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Research Article

Allelopathic Effect of Lemon Plant Parts on the Seedling Germination and Growth of Lettuce and Cabbage

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Abstract

Secondary metabolites produced by different plants might inhibit or stimulate the growth and development of other plants present in its vicinity, this kind of interaction among plants termed as allelopathy. Lemon (*Citrus limon*) peels, flowers, and leaves were chosen for test extract preparation to check the seed germination and seedling growth of lettuce (*Lactuca sativa L.*) and cabbage (*Brassica oleracea L.* var. *capitata* L. f. *alba* D C.) and to observe their allelopathic potential. The experiment was conducted in sterilized Petri dish; germination of seeds was observed for ten days and the shoot length, root length and number of germinated seeds was evaluated. The effects of different concentrations of aqueous extract were compared to distilled water (control). The results showed maximum seed germination in control whereas all the test extracts showed very low germination percentage except lemon flower extract on cabbage seeds showing 80% seed germination. Root and shoot lengths were also reduced in all the test extracts except lemon flower extract against cabbage seeds which showed stimulated shoot growth. It may be concluded that lemon plant produces allelotoxins that can inhibit the growth of other crops in its vicinity. Although lemon flower extract showed the stimulatory effect on cabbage seeds, the detailed confirmatory study is necessary.

INTRODUCTION

In nature, plants species grow together and interact with each other by inhibiting or stimulating the growth and development through different interactions. A special form of competition among plants is known as allelopathy. The term allelopathy was introduced by an Austrian scientist Hans Molisch in 1937, and is derived from two Greek words "*allelon*" which means "to each other", and "*pathos*" which means "to suffer" and denote the injurious effect of one plant upon the other [14]. However, in 1996, the International Allelopathy Society defined allelopathy as *"Any process involving secondary metabolites produced by plants, micro-organisms, viruses, and fungi that influence the growth and development of agricultural and biological systems (excluding animals), including positive and negative effects"*[17].

Allelopathic interactions are primarily based on the synthesis and release of secondary metabolites (known as allelochemicals or allelotoxins) by higher plants that initiate a wide array of biochemical reactions, which induce several biological changes. Allelopathic chemicals can be present in any part of the plant: leaves, flowers, roots, fruits, seeds, rhizomes, pollens or stems. They can also be found in the surrounding soil. Allelochemicals are released into the environment by root exudation, leaching from aboveground parts and volatilization and by decomposition of plant material [13]. The toxic chemicals may interfere with germination of seeds, inhibit shoot or/and root growth; they may inhibit nutrient uptake, or they may attack a naturally occurring symbiotic relationship thereby destroying the plant's valuable source of a nutrient. A variety of crop and weed species have been reported to possess allelopathic activity on the growth of other plant species [9,21].

The research and development in allelopathy are of urgency for the improvement of agriculture, forestry and the global environment, because allelopathy majorly deals with invasive or exotic and native weeds, and it was found that plants with allelopathic property can be potentially used as natural herbicides [2,3,8,14]. On the other hand, allelopathic crops are also found to keep hampering agricultural practices and bring about environmental degradation [2,3]. The allelochemicals are considered to be more biodegradable than traditional herbicides; however, allelochemicals may also have undesirable effects

on non-target species. Thus, necessary ecological studies are required before widespread use of allelochemicals.

Earlier studies suggest that certain plants which are traditionally used for their medicinal values show some allelopathic effects, too. For example, Basil (*Ocimum basilicum* L.) on some crops [19], neem and other medicinal plants on mung bean [10]. *Citrus lemon* is an important medicinal plant [11] as well as used in a wide variety of food and drinks and as a cleaning agent [12]. In northeast India, lemon is commonly grown in vegetable and kitchen gardens. Thus, there is a chance of it affecting crops present in the vicinity. Keeping the above point in view, in the present work was undertaken to test the allelopathic potential of parts of the lemon plant on seed germination and seedling growth of two important food crops lettuce (*Lactuca sativa* L.) and cabbage (*Brassica oleracea L.* var. *capitata* L. f. *alba* D C.).

MATERIAL AND METHODS

Collection of Plant Materials

The experiment was conducted during January and February 2014, and the lemon plant parts were collected from Pailapool, a village in the Cachar district of Barak Valley, Assam, and India a day before performing the experiment. Lemon plant parts i.e. fruits, flowers, and leaves were collected from the same plant from a residential vegetable garden. For the germination experiment, the seeds of lettuce and cabbage were procured from a reputed vendor in an agricultural fair held in Guwahati, Assam, India.

Based on earlier reports of the allelopathic property of leaf extracts in comparison to other parts. The extracts were then tested for their allelopathic potential on the germination and seedling growth of lettuce (*Lactuca sativa L.)* and cabbage (*Brassica oleracea L.* var. *capitata* L. f. *alba* D C.).

Preparation of Plant Extract

Lemon extracts were prepared from leaves, peels and flowers buds [5,10]. The lemon plant parts were first washed with running tap water and are air dried. Two (2) grams of each of lemon peel, flowers and leaves were weighed and grinded separately using mortar and pestle. To each, 10 ml of warm distilled water (37^0C) was added, making a 20% solution. The samples were transferred to a test tube and are thoroughly mixed using a vortex mixer.

Seed Inoculation

Clean and sterilized Petri dishes were taken, and a piece of filter paper is placed over it. The filter paper in the Petri dishes was moistened by adding the peel extract, flower extract, and leaf extract accordingly which acts as the medium for growth. The control plates were moistened by distilled water but not overdamped. Forty (40) lettuce seeds of similar size were counted and positioned on the filter papers of each dish, ensuring that the seeds do not touch each other. Each petri dish was then wrapped with parafilm so that the dishes don't dry out and also to prevent infection (Figure 1). The Petri dishes were then incubated at 16 hr photoperiodic chamber at 25 ± 1°C. The same procedure was repeated for cabbage seeds, where thirty-five (35) seeds were accommodated in each dish.

Seedling germination and growth

Germination test was based on the visual appearance of seedlings in the Petri dishes for ten days after their inoculation. Seeds were considered germinated upon radicle emergence, and the germination was determined by counting the number of germinated seeds regularly for ten days. Lengths of roots and shoots were measured using ruler centimeter on day 10 and the germination percentage was calculated (Raun et al., 2002).

Data analysis

SPSS 16.0 was used to analyze the statistical data. Descriptive statistics calculates the means of all replicates with standard error and deviations. Comparison of germination rate between treatments was tested using a Chi-square test.

RESULTS

Germination percentage

The effect of aqueous extract of three test parts of lemon plants on the germination of lettuce and cabbage is shown in Tables 1 and 2. The results of the experiment showed that the germination percentage of the test extracts varied significantly from the control where the germination rate for cabbage are 85.7%, and lettuce seeds are 72.5%. The lowest germination rate was noted in cases leaf against lettuce and cabbage seeds, and the result was found to be significant at $P < 0.001$. Interestingly, cabbage was not very sensitive to lemon flower that showed 80% seedling germination.

Root length

The allelopathic treatments significantly influenced root length. The control had the longest root length of 2.9 ± 0.213 cm. The most effective reduction among all treatments was observed

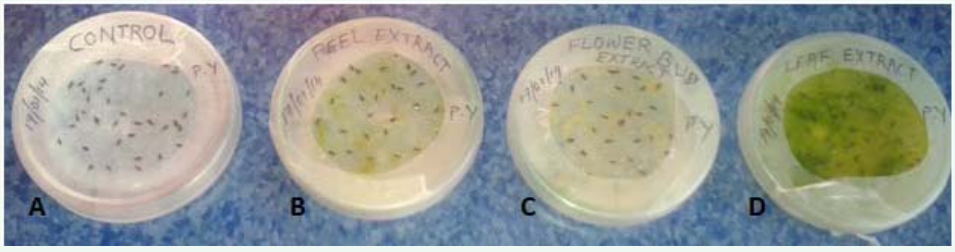

Figure 1 Seed inoculation on control and lemon plant extracts.
A) Control; B) Peel extract; C) Flower bud extract and D) Leaf extract

Table 1: Effect of extract of lemon plant parts on the seedling germination lettuce.

Treatment (n=40)	Seeds germinated	Seeds not germinated
Control	29	11
Peel	25	15
Flower	3	37
Leaf	3	37

Table 2: Effect of lemon plant extracts on the seedling germination of cabbage.

Treatment (n=35)	Seeds Germinated	Seeds not germinated
Control	30	5
Peel	5	30
Flower	28	7
Leaf	6	29

in both lemon flower and leaf extract against lettuce where the mean difference was observed as 2.4 ± 0.093 cm and 2.4 ± 0.046 cm respectively (Table 3). Cabbage roots were more sensitive to lemon leaf, and peel is attaining a maximum length of 0.6 ± 0.105 cm and 1.6 ± 0.320 cm, both having a mean difference of more than 6 cm (Figure 2, Table 4).

Shoot length

The lettuce seedlings were significantly affected by all the plant treatments (Table 3). Therefore, the order of severity of lettuce seeds can be given as flower>leaf>peel>control. Flower bud extract and leaf extract significantly decreases the shoot growth having a mean difference of 2.9 ± 0.082 cm and 2.5 ± 0.115 cm with the control treatment. Although lettuce seed attains shoot height of 2.3 ± 0.179 cm in peel extract, the result was also found to be significant at P<0.001. Interesting results have also been observed in the case of cabbage, where the maximum shoot growth of 5.8 ± 0.402 cm was monitored in the lemon flower extract (Table 4). In the case of peel extract, the average shoot length of few seeds that germinated had a greater length (3.8 ± 0.396 cm) which indicates that lemon flower and to some level lemon peel have some stimulatory effect on cabbage shoot development. The highest reduction in the shoot and root length of lettuce seeds were observed in the flower bud and leaf extract of lemon. Moreover, the result from cabbage seeds directly points at leaf extract. The experiment thus infers that, that the allelopathic substances may be present in a leaf of lemon whereas stimulatory response was obtained in the case of the flower against cabbage growth.

Seedling appearance

In both the cases, plants in the control grew very well, spreading leaves and showed no sign of abnormalities. The plantlets in all the test extracts (excluding flower extract against cabbage) showed chlorosis, rot, burnt root tips or abnormal growth indicating the adverse effect of allelopathy. Seedlings in flower extract were very healthy, and maximum seeds showed germination.

DISCUSSION

Obtained results make it clear that lemon exerted allelopathic effects on lettuce and cabbage seeds thus influencing their germination and root and shoot development. Earlier experiments on allelopathy have revealed that the inhibitory allelopathic effect of leaf extract was more potent than that of other vegetative parts [5,10], which is in corroboration with our results in lettuce seed but in the case of cabbage seeds peel extract had the highest inhibitory effect. Lemon leaves firstly inhibited the germination, and even those germinated were unhealthy and not able to survive [2,3], concluded that the crucifer species like *Raphanus sativus* L., *Brassica campestris* L. and *Brassica oleracea* L. were sensitive to leaf aqueous extract of *Parthenium hysterophorus* [2,3]. Several researchers performed experiments on lettuce seeds germination against lemon peel extract which resulted in 0% seed germination [16], but the present study showed that the lemon peel extract possessed inhibitory effect on lettuce and showed 62.5% seed germination, but the seedlings did not grow normally and had retarded and stunted growth. These findings

Table 3: Effect of lemon plant parts extract on root and shoot of lettuce seeds.

Treatment (n=40)	Shoot length (cm)	P value	Root length (cm)	P value
Control	3.8±0.301	-	2.9±0.213	-
Peel	2.3±0.179*	0.003	0.8±0.098*	<0.001
Flower	0.9±0.219*	<0.001	0.5±0.120*	<0.001
Leaf	1.3±0.416*	<0.001	0.5±0.167*	<0.001

Values are mean ± standard deviation of forty replicates; *= significant at P<0.01; compared with control.

Table 4: Effect of lemon plant parts extracts on root and shoot of cabbage seeds.

Treatment (n=35)	Shoot length (cm)	P value	Root length (cm)	P value
Control	3.5±0.201	-	7.9±0.582	-
Peel	3.8±0.396ns	0.521	1.6±0.320*	<0.001
Flower	5.8±0.402*	<0.001	4.6±0.278*	<0.001
Leaf	1.1±0.330*	<0.001	0.6±0.105*	<0.001

Values are mean ± standard deviation of thirty-five replicates; ns= non-significant; *= significant at P<0.01; compared with control.

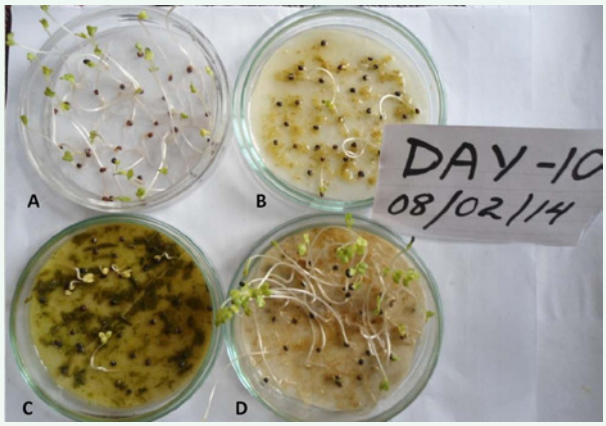

Figure 2 Germination and seedling growth observed on cabbage seeds (after 10 days of inoculation).
A) Control; B) Peel extract; C) Leaf extract and D) Flower bud extract

are in accordance with previous reports which concluded that sometimes seed germination is not inhibited, but the process may be delayed, cotyledon and root size are diminished, or radicle or seedling development is abnormal [4,7,18]. However, lemon peel extract had a higher inhibitory effect on cabbage seedlings and resulted in only 14.3% of seed germination. Microbial growth was also observed in the lemon peel dish. Some white mould covering some ungerminated seeds was seen in peel extract, too. The mould might also have contributed to the inhibition of seed germination. Plants became chlorotic and eventually died in the presence of allelotoxins, burnt root tips and rooting of plantlets were observed in all the test extracts, except flower extract against cabbage seeds in our study.

The positive or stimulatory allelopathic effect was noted in the case of the lemon flower against cabbage seeds. Although germination was delayed by 3-5 days but by day 7, most of the seeds germinated and by day ten the shoot length was evaluated to be greater than that in control. Also, in the case of lemon peel, although the allelochemicals reduced the rate of germination of cabbage seeds but it could not further affect the length of shoots as their shoot length was higher than that of control.

It has been observed that some whitish substance occurred in the control (for cabbage) which is assumed to be either mould or root hair. If it was mould then, it might have been the reason for inhibition of shoot length. Several authors previously concluded that allelopathy and stress interact under natural conditions (Einhellig, 1996; Inderjit & Nishimura, 1999). This implies that the results of an experiment designed to investigate allelopathic activity are likely to be strongly influenced by the test conditions. Under laboratory conditions, which is typically less stressful than field conditions, the allelopathic effect might be reduced (Romeo & Weidenhamer, 1998). A previous study suggested that the lemon plant contains an allelochemical leptospermone which exerts allelopathic effects on other plants growing nearby the lemon plant (Ambika, 2013). This is the causal factor of the growth response observed.

CONCLUSIONS

Results of the present study suggest that seed germination of both lettuce and cabbage can be inhibited by lemon peel, flower, and leaf extracts. The growth of root and shoot was inhibited in the presence of all the test extracts in the case of lettuce. In the case of cabbage, lemon leaves extract showed inhibitory effect on root and shoot growth. However, the length of cabbage shoots in peel and flower extracts were higher than that of control with reduced root length. It can also be concluded that the leaf extract had the highest inhibitory allelopathic effect on both lettuce and cabbage seeds. So, planting lettuce and cabbage in an area where lemon is grown may not be recommended as the yield of lettuce and cabbage might be reduced. The lemon plant can also be used as an important source of natural herbicide to control weeds in a crop field. However, further confirmatory studies are vital before recommending such practices.

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