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Allelotoxic activity of *Eucalyptus rostrata* Schlecht. on seed germination and seedling growth of *Chenopodium album* L. and *Portulaca oleracea* L.

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Key words: Allelopathy, *Eucalyptus, Chenopodium, Portulaca,* seed germination, seedling growth.

Abstract

Eucalyptus rostrata is known to contain various substances that are allelopathic. The allelopathic effect of leaf (ERLAE) and bark (ERBAE) of *Eucalyptus rostrata* aqueous extracts was evaluated for their potential uses as control on seed germination and seedling growth of two noxious weeds; *Chenopodium album* and *Portulaca oleracea*. The biological activity of the two types of extracts on germination efficiency differs with respect to the type of extract and the recipient species. Assertively, the consequence of the extracts on the two weeds was in the following order: ERLAE > ERBAE and almost, the action was highly effective and significant in *C. album* compared to *P. oleracea*. The reduction in plumule and radical lengths for the two recipient plants in the present study may be attributed to the reduced rate of cell division and cell elongation due to the presence of allelochemicals in the aqueous extracts. In conclusion, the obtained results in the present study may share in solving the problem of heavy use of synthetic herbicides via application and trying of a number of promising medicinal plants rich in allelochemicals such as *E. rostrata*.

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Introduction

Modern conventional agriculture requires a high degree of inputs and technology. The herbicidal control of weeds is a costly affair and also deteriorates the quality of soil, water, animal and human health, and food (Doran and Safley, 1997; Narwal, 2006). The excessive use of herbicides can also, in some circumstances, lead to the development of resistance to synthetic chemicals and an increase in the number of herbicide-resistant weeds (Duke *et al*., 2002; Bhowmik and Inderjit, 2003; Batish *et al*., 2007). Therefore, economic and environmental constraints of crop production systems have stimulated interest in alternative weed management strategies (Anaya, 1999; Mortensen *et al*., 2000; Singh *et al*., 2001).

Allelopathy is an important mechanism of plant interference mediated by the additional phytotoxins to the environment; chemicals with allelopathic potential are present in virtually all plants as in most tissues. Under appropriate conditions, these chemicals may be released into the environment, in sufficient quantities to affect neighboring plants (Tahir, 2011). Thus, allelopathy interactions between plants and other organisms may become an alternative to synthetic herbicides and other pesticides (Razavi, 2011). Allelopathy plays an important role in agroecosystems and offers the potential for selective biological weed management by the production and release of allelochemicals from leaves, flowers, seeds and roots of living or decomposing plant materials (Weston, 1996).

Agroforestry is the integration of trees and shrubs into farming landscapes to increase the farm productivity and sustainability of farming systems (Fikreyesus *et al*., 2011). In Egypt, *Eucalyptus* is commonly cultivated tree species widely used as shelterbelts for irrigated agricultural lands in the north western region. El-Darier (2002) reported that large area of ground surface beneath of eucalyptus remains bare and is limited understory vegetation growth. The leaves of *E. rostrata* has shown allelopathic activity representative of a wide variety of plants capable of establishing gradients of toxicity in an otherwise uniform environment. Such gradients drastically alter the species composition and thus are highly important to the study of vegetative composition. (Inouye *et al*., 2001). The allelopathic compatibility of crops with trees should be checked that trees have not harmful effects on associated crops. It has been shown that *Eucalyptus* species have strong allelopathic activity among several examined plants (Gliessman, 2007).

The crops near the *eucalyptus* often suffer from disturbances on the morphological, physiological, and biochemical levels due to the suppressive effects caused by the leached organic matter; mainly phenolics that enter the soil (El-Darier, 2002). Such inhibition may be due to the severe effects of allelochemicals on the rate of water uptake and subsequent transpiration capacity which are confirmed by welting of watermelon individuals in the field or by altering its metabolism and mobilization of storage compounds during germination (El-Darier and Youssef, 2004).

The aim of this experiment was to determine the allelopathic effect of *Eucalyptus rostrata* aqueous *extract* obtained from the leaves and bark and their potential uses as control on weed seed germination and seedling growth of two weeds; *Chenopodium album* and *Portulaca oleracea*.

Materials and methods

The study work was achieved during year 2013. One medicinal plant species (*Eucalyptus rostrata* Schlecht.; donor species) was used in this investigation to study its allelotoxic potential on seed germination, and some growth parameters of two weeds (*Chenopodium album* L. and *Portulaca oleracea* L.) dominant in *Zea mays* L. fields.

Plant Materials

Leaves and bark of the donor species have been collected from El-Behira governorate 50 km south of Alexandria city during the vegetative stage**.** The plant materials were dried in shade then ground in a Wiley Mill to coarse uniform texture and stored in glass jars

until use. Seeds of the two weeds and crop species were purchased from the International Research Center, El-Dokki, Giza, Egypt.

Preparation of Donor Species Aqueous Extract

Dried powder of leaves and bark of *Eucalyptus rostrata* (50 g for each) were extracted with 300 ml distilled water. The extract was conducted in dark for 24 h at 25°C. The supernatant was taken and centrifuged at 3000rpm for 15 minutes; this would be full strength concentration (100%). The extracts were prepared no more than 48 h in advance and were kept in a refrigerator at 5°C until used and the purified extract was adjusted to pH 6.8 with 1M HCl. Series of dilutions were prepared from the stock solution (5, 10, 20 and 40% besides the control) and were tested for their effects on germination parameters, and seedling growth of *Chenopodium album* L. and *Portulaca oleracea* L.

Germination Bioassay

Petri-dish experiment was applied to investigate the potential allelotoxic effects of the target species aqueous extract on germination percentage (GP), inhibition percentage (IP), seed germination index (SGI), energy of germination (GE) and plumule (PL) and radical (RL) lengths of the two weedy species. Twenty seeds of each weed were arranged in 9-cm diameter Petri-dishes lined with two discs of Whatman No.1 filter paper under normal laboratory conditions with day temperature ranging from 19-22°C and night temperature from 12-14°C. 10 ml of the respective donor species aqueous extract (5, 10, 20 and 40) or distilled water as control were added daily to three replicates in a randomized complete block design. Before sowing, the seeds were immersed in 2% CHLOREX for 2 minutes then rinsed four times with distilled water. Finally, the seeds were soaked in aerated distilled water for 24 hours.

Germination percentages and PL and RL lengths were recorded every day for seven successive days.

Calculations

1. Inhibition percentage (IP) of the donor species extract was expressed as a percentage of growth inhibition of the test species in different concentration levels with respect to water control. Higher values indicate lower toxicity (Cayuela *et al.*, 2007).

Inhibition percentage (IP) = [1- (allelopathic/control) 100]

2. Seed germination index (SGI) was calculated according to the following equation (Scoot *et al.*, 1984).

$$SGI = \Sigma Ti\ Ni/S$$

Where,

Ti= is the number of days after sowing

Ni= is the number of seeds germinated on day i

S= is the total number of seeds planted

3. Energy of germination (GE**)** was recorded according to Farooq *et al.* (2005) at the 4th day after sowing. It is the percentage of germinating seeds (GP) four days after sowing relative to the total number of seeds tested (TNST).

$$GE = GP\ (4^{th}\ day)/TNST$$

Statistical Analysis

All the data of the present study were subjected to standard one-way analysis of variance (ANOVA) and student's t-test (t-value < 0.05 was considered as significant) using the COSTAT 2.00 statistical analysis software manufactured by CoHort Software Company (Zar, 1984).

Results

The germination percentage (GP) of *Chenopodium album* and *Portulaca oleracea* was considerably decreasing with increasing the concentrations of *Eucalyptus rostrata* leaf (ERLAE) and bark (ERBAE) aqueous extracts. The percentage decreased from 95% at the control to 55 and 65% for *C. album* and to 50 and 70% for *P. oleracea* at 40 % concentration level for ERLAE and ERBAE, respectively after seven days from sowing (Figure 1). It is worth to mention that the effect of ERLAE was higher than that exhibited by ERBAE. However, data indicated that the two types of extracts exerted a significant strong suppressive effect

on the germination of the seeds of the two recipient species.

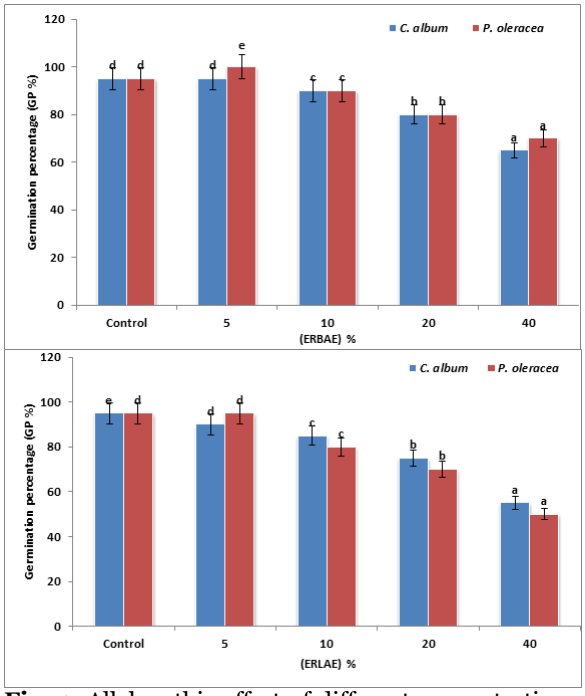

Fig. 1. Allelopathic effect of different concentrations of Eucalyptus rostrata leaves (ERLAE) and bark (ERBAE) aqueous extracts on germination percentage of Chenopodium album and Portulaca oleracea in germination bioassay (data are the means of three replicates).

*P. value (between ERLAE and ERBAE at the seventh day) for C. album 0.0171 and P.oleracea 0.0267.

*P-value was considered a significant if it ≤ 0.05 probability level according to paired t-test.

Different letters within each column for the two types of extract indicate a significant difference at probability level ≤ 0.05 according to ONE-WAY ANOVA

High significant correlations were calculated from simple linear regression obtained by plotting seed germination percentage of the test species versus the different concentrations of ERLAE and ERBAE (Figure 4) as evidenced by the high values of the coefficient of determination (R^2), which found to be 1.0 and 0.994 for *C. album* and 0.92 and 0.954 for *P. oleracea*, respectively.

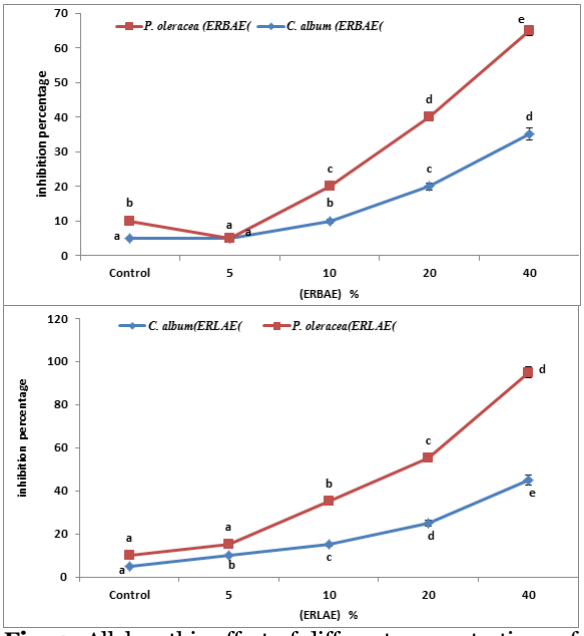

Fig. 2. Allelopathic effect of different concentrations of Eucalyptus rostrata leaves (ERLAE) and Bark (ERBAE) aqueous extracts on inhibition percentage (IP) of Chenopodium album and Portulaca oleracea in germination bioassay (data are the means of three replicates).

*P. value (between ERLAE and ERBAE at the seventh day) for C. album 0.0171 and P.oleracea 0.0267.

*P-value was considered a significant if it ≤ 0.05 probability level according to paired t-test.

Different letters for each line for the two types of extract indicate a significant difference at probability level ≤ 0.05 according to ONE-WAY ANOVA

Seed germination index (SGI) and energy of germination (GE) are illustrated in (Table 1). The value of SGI moderately decreased as ERLAE and ERBAE concentration increased. This reduction was statistically ($p \leq 0.05$) significant as evaluated by ANOVA test. At control level, a value of about 20.3 was decreased to 10.2 and 12.6 for *C. album* and from 18.5 to 9.3 and 12.1 for *P. oleracea* at 40% concentration level for both types of extracts, respectively. Concerning GE, the value decreased extensively as ERLAE and ERBAE concentration increased. This reduction was statistically ($p \leq 0.05$) significant. GE started with a value of about 0.7 at control level which decreased to 0.2 and 0.4 for *C. album* and from 0.6 to 0.2 and 0.2 for *P. oleracea* at 40% concentration level for the two extracts, respectively.

Table 1. Allelopathic effect of different concentrations of Eucalyptus rostrata leaves (ERLAE) and bark (ERBAE) aqueous extracts on seed germination index (SGI) and energy of germination (GE) of Chenopodium album and Portulaca oleracea (weed species) in germination bioassay (data are the means of three replicates).

	C.album	***P. oleracea***	***C.album***	***P.oleracea***
	SGI		**GE**	
***Eucalyptus rostrata* leaves aqueous extract (ERLAE)**				
Control	20.3^{e}	18.5^{e}	0.7^{a}	0.6^{a}
5	19.0^{d}	17.5^{d}	0.6^{a}	0.5^{a}
10	16.1^{c}	13.8^{c}	0.4^{a}	0.4^{a}
20	13.9^{b}	11.7^{b}	0.3^{a}	0.2^{a}
40	10.2^{a}	9.3^{a}	0.2^{a}	0.2^{a}
***Eucalyptus rostrata* bark aqueous extract (ERBAE)**				
Control	20.3^{e}	18.5^{e}	0.7^{a}	0.6^{a}
5	20.1^{d}	18.5^{d}	0.7^{a}	0.6^{a}
10	19.2^{c}	16.1^{c}	0.6^{a}	0.5^{a}
20	16.3^{b}	13.6^{b}	0.5^{a}	0.3^{a}
40	12.6^{a}	12.1^{a}	0.4^{a}	0.2^{a}
P. value*	**0.0155**	**0.0158**	**0.0120**	**0.0124**

*P-value was considered a significant if it ≤ 0.05 probability level according to paired t-test.

Different letters within each column for the two types of extract indicate a significant difference at probability level ≤ 0.05 according to ONE-WAY ANOVA

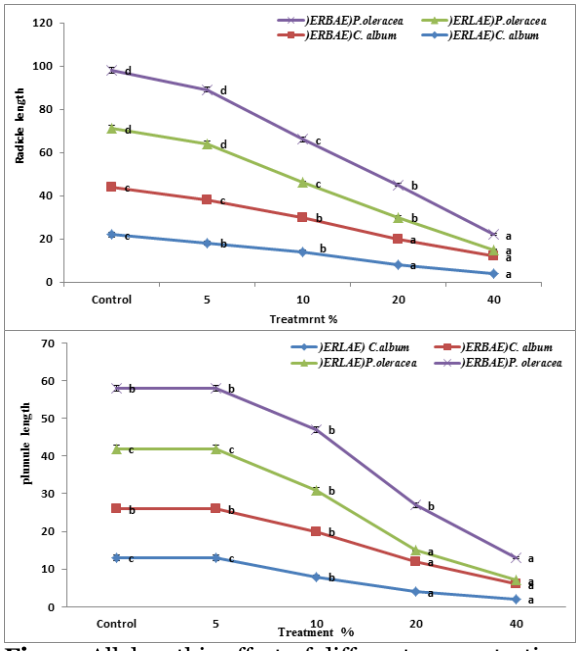

Fig. 3. Allelopathic effect of different concentrations of Eucalyptus rostrata leaves (ERLAE) and bark (ERBAE) aqueous extracts on plumule length (PL) (cm) and radicle length (RL) (cm) of Chenopodium album and Portulaca oleracea (weed species) in germination bioassay (data are the means of three replicates).

*P. value (between ERLAE and ERBAE at the seventh day) = 0.0069 and P.oleracea 0.0398

*P-value was considered a significant if it ≤ 0.05 probability level according to paired t-test.

Different letters for each line for the two types of extract indicate a significant difference at probability level ≤ 0.05 according to ONE-WAY ANOVA

The allelopathic potential of the different concentrations of ERLAE and ERBAE on plumule length (PL) of the two test species is illustrated in (Figure 3). Generally, all concentrations of ERLAE and ERBAE reduced PL. The length was visibly reduced from 13 cm for control to 2 and 4 cm for *C. album* and from 16 cm for control to 1 and 6 cm for *P. oleracea* for the two types of extracts at 40% concentration respectively after seven days from the beginning of the experiment. The regression lines between PL and the different concentrations of the

two types of extracts confirmed the different effects of the different types of extracts for the two recipient species (Figure 5 and 6).

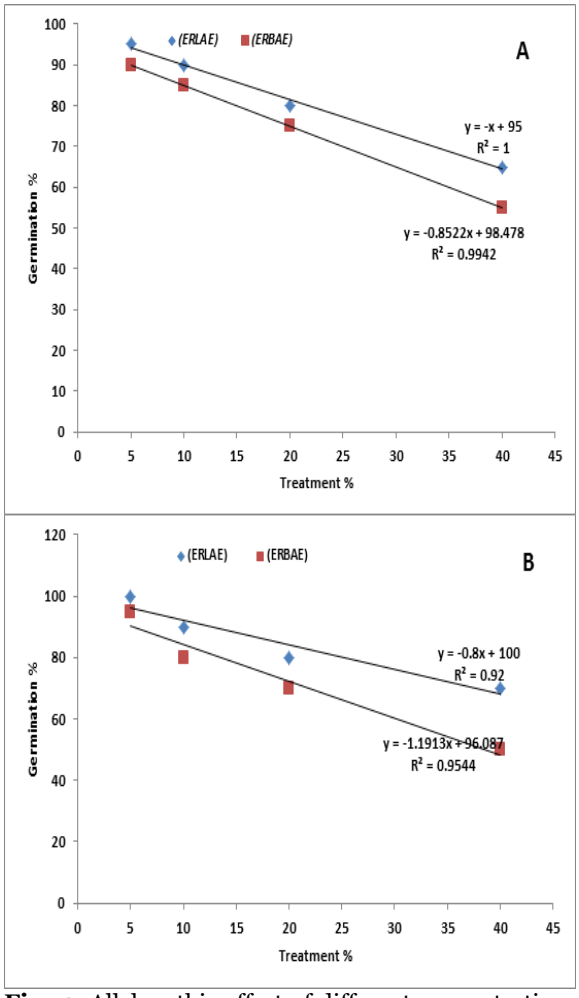

Fig. 4. Allelopathic effect of different concentrations of Eucalyptus rostrata leaves (ERLAE) and bark (ERBAE) aqueous extracts on germination percentage of Chenopodium album (A) and Portulaca oleracea (B) in germination bioassay (data are the means of three replicates).

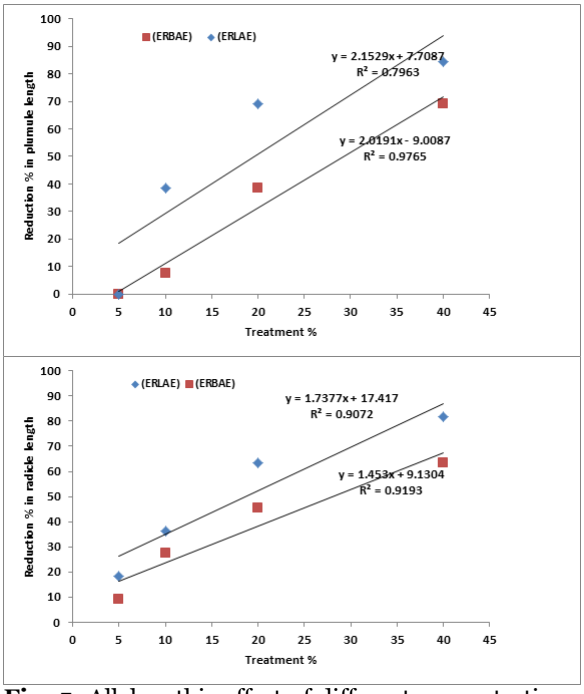

Fig. 5. Allelopathic effect of different concentrations of Eucalyptus rostrata leaves (ERLAE) and bark (ERBAE) aqueous extracts on the reduction percentage (relative to control) in plumule length (PL) (cm) and radicle length (RL) (cm) of Chenopodium album in germination bioassay (data are the means of three replicates).

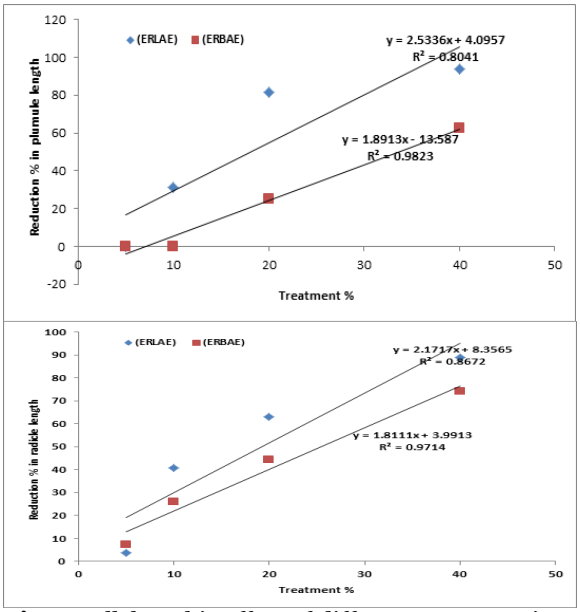

Fig. 6. Allelopathic effect of different concentrations of Eucalyptus rostrata leaves (ERLAE) and bark (ERBAE) aqueous extracts on the reduction percentage (relative to control) in plumule length (PL) (cm) and radicle length (RL) (cm) of Portulaca oleracea (weed species) in germination bioassay (data are the means of three replicates).

The corresponding allelopathic effects of ERLAE and ERBAE on the radical length (RL) of the two recipient species were illustrated in Figure 3. Data demonstrated that the RL was significantly decreasing upon applying different concentrations of the two types of extracts. The length decreased from 22 cm at control to 4 and 8 cm for *C. album* and from 27 cm at control to 3 and 7 cm for *P. oleracea* at 40% concentration level for the types of extracts respectively. Figure 5 and Figure 6 confirmed the different effects of the different types of extracts on RL through the application of the regression analysis resulting in high values (0.907 and 0.919 for *C. album* and 0.867and 0.971 for *P. oleracea*) of the coefficient of determination (R^2).

Discussion

Weeds are one of major constraints to plant production worldwide. Weeds affect crop growth and production that may be significantly reduced when weeds compete with them for light, water and minerals (Hussein, 2001). Existing weed control methods are either expensive or hazardous. Although herbicides available in the market may offer effective control of several weeds but environmental damages occur (Zhu and Li, 2002); herbicide resistance development among weeds (Heap, 2008) and health concerns due to over and misuse of synthetic herbicides (Kudsk and Streibig, 2003) all those has led the researchers to focus on alternative weed management strategies (Jabran *et al.,* 2008). Allelopathy provides a relatively cheaper and environmental friendly weed control alternative. This can be considered as a possible alternative weed management strategy (Cheema *et al*., 2000b). Natural plant products play a variety of physiological roles and thus can serve as a source of novel herbicides (Duke *et al*., 2000). Moreover, the use of allelopathy of the medicinal plants has been suggested as a viable option for alternative weed management under sustainable agriculture (Fujii *et al.,* 2003; Hong *et al.,* 2003; Batish *et al.,* 2007; Zeng *et al.,* 2009; Salhi *et al.* 2011and 2014; El-Darier *et al*., 2014).

Water-soluble toxins in the litter of *Eucalyptus rostrata* inhibited herbaceous growth in the laboratory, greenhouse and field (Moral and Muller, 1970). The oil quaintly hydrodistillated from *E. rostrata* ranged from 0.63 % up to 1.59% depending on season and location (Grbović *et al.,* 2010). Fadel *et al.* (1999) reported that *E. rostrata* essential oils from Mali, Mozambique, Nigeria, Egypt and Iran contain 1,8-cineole as the main compound. Antioxidant activity of an extract from the leaves of *Eucalyptus rostrata* was the highest among 16 *Eucalyptus* species (Okamura *et al.,* 1993).

The present study investigates the action of leaf (ERLAE) and bark (ERBAE) extracts of *E. rostrata* on some germination parameters and seedling growth of *Chenopodium album* and *Portulaca oleracea* under laboratory conditions. The biological activity of the two types of extracts differs with respect to the type of extract and the recipient species. Confidently, the consequence of the extracts on the two weeds was in the following order: ERLAE > ERBAE and almost, the action were highly effective and significant in *C. album* compared to *P. oleracea*. Inhibiting emergence and growth of rice plant via application of nettle leaf and goose foot aqueous extracts may be attributed to the disturbance in the activities of peroxidase, alpha-amylase and acid phosphates (Alam and Islam, 2002). Caution should be taken regarding the ecological implications of the data, because phytotoxicity of the different types of extracts is influenced by biotic and abiotic factors. Previously, Moral and Muller (1970) reported that caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid and gallic acid extracted from *E. rostrata* does not inhibit annual herbs on sand soil rather than poorly drained, shallow, and high in colloidal content, where toxin concentrations could rise to significant proportions. On the other hand, the aqueous extract and crud powder of *Eucalyptus rostrata* significantly suppressed seed germination of *Vicia faba* and *Zea mays* under different concentrations (El-Darier, 2002).

Ahmad (2012) showed that donor species; *Artemisia monosperma, Peganum harmala* and *Silybum marianum* extracts exhibited allelopathic action on the recipient species *Chenopodium album* as weed species and *Triticum aestivum* as crop species. The aqueous extract of all donor species have an inhibitory effect on the diurnal germination percentage of *C. album* seeds. Additionally, reserve mobilization, seems to be delayed or decreased under allelopathy stress conditions (Ahmad *et al*., 2011). Similar results were obtained by El-Darier and Zein El-Dien (2011) on *Lycopersicon esclentum* and El-Darier *et al*. (2014) on *Vicia faba* seeds. They found a gradual increase of inhibition percentage of the two crop species as a response to the higher concentration levels of *Medicago sativa* aqueous extract. Maharjan *et al*. (2007) found that seed germination of *Raphanus sativus, Brassica campestris* and *Brassica oleracea* was completely inhibited at >2% leaf extract of *Parthenium hysterophorus*. To go through with this, Assaeed (2003) reported that the aqueous extracts prepared from the leaves and inflorescence of *Artemisia monosperma* negatively affected seed germination of some species of sandy habitat in a bioassay experiment. Gholami *et al*. (2012) reported that the highest germination percentage (GP) of *Portulaca oleraceae* and *Chenopodium album* were occurred in control treatment and GP were decreased when the increase in extract concentrations of *Crocus sativus* and *Peganum harmala*. *C. album* was more sensitive to application of plant extracts than *P. oleraceae*. The same was obtained by Tanveer *et al*. (2010) on the effect of *Euphorbia helioscopia* aqueous extract on seed germination of *Triticum aestivum, Cicer arietinum*, and *Lens culinaris*.

In the present study, at high concentration of the extracts (40%) the germination energy (GE) decreased by a percentage of about 28% and 56% relative to control for ERLAE and ERBAE, respectively for *C. album* and by 33% for both types of the extracts with respect to *P. oleracea*. This could occur only when some allelochemicals present in the extract prevented growth of the embryo, or caused death. Jankowska *et al*. (2009) found that the germination energy of *Lolium westerwoldicum* seeds was inhibited as response to the high concentration level of *Taraxacum officinale* extract. Additionally, the extract of *Parthenium hysterophorus* induced a variety of chromosomal aberrations in dividing cells, which increased significantly with increasing concentrations and durations of exposure (Rajendiran, 2005).

The reduction in plumule and radicle lengths for the two recipient plants in the present study may be attributed to the reduced rate of cell division and cell elongation due to the presence of allelochemicals in the aqueous extracts (Javaid and Anjum, 2006). Khan *et al*. (2008) revealed that aqueous extract of *E. camaldulensis* at various concentration levels inhibited the germination, reduced fresh weights and dry weights of wheat seedlings. Its effectiveness suggests that its leaves may act as a source of allelochemicals after being released into soil or after decomposition.

Several studies had shown that compounds of plant origin, such as allelochemicals, affect mitotic activity of growing roots (Rizvi *et al*., 1992; Einhellig, 1996). Such an inhibitory effect on mitotic may directly decrease plant growth, and so mitotic activity can be used to evaluate root growth resulting from cell division of meristematic cells and cell expansion in the elongation zone of roots (Dayan *et al*., 2000). Abu Romman *et al*. (2010) reported that the allelopathic effect of *Euphorbia hierosolymitana* reduced germination percentages, radical and coleoptile growth of wheat seedlings. Similarly, Bhatt *et al*. (1994) asserted that the bark and leaf extract of *Quercus glauca* and *Q. leucotricophora* significantly reduced germination, plumule and radical length of wheat.

In conclusion, the obtained results in the present study may share in solving the problem of heavy use of synthetic herbicides via application and trying of a number of promising medicinal plants rich in allelochemicals such as *E. rostrata*.

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