechanisms of essential oils to inhibit seed germination is not known. Essential oils from cinnamon (*Cinamomum zeylanicum* L.) and red thyme (*Thymus vulgaris* L.) inhibits the potato sprout growth by killing the meristematic cells (41). Genotoxicity tests (Comet assay and Micronuclei test) indicated that essential oils from lavender (*Lavandula* spp.) significantly damaged the DNA in seedlings of *Vicia sativa* L. (10,37,47). After germination, essential oils may interfere with some biochemical process or mitosis that inhibits the cotyledon growth (8,17,33). An important point highlighted by our study was that the relation between oil concentration and inhibitory ability was not always dose-dependent for all weed species. In particular, this effect was noticed for red root pigweed and common lambsquarter, it has also been reported on the germination of common vetch and black nightshade (26) (Table 1). The inhibition of germination of redroot pigweed was inhibited by essential oils is important, as it is major weed and has developed resistance to herbicides with different modes of action (29). Using essential oils an alternative weed management system appears to be possible (1,9,15,19,28,37,44). Our data suggest that the concentration of essential oils plays a pivotal role in their eventual field application. Germination rates of common lambsquarter, wild mustard and black nightshade were reduced by over 50% at relatively low concentrations of essential oils. In pot culture, the germination inhibition rate was recorded at 30<sup>th</sup> days after essential oil application, till then the weeds have developed 3 true leaves and were going to compete with crops. Being able to control weed emergence up to this stage is an important result. This is useful information in an Integrated Weed Management System (IWMS) framework. The combined action of diverse weed control measures could make feasible

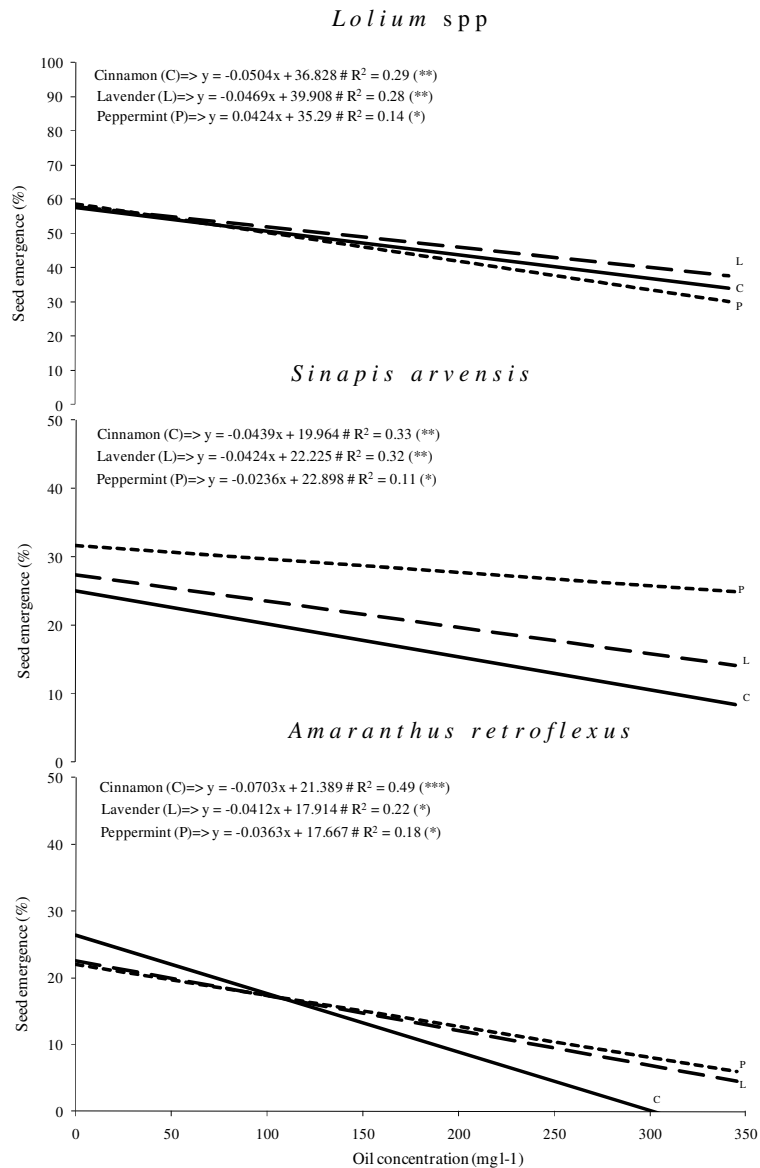


Figure 2. Regression lines of seed germination and oil concentration in three weed species treated in semi-controlled condition with cinnamon, lavender, and peppermint essential oils. \*, \*\*, \*\*\* = significant  $P \leq 0.05$ ,  $P \leq 0.01$ ,  $P \leq 0.001$ , respectively; NS = Not significant

application of essential oils in IWMS (1,15,36,43). For example, the application of an essential oils at low concentration could inhibit weed emergence long enough during the early stages of crop growth for the crop canopy to develop. Further weed control could be done mechanically. Another aspect of interest for an IWMS is the low susceptibility of ryegrass compared to others test weeds. Ryegrass is a monocotyledonous species, while all others weeds are dicotyledonous, a selective use at the lower concentration of essential oils could be hypothesized. The identification of lethal dose of essential oils could be useful in field conditions. However, additional work is required to determine the mechanism of herbicidal action and to develop a formulation that could be used under field conditions. Besides the common belief that essential oils are highly volatile and their persistence under field conditions needs to be improved, however, the bioconversion of such compounds by the environment and microorganisms needs to be studied. It is known that bioconversion of essential oils by non-specific enzymes produces less harmful compounds (18). In addition the low persistence due to their biodegradability and easily catabolism in the environment should be re-considered as the natural products are being used in sustainable organic farming (10,11,19,38,43,46). In fact the low persistence in soil and water gives low or no toxicity against vertebrates, fishes, birds and mammals is important for plant protection (15,23,27,43,48). Last but not least their effects on crop species must be investigated. Further field research is necessary to develop an appropriate technology of essential oil application to inhibit the weed seed germination and its effects on crop plants.

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