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Research Paper

Allelopathic Effects of Some Organic Mulch Extracts on Seed Germination and Early Growth of Some Ornamental Plants

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Abstract

Mulches have significant environmental, aesthetic and functional benefits. However, there are arguments that some organic types of mulches prevent seed germination and the growth of plants due to their allelopathic effects. This study examined the allelopathic influence of the extracts of some organic mulches on seed germination percentage, and growth of the seedlings of some ornamental plants in two interrelated experiments. In the first experiment, allelopathic effects of six organic mulches including pine wood chips of the plane tree, pine cones, pine leaves, pine barks, sawdust, and grass clippings of *Festuca arundincea* were examined on germination and early growth of lettuce seeds as an indicator species. The second experiment was designed based on the results of the first experiment so that the extracts of the mulches with the lowest germination rates were selected and their effect on seed germination of five flowering plants of *Alyssum maritimum*, *Celosia argentea*, *Tagetes* sp., *Zinnia* sp. and *Rudbeckia* sp. were evaluated. The results showed that pine leave extracts had inhibitory effects on germination of lettuce seeds (in the first experiment) and also showed significant and strong inhibitory effects ($P<0.01$) on seed germination, radicle length, plumule length, plumule fresh and dry weights of all seasonal flowers. Based on these results, pine leaves after being released in the soil or after decomposition may act as a source of allelochemicals and should be used by care as mulch in landscaping at least if it is combined with ornamental plants.

Keywords: Allelopathy, Flowering plant, Germination, Mulch, Urban landscape.

INTRODUCTION

Water scarcity is an environmental constraint for agricultural production (Farooq *et al.*, 2009). In recent years, due to limited water resources, to increase the efficiency of water consumption and its optimal consumption, the use of various types of mulch is considered as one of the important principles of water-wise landscaping (Ellefson and Winger, 2004).

Mulches provide low maintenance and homogenous background in urban landscapes, reducing soil moisture evaporation rates, soil erosion, and weed control, balancing soil temperature and providing nutrients for soil (Singer and Martin, 2008). Lead and cadmium, which are the most common contaminations in urban areas, can be removed by mulches from eucalyptus, pine, populus and cedar leaves (Salim and Abu El-Halaw, 2002).

The word allelopathy, from the Latin word of allelon which means 'of each other' and pathos, which means 'to suffer', refers to the chemical inhibition of one species over the other (Weir *et al*., 2004). Direct or indirect stimulatory or inhibitory effects of a plant on another through the release of chemical compounds into the environment are referred to as allelopathy (Saberi *et al*., 2013). Such allelopathic effects can be useful in some environmentally friendly techniques in controlling the weeds and help in reducing the economic and environmental costs of using herbicides because herbicides create environmental pollutions (Awan *et al*., 2012). Such chemicals may be released to the environment through exudation from the roots, washing from leaves, stems, flowers, fruits and seeds (Babaahmadi *et al*., 2013), and volatilization or decaying from the plant tissues (Mubeen *et al*., 2011; Qin *et al*., 2011). In other words, allelochemicals escape from plants in different ways. Four major methods by which allelochemicals are released from the mother plants could be summarized as 1) Leaching, in this way inhibitor components are produced by dead or alive parts of the plants; 2) Volatilization, by which terpene components are released from the leaves of some plant species; 3) Decomposition, in this method allelochemicals are released from plant residues; and 4) Exudation, in this way high quantities of organic compounds release from roots of several crops and non-crop species which act as inhibitors for the growth of other plants (Moosavi *et al*., 2011; Ankita and Chabbi, 2012). Chemically, allelochemical compounds have open-chain molecular structures (Ayyaz Khan *et al*., 2008; Saberi *et al*., 2013), and they are water-soluble chemicals (Babaahmadi *et al*., 2013; Sarkar *et al*., 2012). These are secondary metabolites (Morikawa *et al*., 2012) that have roles in plant-plant, plant-soil, plant-disease, plant-insect and plant-predator interactions that may be beneficial or detrimental to plants (Saberi *et al*., 2013; Ayyaz Khan *et al*., 2008). Also, they may result in a reduction in growth and yield through negative interactions with important physiological processes such as changes in the cell wall structure, the activity of some enzymes (Ayyaz Khan *et al*., 2008; Saberi *et al*., 2013), and prevention of cell division (Saberi *et al*., 2013; Qin *et al*., 2011). According to Weir *et al*. (2004) also allelochemicals can act on plants in some ways: 1. One of the best-characterized phytotoxic mechanisms induced by allelochemicals is the inhibition of photosynthesis and oxygen evolution through interactions with components of photosystem II (PSII), 2. Decrease in enzymatic activity, which might be caused by general protein damage leading to decreased enzymatic activity, 3. Disruption of amino acid metabolism, 4. Disruption in normal metabolic processes such as inhibiting polar auxin transport and inhibiting the peroxidase and oxidase catalyzed oxidation of auxin (Weir *et al*., 2004).

There are several landscape components such as mulches and bedding flowers, each of them has effects on urban landscaping. For example, mulches are any natural or synthetic substances that cover the soil surface in landscapes, and protect and promote soil quality and generally have a thickness between 1 to 4 inches (Iies and Dosm, 1999). Flower beds also provide year-round beauty, color, and fragrance to urban landscapes (Bromley, 2015) and despite their need for high maintenance and resources such as water; it is hard to convince people to remove them from urban landscaping. Therefore, strategies are discussed to combine these two landscape elements to achieve lower input and maintenance required landscapes. Very limited research works have been conducted in this area from which the work by Pakdel (2010) and Iise and Dosmann (1999) can be discussed. It has been reported that Allelopathy plays an important positive role in the weed-controlling behavior of many organic and living types of mulches (Duryea *et al*., 1999). However, it might have an inhibitory effect on germination and seedling growth of ornamental flowers. This study was designed to firstly investigate the presence or absence of allelopathic effects of some organic mulches. It also directly examined such effects on seed germination of some ornamental flowers commonly used in urban landscapes through some laboratory tests.

MATERIALS AND METHODS

Plant materials and preparation of the extracts

The experiments were conducted in two trials at the Department of Horticulture and Landscape, Ferdowsi University of Mashhad, Iran, during 2018. Mashhad is the second biggest city in Iran and is located in the northeast of the country (36° 18' 38.5164" N and 59° 35' 58.0452" E.) with a semi-arid climate, cold winters, and hot dry summers. The average annual rainfall is about 255 mm, with very low rainfall events in springs and autumns. The average minimum and maximum mean annual temperatures are 4˚c and 22˚c, respectively (National Centers for Climatology, 2019). Organic mulches including wood chips of the plane tree, pine cones, pine leaves, pine bark, sawdust, and grass clippings of *Festuca arundincea* were used as the mulch types (Fig. 1). These materials were sourced from local materials available in adjacent natural environments. The extracts of these mulch types were obtained in the laboratory. To make the extracts, 125 ml of tightly packed mulch of each type were placed in a 1000 ml beaker with 875 ml of distilled water. The mulch was soaked after 72 hours at 25ºC and each extract was filtered with a funnel and a filter paper.

Experiments

In the first experiment, we used a common method to measure the allelopathy by extracting water-soluble chemicals from the mulches and then applying these extracts for the germination of lettuce seeds. This method has been previously used by Duryea *et al*. (1999). Twenty-five lettuce seeds were placed in each Petri dish and 4 ml of each extract was applied to the seeds located on a filter paper in each Petri dish. The Petri dishes were placed in laboratory conditions at 25 ºC. At the end of the experiment, we recorded the number of germinated seeds of each mulch extract. The experimental design was a completely randomized design with seven treatments and three replications.

In the second experiment, based on the results of the first experiment the extracts that had the lowest germination rates were selected and their effect on seed germination of five bedding flowers: *Alyssum maritimum*, *Celosia argentea*, *Tagetes* sp., *Zinnia* sp. and *Rudbeckia* sp. were evaluated. The measuring methods in this experiment were similar to the methods used in the first experiment. Distilled water was applied as control. The first experimental design was a completely randomized design with the mulch extracts as the only factor. The second experiment was factorial based on a completely randomized design with two factors containing different flowering plants and the use of the extracts or non-use as the factors with three replications.

Measurements

Average radicle length, average plumule length, fresh and dry weights of radicles, and fresh and dry weights of plumules (Hartmann *et al*., 2002), were measured in this experiment. The germination percentage was recorded every day, starting from the first day after the seeds were initially placed in the Petri dishes (Khayyat *et al*., 2014). The germination percentage was measured using the following formula:

$$GP = (Ni / N) \times 100 \qquad (1)$$

Where; PG is the percentage of germination, Ni, is the number of germinated seeds in the last day of counting, and N is the total number of seeds. Also, the germination rate (RS) was measured using the method explained by Khan and Yungar (1997). The formula is as follows:

$$RS = \sum^{n} Si/Di \qquad (2)$$

in which RS is germination rate (number of germinated seeds per day), Si was the number of transduced seeds in each count and Di was number of the days until counting the nth number of germinated seeds.

Average germination time (MGT) was also calculated based on the method of Matthews and Khajeh-Hosseini (2007). The formula is as follows:

$$MGT = \frac{A1D1+A2D2+AnDn}{A1+A2+An} \qquad (3)$$

In which A is the number of germinated seeds each day, D is the number of the days of germination.

Fig. 1. A-F) The mulch types used in this experiment, G and H) Experimental trials of this study along with the control treatments.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) and comparison of the means was performed using LSD tests. The analyses were performed with the statistical software package of JUMP. V.8.

RESULTS

Experiment 1

The ANOVA analysis showed that there were significant differences between the mulch types on germination percentage, average germination, germination rate, and average radicle and plumule lengths (Table 1).

Using the extract of pine leaves significantly decreased the germination percentage of lettuce as a sensitive plant compared with the control treatments, whereas the extracts of pine bark, cone, and wood chips showed a 100% seed germination. Average germination, germination rate, average radical length, and average of plumule length of lettuce seeds were zero when extract of pine leaves was used. Using the extract of grass clipping, pine bark and pine cone as the irrigation water led to the increment of germination rate of lettuce seeds, compared with the control, respectively. The extracts of sawdust, wood chips, and distilled water were associated with the lowest germination rates (Table 2).

The average radicle length of the lettuce seeds was significantly reduced by treatment with the extract of wood chips, grass clipping, sawdust, and pine bark compared to the control, respectively. The average radicle length was highest on lettuce plants when the extract of pine cones was applied (Table 2).

Table 1. Analysis of variance of the effect of different extracts of mulches on the studied traits in lettuce.

S.o.V	df	Germination percentage	Average germination	Germination rate	Average radicle length	Average plumule length	Fresh weight of radicle	Dry weight of radicle	Fresh weight of plumule	Dry weight of plumule
Mulch extraction	6	4105.76**	1904.43**	0.83**	12.44**	8.64**	0.00ns	0.00ns	0.02ns	0.00ns
Error	14	13.48	137.37	0.02	0.25	0.18	0.00	0.00	0.04	0.00
CV (%)		18.18	11.13	18.11	19.36	14.95	22.04	23.42	19.01	22.26

** and ns: Significant at $P < 0.01$ and insignificant, respectively.

Table 2. Effect of different extracts of mulches on germination (%), average germination, germination rate, the average radicle length, and the average plumule length of the seeds of lettuce.

Extractions	Germination (%)	Average germination (day)	Germination rate (n/d)	Average radicle length (mm)	Average plumule length (mm)
Wood chips	100.00a	83.33a	1.10c	3.24c	4.69ab
Pine cone	100.00a	50.00b	1.28bc	5.70a	5.21a
Pine leaf	0.00c	0.00c	0.00d	0.00e	0.00e
Pine bark	100.00a	50.00b	1.45ab	0.31e	3.37d
Sawdust	92.33b	31.43b	1.11c	2.01d	4.17bc
Grass clippings	92.00b	35.76b	1.63a	2.86cd	3.76cd
Distilled water (control)	100.00a	50.00b	1.08c	4.15b	3.14d

In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Experiment 2

The analysis of variance for germination percentage showed that there was a highly significant difference between different flowers. Analyses of variance also showed that using the extracts made significant differences in the measured factors compared to the control treatments (Table 3).

The data shows that the germination of the seeds severely reduced by the allelochemicals (Table 4). Pine leaf extracts significantly inhibited seed germination in all of the bedding flower types compared to distilled water.

Also, the results indicated that the extract of pine leaf had significant effects on the average plumule and radicle length and fresh and dry weight of plumules (Table 5).

Table 3. Analysis of variance of the effect of different extracts of mulches and bedding flower on the studied traits.

S.o.V	df	Percentage	Average germination	Germination rate	Average radicle length	Average plumule length	Fresh weight of radicle	Dry weight of radicles	Fresh weight of plumules	Dry weight of plumules
Mulches extractions (M)	1	39385.63**	803.52**	75.30**	35.58**	49.20**	0.01*	0.00*	0.53**	0.00**
Bedding (B)	4	634.63**	12.97**	0.22*	2.10**	3.89**	0.01ns	0.00n.s	0.14**	0.001**
M3B	4	705.90**	14.39**	0.22*	2.10**	3.89**	0.01ns	0.00ns	0.14**	0.001*
Error	20	38.17	0.78	0.39	0.07	0.03	0.01	0.00	0.01	0.00
CV (%)		16.62	23.17	16.59	16.45	26.50	15.23	22.47	16.79	25.85

*, ** and ns: Significant at $P < 0.05$, $P < 0.01$ and insignificant, respectively.

Table 4. Effect of the extract of pine leaves on average germination and germination rate of some bedding flowers.

Bedding flowers	Seed germination(No.)		Average germination(day)		Germination rate(n/d)	
	Distilled water	Pine leaf	Distilled water	Pine leaf	Distilled water	Pine leaf
Alyssum maritimum	100.00a	0.00d	14.28a	0.00d	2.82c	0.00d
Celosia argentea	90.66a	0.00d	12.95a	0.00d	3.04bc	0.00d
Tagetes sp.	66.66b	0.00d	9.52b	0.00d	3.36b	0.00d
Zinnia sp.	62.33b	0.00d	8.90b	0.00d	2.87c	0.00d
Rudbeckia sp.	49.33c	0.00d	7.04c	0.00d	3.73a	0.00d

In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

Table 5. Effect of the extract of pine leaves on average radicle length, the average plumule length, fresh and dry weight of plumule of some bedding flowers.

Bedding flowers	Average radicle length (mm)		Average plumule length (mm)		Fresh weight of plumule (g)		Dry weight of plumule (g)	
	Distilled water	Pine leaf	Distilled water	Pine leaf	Distilled water	Pine leaf	Distilled water	Pine leaf
Alyssum maritimum	1.03[c]	0.00[d]	2.64[b]	0.00[d]	0.05[c]	0.00[c]	0.002[c]	0.00[c]
Celosia argentea	2.25[b]	0.00[d]	1.74[c]	0.00[d]	0.06[c]	0.00[c]	0.004[c]	0.00[c]
Tagetes sp.	3.28[a]	0.00[d]	2.76[b]	0.00[d]	0.43[b]	0.00[c]	0.02[b]	0.00[c]
Zinnia sp.	3.40[a]	0.00[d]	4.99[a]	0.00[d]	0.73[a]	0.00[c]	0.04[a]	0.00[c]
Rudbeckia sp.	0.92[c]	0.00[d]	0.63[d]	0.00[d]	0.04[c]	0.00[c]	0.0003[c]	0.00[c]

In each column, means with similar letter(s) are not significantly different ($P < 0.05$) using the LSD test.

DISCUSSION

The effects of mulch vary depending on the type of mulch material used, therefore, standardization of mulch materials is very important for better growth and optimal performance of plants (Daniele *et al*., 2019). Despite the positive effects of organic mulches such as weed control, moisture and temperature amelioration and water-saving (Daniele *et al*., 2019), these materials might, in some cases, be sources for allelopathic effects. Such effects may hinder the germination and growth of landscape plants adjacent to or combined with them.

In this study, pine leaf extract showed the allopathic effect on bioassay species germination when lettuce seeds were used as an indicator species. Duryea *et al*. (1999) showed that water extracts from fresh eucalyptus, *Melaleuca* and pine straw mulches inhibited the germination of lettuce seeds. Pine bark extracts also had a slight inhibitory effect on seed germination of lettuce. This finding is consistent with the results of our study.

Based on the results of this study also, it was confirmed that pine leaf extract had a significantly higher reduction on seed germination of the bedding flowers of *Alyssum maritimum*, *Celosia argentea*, *Tagetes* sp., *Zinnia* sp. and *Rudbeckia* sp. Among the mulches, allelopathic properties could have two possible impacts: 1) A mulch might inhibit the germination of weed seeds, or 2) A mulch might inhibit the growth of landscape plants (Duryea *et al*., 1999). Allelochemicals might inhibit seed germination by suppressing the synthesis of gibberellins (Zhang and Fu, 2009; Zhang and Fu, 2010), indole acetic acid (Saberi *et al*., 2013; Zhang and Fu, 2010), and the mitotic activity of young cells resulting in the inhibition of seed germination (Sarkar *et al*., 2012). Allelopathic effect of these compounds in interaction with plants is often observed to occur early in the life cycle of the plants causing inhibition of seed germination and/or seedling growth (Ankita and Chabbi, 2012; Fallah Toosi and Baki, 2012). Stop in germination process during germination period may also be because of changes in enzyme activities which restrict the conversion of nutritive compounds and delay or stimulation in using nutritive matter can cause lack of production of respirable vesicles and finally can result in lack of ATP in the seeds exposed to allelochemical compounds (Saberi *et al*., 2013; Weir *et al*., 2004).

Application of the extract of grass clippings, pine barks, and pine cones as irrigation water increased the seed germination rate of lettuce compared to the control. Sawdust, wood chips, and distilled water have the least germination. During seed germination, there is a rapid increase in glycolytic activity linked to an increased rate of respiration. This glycolytic activity is necessary to mobilize stored carbohydrates to provide the seeds with the reducing power, ATP, and carbon products required for the biosynthesis of the roots and aerial parts of the emerging seedling (Weir *et al*., 2004). Weir *et al*. (2004) stated that metabolic energy can be restricted by disorder in the respiration rate and consequently can decrease the early growth of seedlings. Radicle and plumule were the first organs that emerged from the seeds and exposed to allelopathic matters; therefore, it can be predicted that their growth can be decreased when they are exposed to allelopathic compounds. The decrease in plumule length can cause by inhibition in cell division and elongation or a decrease in hormones such as acetic acids and gibberellins. Allelochemicals can also decrease the amount of fusion auxin in roots. These compounds decrease the growth by hindering nutritive absorption and or interfering in respiration. According to Saberi *et al*. (2013), allelochemicals influence germination and early growth of seedlings in two ways. First, they hinder cell division, and second, they inhibit the elongation of the cells (Saberi *et al*., 2013). Aqueous extracts of *Brassica napus*, *B. rapa* and *B. juncea* inhibited the seed germination and seedling growth of sunflowers (Jafariehyazdi and Javidfar, 2011). In another experiment, the growth of *Lactuca sativa* was evaluated using the sandwich method. The results showed that *Inula falconeri* and *Inula koelzii* decreased the growth of lettuce. Applying 50 mg dried leaf extract of these plants showed a high inhibitory effect on the growth of the root and hypocotyl of *Lactuca* sp. (Latif Khan *et al*., 2009).

Babaahmadi *et al*. (2013) represented that hydro-extract of yarrow (*Achillea willhelmsi* and *A. milifolium*), Sage (*Salvia officinalis*), Catnip (*Nepeta cataria*) and Tansy (*Tanacetum vulgare*) reduced the seed germination of *Alyssum hirsutum* and *Amaranthus retroflexus*.

In the current experiment, the average radicle length of lettuce seeds was significantly reduced by the extract of wood chips, grass clippings, sawdust, and pine barks compared to the control. Fikreyesus *et al*. (2011) also reported that leaf, root, bark, and fruit extracts of *Eucalyptus camaldulensis* showed an inhibitory effect on germination and root and shoot elongation of tomato plants. Qin *et al*. (2011) also showed that nut solution decreased seed germination of tomato. In another experiment, it was found that toxic compounds were released from pine needle tissues. The amount of growth inhibition depended on the type of pine needles (fresh pine needles, senesced pine needles, and decaying pine needles). The highest reduction in root and shoot growth was observed in tall fescue and Bermuda grass from the fresh pine needles. Root and shoot growth of oat was inhibited with all needle types. Pine needle extracts exhibited toxic effects that influenced germination and radicle elongation of the turfgrasses (Nektarios *et al*., 2005). The findings of these researchers confirm our results. It is noteworthy that the concentration of the chemicals that are often tested in the laboratory may not be the same as those accumulated or leached out in the field. Therefore, it might be more appropriate to further investigate these results in a field experiment.

CONCLUSIONS

The results of this study showed that the use of the extracts of grass clippings and pine bark increased the germination rate of lettuce compared to the control, while the extracts of sawdust, wood chips and distilled water and pine leaves resulted in lower germination rates. It was also confirmed that pine leaf extract significantly reduced seed germination of the bedding flowers of *Alyssum maritimum*, *Celosia argentea*, *Tagetes* sp., *Zinnia* sp. and *Rudbeckia* sp. Therefore, it is recommended to use this mulch type with care due to its allopathic effects in urban green spaces.

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