ASSESSMENT OF ALLELOPATHIC EFFECTS OF EUCALYPTUS (*Eucalyptus Camaldulensis* L.) PLANT PARTS ON SEED GERMINATION AND SEEDLING GROWTH OF WHEAT (*Triticum aestivum* L.)

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Abstract: *The study was conducted to investigate the potential allelopathic effect of Eucalyptus camaldulensis leaf, bark and fruit aqueous extract on germination of Triticum aestavium in Petri-dish experiments. In addition, the allelopathic effect was demonstrated on growth and some metabolic changes of T. aestavium L. seedlings grown in a pot experiment. The results suggested that E. camaldulensis leaf, bark and fruit aqueous extract appeared to have inhibitory allelopathic effect on the recipient species compared to control. The germination percentage and growth parameters of T. aestavium were significantly reduced gradually with the increase of aqueous extract concentration levels. However the reduction was varied and could be parts of the donor species and concentration dependent.Results also revealed the highest inhibitory effect on seedling growth was exhibited by E. camaldulensis at 70% concentration. Furthermore, the growth parameters, photosynthetic pigment contents, total carbohydrate, crude protein content (%) and nitrogen content (%) of T. aestavium seedlings were significantly decreased with the increase of aqueous extract concentration levels. Among the three parts of the donor species the leaf aqueous extract had highest inhibitory effects on germination and other growth parameters. Additionally, the highest values of total phenolics content were found in E. camaldulensis leaf. These findings indicate that wheat sown in fields which had leaf, bark and fruit litter of E. camaldulensis will be adversely affected regarding germination, growth and ultimately resulting in lower yields of wheat.*

***Keywords**: Eucalyptus camaldulensis, Triticum aestavium , allelopathy , seed germination.*

1. INTRODUCTION

Allelopathy is a natural ecological phenomenon in which different organisms affect the functioning of other organisms in their vicinity, negatively or positively by releasing secondary metabolites [1]. The main principle in allelopathy arises from the fact that plants produce thousands of chemicals; and many of these chemicals are released by leaching, exudation, or decomposition processes. Subsequently, some of these compounds which are known as allelochemicals alter the growth or physiological functions of receiving species [2]. Plants or organisms that release these compounds are called 'donor species', while those that are influenced in their growth and development are called 'target or recipient species'. Research conducted in the last half of the twentieth century demonstrated growth inhibition by allelochemicals that influenced vegetation patterns, rate and sequences in plant succession, weed abundance, crop productivity, and problems in replanting fruit and other crops [3]. In the past two decades, much more work has been done on plant derived compounds as environmentally safe alternatives to herbicides for the weed control [4]. In this regard, the use of crops having allelopathic properties can reduce the dependency on synthetic herbicides and increase crop yields [5]. Eucalyptus belongs to the family Myrtaceae. It is widely planted evergreen genra grow under a wide range of climatic and edaphic conditions in their natural habitats [6]. It is a large perennial woody tree having distinctive glaucoushue and its one of the most important plants used to prevent soil erosion in farmland as windbreak. Mainly it is cultivated for its timber, fuel and paper pulp, but it has also wide range of medicinal properties, and it is a source of essential oil used in medicines and perfumes. Its oil has pesticidal, nemticidal and insecticidal activity [7]. Many studies have evaluated the allelopathic effects of *Eucalyptus* species and confirmed the strong inhibitory effects of eucalyptus extracts on some crops [8]. It was reported that where *Eucalyptus* stand is replaced by the agricultural crop, that crop will not grow well, at least for a number of years [9]. The allelopathic effect of *Eucalyptus* spp. has been attributed to the production of several volatile terpenes and phenolic acids. Phytotoxic phenolic substances in leaf extracts of *E. globulus* produce volatile terpenoids that have inhibitory effects on germination and seedling growth of various crops [10]. Phenolic acids and volatile oils released from the leaves, bark, and roots of certain *Eucalyptus* spp. have deleterious effects on other plant species [11]. Many studies have evaluated the allelopathic effects of *Eucalyptus* spp. and confirmed the strong inhibitory effects of *Eucalyptus* extracts on some crops

[8]. In this study we investigated the allelopathic effect of *Eucalyptus camaldulensis* leaf, bark and fruit aqueous extract on germination, growth and some metabolic products of *Triticum aestavium* seedlings.

2. MATERIALS AND METHODS

2.1. Plant sampling and preparation of extracts

The Fresh samples of leaves and dry samples of bark and fruits were collected from the *E. camaldulensis* L. trees growing beside the road in the University of Omar al-mukhtar university, Bayda. Libya. during the 2013. The collected samples were washed and dried in the oven at 70^0C for 72 hr and then powdered. Different concentrations of aqueous extract (5, 10, 20 and 40% w/v) were prepared at room temperature (20 ±2ºC) for 24 hours. The mixture was filtered through four layers of cheesecloth and kept at 5 ºC in the dark until use, in addition to the control (distilled water).

2.2. Petri Dishes Experiments

Twenty five seeds of the recipient species (*T. aestivum* L. Local variety) were arranged in 9-cm diameter Petri-dishes on two discs of Whatman No.1 filter paper under normal laboratory conditions with day temperature ranging from 20-23ºC and night temperature from 14-16ºC. Five milliliters of each level of the donor species aqueous extract concentrations were added to each replicates. Before sowing, the seeds were surface sterilized by soaking for two minutes in 5% sodium hypochlorite, then, thoroughly washed with tap water for several times followed by distilled water. Treatments were arranged in a completely randomized design with four replications. Seeds were considered germinated when radicle length was 2 mm and the number of germinated seeds was recorded daily, while germination percentage, radicle, plumule length and the dry weight of seedlings were recorded after 7 days at the end of the experiment.

2.3. Pot Experiments

Pot experiments were conducted to study the Allelopathic effect of *E. camaldulensis* leaf, bark and fruit aqueous extracts (70%) on growth and some metabolic products of wheat seedlings. Five seeds of the recipient species were sown in plastic pots (15 cm in diameter) with about 600 g clay soil. The pots were kept in the greenhouse conditions, and when the seedlings were about 12 cm in length, they were thinned down to three per pot. Pots were arranged in two groups, seedlings of the first group were irrigated with *E. camaldulensis* leaf, bark and fruit aqueous extract (70%), and the other group was irrigated with tap water as an absolute control treatment. Pots kept near the field capacity. Pots were arranged in a complete randomized design with 3 replicates for each treatment. After 50 days of sowing plants were harvested for further analysis. At the end of the pot experiment shoot and root length, and dry matter yields of the shoots and root of (*T. aestivum*) seedlings were determined. Leaf area was determined according to [12]. The photosynthetic pigments were determined by spectrophotometric method as described by [13], The total carbohydrates were determined by the method of anthrone sulphoric acid which was carried out by[14], The crude protein content were determined by the method as described by [15] and nitrogen content was estimated spectrophotometrically using the Nessler's method as described by [16]. The concentration of phenolics in plant extracts was determined using spectrophotometric method [17].

2.4. Statistical analysis

The experimental results were subjected to one way-analysis of variance and the means were separated by the least significant difference, LSD, using Co-stat program.

3. RESULTS AND DISCUSSION

The allelopathic effects of the leaf, bark and fruit aqueous extract of *Eucalyptus camaldulensis* on the germination percentage (GP), plumule and radicle length and seedling dry weight of *Triticum aestavium* are represented in Tables 1, 2 and 3 respectively. The data demonstrated that the GP and the growth parameters of the recipient species was significantly affected by applying the different concentrations of the leaf bark and fruit aqueous extract of *Eucalyptus camaldulensis*. Commonly, GP and the growth parameters decreased with the increase in treatment concentrations. Compared to control, reduction increased as the concentration of the donor species increased. The reduction was varied and was parts of the donor species dependent. 40% concentration level had the greatest effect (Tables 1, 2 and 3). It had been demonstrated that the main mechanism of allelochemicals action was the inhibition of specific enzyme activities [18]. Hydrolytic enzymes seemed to be one of the most sensitive enzymes to allelopathy stress, which may decrease activity, and hence delay seed germination. Germination of seeds depended on amylase activity that regulated starch breakdown which was necessary for supplying substrates to respiratory metabolism and the release of energy [19]. Additionally, the inhibition is probably due to the presence of phenols in the root of the *Eucalyptus camaldulensis*. Phenols have been reported to interfere with the activities of respiratory enzymes in seed germination, thereby causing inhibitory effect on its germination [20]. It has been suggested by [21] (2010) that alteration in the synthesis or activities of gibberellic acid in the seed could be due to the presence of phenolic compounds. Similar observations had been reported using several donor species, such as *Eucalyptus globules* [22]. It was reported that the aqueous extracts strongly suppressed germination and seedling growth. Moreover, roots were more sensitive to the allelochemicals than shoots and the response to phytotoxins was concentration-dependent [23]. Furthermore, allelochemicals could inhibit the elongation, expansion and division of cells which were a prerequisite for seedling growth [24]. Similar findings were also reported by [25] (2004) who found that eucalypt oil significantly reduce the seed germination, the seedling growth, as well as the chlorophyll content and the cellular respiration of the tested plants. Among the three parts of the

Table (1): Allelopathic effect of *E. camaldulensis* leaf aqueous extract on germination percentage and some growth parameters of *Triticum aestavium* L.

Conc.	G%	plumule length (cm)	Radical length (cm)	Seedling fresh wt. (mg/seedlings)	Seedling dry wt. (mg/seedlings)
Control	100	4.9	5.5	2.1	1.0
5%	78	3.3	4.8	1.7	0.9
10%	70	2.6	3.6	1.6	0.8
20%	44	1.8	1.6	1.4	0.7
40%	18	0.1	0.1	1.2	0.6
L.S.D 5%	--	0.7	0.7	0.6	0.1

Table (2): Allelopathic effect of *E. camaldulensis* bark aqueous extract on germination percentage and some growth parameters of *Triticum aestavium* L.

Conc.	G%	Plumule length (cm)	Radical length (cm)	Seedling fresh wt. (mg/seedlings)	Seedling dry wt. (mg/seedlings)
Control	100	9.7	9.8	6.4	0.7
5%	88	8.5	8.4	6.2	06
10%	71	5.8	8.0	5.6	0.5
20%	71	4.3	4.8	4.4	0.4
40%	57	1.1	3.4	2.2	0.3
L.S.D 5%	--	1.1	1.0	0.1	0.1

Table (3): Allelopathic effect of *E. camaldulensis* fruit aqueous extract on germination percentage and some growth parameters of *Triticum aestavium* L.

Conc.	G%	plumule length (cm)	Radical length (cm)	Seedling fresh wt. (mg/seedlings)	Seedling dry wt. (mg/seedlings)
Control	100	10.8	11.8	3.8	0.8
5%	87	7.3	6.6	3.4	0.6
10%	85	2.7	3.7	2.5	0.5
20%	54	1.7	2.3	1.9	0.4
40%	26	0.4	1.2	1.2	0.3
L.S.D 5%	--	0.6	0.7	0.2	0.01

donor species the leaf aqueous extract had highest inhibitory effects on germination and other growth parameters; that was followed by fruit aqueous extract. Least effect was shown by bark aqueous extract on the recipient species. Highest inhibitory effect on seedling growth was exhibited by *Eucalyptus camaldulensis* at 40% concentration; that was followed by 20% concentration (Tables1, 2 and 3). Under pot experiment conditions, Data presented in Table 4 show that the extract application decreased the growth parameters of wheat seedlings as compared with the control. The reductions in seedlings root and shoot length, leaf-area and dry weight may be attributed to the reduced rate of cell division and cell elongation due to the presence of allelochemicals in the donor species aqueous extract [26]. Several studies had shown that compounds of plant origin, such as allelochemicals, affected mitotic activity of the growing roots [27]. The content of the photosynthetic pigments (Chl. a, Chl. b and Carotenoids) of wheat seedlings treated *Eucalyptus camaldulensis* leaf, bark and fruit aqueous extract was decreased as compared with the control (Table 5). This reduction was more prominent in chlorophyll b accumulation for the different aqueous extracts (Table 5). The decrease in photosynthetic pigment contents of the studied recipient species may be related to the inhibitory effect of the released allelochemical substances from the donor species on the synthesis of the pigments and/or the structure of chloroplasts. [25] (2004) concluded that the decrease in photosynthetic pigments was attributed to the modification in the integrity of chloroplast and thylakoid membranes in response to allelochemicals. Generally, nitrogen content (%), total carbohydrates and crude protein content of wheat seedlings significantly decreased due to the apparent allelopathic action of *Eucalyptus camaldulensis* leaf, bark and fruit aqueous extract compared the control (Table 6). The decrease in total carbohydrates content of the recipient species, under this study, may be attributed to the inhibitory effect of the released allelochemical substances (from the donor species) on the synthesis of photosynthetic pigment, and hence, on photosynthetic activity. [28] (2004) reported that the decrease of total carbohydrates content of (*Sinapis alba*) under allelochemicals stress may be related to the reduction of the photosynthetic activity, leaf area and the stomatal frequency, as well as, the inhibitory effect on the photosynthetic apparatus which may result in a decrease of CO_2 diffusion and assimilation. In addition, Inhibition of photosynthesis and other impaired metabolic activities may be resulted in a decrease of protein synthesis and/or the stimulation of the protein degradation. Therefore, the decline in total protein content may be related to the enhancement of protein degradation, and/or alteration in the incorporation of amino acids into protein, hence, reduce the growth of target species. It had been demonstrated that allelopathic substances stress generally induced a marked decrease in total protein content in several target plant species, including *Lactua sativa* [29] and *Triticum durum* [30].

Table (4): Allelopathic effect of *E. camaldulensis* leaf, bark and fruit extracts on shoot, root length, leaf-area and dry weight of *Triticum aestavium* L.

Treatments	Shoot length cm/pot	Root length cm/pot	Leaf area cm^2	Dry weight (g/pot)	
				Shoot	Root
Control	39.3	35.1	28.0	1.2	0.5
Leaf extract	25.3	17.5	23.0	0.6	0.4
Bark extract	27.5	24.3	25.5	0.5	0.2
Fruit extract	27.1	25.5	17.5	0.4	0.3
L.S.D. 5%	1.2	1.0	1.3	0.4	0.06

Table (5): Allelopathic effect of *E. camaldulensis* leaf, bark and fruit extracts on chlorophyll (a), (b) and carotenoids contents of *Triticum aestavium* L.

Treatments	Chlorophyll (a) mg/g	Chlorophyll (b) mg/g	Carotenoid mg/g
Control	2.5	2.7	2.4
Leaf extract	1.3	1.1	1.0
Bark extract	2.2	2.3	2.0
Fruit extract	1.9	1.2	1.3
L.S.D. 5%	0.6	0.05	0.4

Table (6): Allelopathic effect of *E. camaldulensis* leaf, bark and fruit extracts on shoot and root total carbohydrates, nitrogen content (%) and crude protein content (%)of *Triticum aestavium* L.

Treatments	Total carbohydrate (mg/g)		N%		Crude protein content (%)	
	Shoot	Root	Shoot	Root	Shoot	Root
Control	123.0	132.0	1.2	1.4	7.5	9.4
Leaf extract	41.0	61.0	0.5	0.6	3.8	3.4
Bark extract	84.0	97.0	0.8	1.0	5.0	5.3
Fruit extract	75.0	81.0	0.7	0.8	4.4	5.0
L.S.D.5%	1.9	0.7	-	-	-	-

In the present study, the estimation of total phenolic compounds content in the *E. camaldulensis* parts demonstrated that the highest values of total phenolics content were found in *Eucalyptus camaldulensis* leaf, decreaseing in the fruit, whereas the lowest were detected in the bark (Table 7). Some specific effects of phenolic compounds implicated in allelopathic interactions include inhibition of cell division (coumarin and many alkaloids), modification of cell wall construction (phytohormons and their balance), membrane permeability and function, modification of active transport, inhibition of specific enzymes, germination of pollen, spores and seeds, stomatal movement, pigment synthesis, photosynthesis, respiration (many phenolics and flavonoids), protein synthesis (many phenolics and alkaloids), N_2 fixation, inhibition of nitrifying bacteria, N_2 fixing bacteria, plant water relations, modification of DNA and RNA and complexities of nutrients, allelochemicals may be selective in their actions, or plants may be selective in their response [31].

Table (7): Total phenolic compounds content estimated in the *E. camaldulensis* part

Donor plant part	%wt, dry weight
Leaf	1.67
Bark	0.59
Fruit	0.87

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