Phytotoxicity of introduced Pinus halepensis Miller trees on physiological responses of native Ceratonia silique L. trees in the Green Mountain area, Libya

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This Thesis was submitted in Partial Fulfillment of the Requirements for Master's Degree of Science in Botany

University of Benghazi

Faculty of Sciences

July 2020

University of Benghazi **Faculty of Sciences**

Department of Botany

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بسم الله الرحمن الرحيم

وأنزل الله عليك الكتاب والحكمة وعلمك

ما لم تكن تعلم وكان فضل الله عليك عظيما

صدق الله العظيم

Acknowledgments

Almighty and thank to Allah who gave me subsistence and strength to complete this work.

I would like to express my sincere thanks and great appreciation to:

Prof. Mariam F. EL-Barghathi for her guidance and support during this work. I would also like to **Dr. Tarek A. Mukassabi** for his valuable assistance and help to fulfill my work.

Last but not least, I would like to express my deepest thanks to all my friends in the graduate studies and technicians in the Departm00ent of Botany and thanks to all people who helped me during this journey.

To the soul of my parents, my sister and brother with my love

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Abstract

This research was conducted at Department of Botany, Faculty of Sciences, University of Benghazi in Libya, to examine the phytotoxicity of introduced *P. halepensis* (Aleppo pine) trees on some physiological measures of native *C. siliqua* (Carob) trees in wadi Alkhoof area at AL-Jabel AL-Akhdar in Libya. Therefore, lab experiments were performed using aqueous extracts of the needles and the bark of *P. halepensis* and the soil rhizosphere around it with different distances (0.0, 2.0, and 4.0) with two depths (10 and 20 cm) of each soil type. Needles, bark, and soil rhizosphere were seasonally collected. Needles and bark were collected in the middle of each season (autumn, winter, spring, and summer. Based on the results of needles and bark extracts, it has been decided to use the above mentioned soil types which collected in winter and summer seasons. Different concentrations (0.0, 25, 50, 75, and 100 g / l) were prepared from the extract of each plant organ (needles and bark) and from each soil type, to evaluate their effects on seed germination and early seedling development of *C. siliqua*. Some parameters were carried out for seed germination and for seedling development. For seed germination tests, daily germination percentages,

promotion and inhibition percentages of seed germination, and rate of seed germination were calculated. For early seedling development experiments, fresh parameters with seedling vigor and tolerance indices, and dry parameters with root / shoot ratio, and moisture content percentages in terms of fresh and dry mass were evaluated. Data of all experiments were statistically analyzed.

Results of seed germination under needles extracts collected in different seasons showed that, daily and final cumulative germination percentages were reduced under aqueous extract of *P. halepensis* needles, of autumn collection, whereas, *C. siliqua* seeds were not affected by bark extract of the same season. Reduced cumulative germination percentages of significant low values under higher concentrations of needles and bark collected in winter, spring, and summer seasons.

Rate of carob seed germination was greatly decreased under all concentrations of needles extract (autumn , winter, spring germination rate of the same target plant species. Seed germination rate of the same seeds was not affected by needles and bark extracts of *P. halepensis* collected in summer season.

For early seedling development, the results are as following:

The inhibitory effect on fresh parameters, were of great reductions for *C. siliqua* seedlings grown under different concentrations of *P. halepensis* needles and bark collected in autumn season. For dry mass measurements lower values were obtained for specific shoot length, specific root length, and no effect on root / shoot ratio and moisture content percentages on the basis of fresh and dry mass.

Fresh and dry mass parameters of *C. siliqua* seedlings were all decreased with increasing needles extract levels collected in winter. In early seedlings developed under the influence of various solutions of aqueous extract of *P. halepensis* bark from winter season, only shoot fresh mass was reduced. Root / shoot ratio and moisture content percentages of *C. siliqua* were not affected by the same media of aqueous extract of *P. halepensis* bark.

Fresh parameters of *C. siliqua* with reduced values of root length, seedling length, and root fresh mas, vigor and seedling tolerance indices. Different concentrations of needle (spring collection) aqueous extract of *P. halepensis* had no inhibitory effects on of specific length (shoot and root), dry mass (shoot and root), and root / shoot ratio and moisture content percentages of *C. siliqua* seedlings. *C. siliqua* seedlings developed under treatment levels of aqueous extract of *P. halepensis* bark collected in spring season, showed reductions in shoot length, seedling length, shoot fresh mass, root fresh mass, seedling vigor index, and seedling tolerance index. Whereas, both dry measures and moisture content percentages on the basis of fresh and dry mass were not affected by exogenous application of various treatments of *P. halepensis* bark collected in same season.

All fresh measures of *C. siliqua* were not affected by all different concentrations of summer needles extract *P. halepensis*. But shoot length and both seedling vigor and tolerance indices of the target plant seedling posess low values. Whereas, bark extract had no effect except, for shoot fresh mass which was reduced under all concentrations of the same extract. Dry mass measures of *C. siliqua* seedlings under the effect of aqueous extract of *P. halepensis* bark of summer collection were not affected.

Furthermore, all types of soil rhizosphere of various distances at different depths collected in different growing seasons (winter and summer) had no effects on seed germination and early seedling development of the native *C. siliqua* plant species. Therefore, for the effects of extracts from different plant organs, it can be concluded that needles extract from autumn, winter, and spring showed more inhibitory effects on seed germination and seedling parameters than that of bark extract.

For seasonal collection, the phytotoxicity exerted by organs collected in winter seasons was more potent compared with other seasons with low potency of summer season.

I. CHAPTER ONE

Introduction

1.1. History of introduced *Pinus halepensis* Miller:

Al-Jabal Al-Akhdar (Green Mountain) region, Libya has the highest species variety and having distinct environmental features associated with evergreen forest along with the Mediterranean basin from the Atlas Mountains to the Levant, and it has an environment similar to other regions in the Greek islands, Southern Europe such as Italy. The number of plant species in Libya is about 2000 species with some species (75) of them that grow only in AL-Jabal AL-Akhdar area. The natural vegetation of AL-Jabal AL-Akhdar region includes most of plant forms that exist in the Mediterranean. The geography of this area includes three classes of different levels of altitude. There is a significant difference in the topography of AL-Jabal AL-Akhdar area which comprises valleys, knolls and plains. These levels differ from each other in their climate. The effect of domestication on the structure of genetic diversity of economically important tree species has received little attention. With varying climate, genetic depletion through plantations and changes in inhabitants' genetic structure can have damaging consequences for the adaptive tree populations to novel environments (Pandey *et al*., 2004).

The genus *Pinus* belongs to the family Pinaceae and includes about 250 species. It is the largest genus of conifers occurring naturally in the northern hemisphere, particularly in the Mediterranean region, Caribbean area, North and Central American, Asia, Europe. The genus *Pinus* has been planted in the temperate regions of the southern Italy. They are evergreen and resinous plants growing to

3-80 m tall with needle-like gray-green leaves that grow in couples. *Pinus halepensis* is the Mediterranean pine species which was first described and classified in 1768 by Phillip Miller. It is believed that, its natural distribution area, is dominantly west-Mediterranean (Trinajstić *et al*., 2011). In France, typically in its Mediterranean part, pine forest areas have also from 360 km² in 1878 to 1050 km² in 1900 while today they encompass more than 2000 km² (Grove and Rackham, 2001), this massive increase of pine forest area is a result of intensive afforestation efforts in the Mediterranean countries. The species that would be selected for afforestation had to be well adapted to the Mediterranean climate and soils and also had to grow reasonably fast in order for afforestation to be effective.

Pinus halepensis Miller is a Mediterranean species distributed along the coasts and in the islands, it prefers warmer calcareous areas like Libya, Italy, Algeria, Greece and Morocco. Genetic diversity of *Pinus halepensis* Mill. was analyzed by Gómez *et al*., (2001). Eastern Mediterranean populations of *P. halepensis* have undergone a different history from those of the Western Mediterranean area. Farm lands are be naturally colonized by pioneer plant species through processes of secondary succession (Chauchard *et al*., 2007; Sheffer, 2012).

***Pinus halepensis* in Libya:** A genus of about 90 species, extensively distributed in the northern hemisphere and tropical regions of the world, *Pinus* is monoecious, evergreen trees or shrubs usually with whorled branches. Twigs of two kinds; long shoots with scale-like leaves; and deciduous short shoots (spurs) with needle-like leaves in clusters of 2 - 5. Staminate cones, catkin-like, axillary, gathered at the base of young twigs, with spirally arranged scales, each bearing two microsporangia. Ovulate cones lateral or sub terminal, cylindrical-ovoid; ovuliferous scales woody, usually spirally arranged, Seeds are winged. Distribution: Native of Canary island, introduced elsewhere as an ornamental plant represented by two species in Libya, these include:

a - *Pinus halepensis*: evergreen three up to 15 m high, with irregular pyramidal, ovoid or flattened crown, loosely branched; bark silvery-grey, becoming reddish-brown. Twigs glabrous. Leaves in pairs, slender, c. 0.7-1 mm wide, bright green. Staminate cones clustered in heads, ovoid to cylindrical. Ovulate cones solitary or in groups of 2-3, reflexed on thick peduncles, oblong or oblong-conical, reddish-brown; scales oblong, flat or rhombic, with small flattish, each bearing 2 ovules. Seeds oblong-ovoid, occasionally compressed, with pale-brown, oblong, membranous wings, 3– 5 times as long as seeds, chromosome number is (2n) = 24.

Branch with mature female cone x 0.25; dwarf shoot with 2 needles ×1.5; mature female cone 0×.5; ovuliferous scales (dorsal view) with 2 winged seeds × 1; ovuliferous scales (ventral view) with apical umbo and basal leaf × 1; winged seed × 2; cluster of male cones 1×.5; single male cone × 5; microsporophyll with 2 microsporangia × 15**.**

1. 2. Plant – plant interactions:

Plant–plant interactions are key forces structuring vegetation assemblages and determining the arrangement of plant communities. Competition and facilitation can occur simultaneously, giving rise to complex interactions that may have mutable outcomes depending on plants life stage and thickness, on the severity of climatic conditions and on indirect interactions with other species. In semi-arid environments, below-ground competition for limiting resources like water and nutrients is particularly intense. However, according to the stress gradient hypothesis, there may be a change towards increasing frequency of positive plant–plant interactions in environments with severe abiotic stress. In water-limited environments, facilitative interactions involving water may occur through hydraulic lift and canopy shading (Prieto *et al.*, 2012). Other facilitative interactions include positive impacts of plant species on soil nutrient availability (Temperton *et al*., 2007). Facilitative processes can prevail over the negative effects of competition in semi-arid environments (Pugnaire *et al*., 2004), but interactions can shift from facilitation to competition within the same plant community due to variations in water availability across years (Tielborger & Kadmon, 2000), which can be very extreme in arid and semi-arid regions. Resource availability determines the outcome of plant–plant interactions as reported by many studies along abiotic stress gradients (Pugnaire and Luque, 2001). The outcome of plant–plant interactions can also be varied along the different life stages of plant species (Soliveres *et al*., 2010). Consequently, there is a need to understand trends in the net balance between positive and negative plant–plant interactions over long time stages that cover different climatic circumstances with their associated resources availability, and during different plant life stages (Butterfield *et al*., 2010). This is especially important in the light of climate change, as the awareness of environmental harshness can be species-specific, and thus, environmental changes can produce shifts in the outcome of plant–plant interactions. Further, it has become a priority in semi-arid Mediterranean ecosystems, which are highly weak to predicted increases in temperature and decreases in rainfall that could produce dramatic changes in the composition and biodiversity of plant communities (McCluney *et al*., 2012). A deeper understanding of the physiological processes affected by plant–plant interactions in the long term can help to formulate better predictions about future shifts in plant community composition and structure under changing environmental conditions and associated variations in resources availability (Moreno-Gutierrez*et al*., 2012).

Single-species plantations may harbour (Brockerhoff *et al*., 2003) or decrease native biodiversity (Humphrey and Patterson, 2000). *Pinus halepensis* has been planted widely in dry and semi-arid areas throughout the Mediterranean basin (Maestre and Cortina, 2004). Positive interactions between plant species, or facilitation, are extensive in natural communities, especially in stressful environments (Bruno *et al.*, 2003). It has been predicted that that the comparative importance of facilitation and competition may vary inversely across gradients of abiotic stress, with facilitation dominating in zones of high abiotic stress. This prediction has been supported by different studies conducted in semi-arid areas (Pugnaire and Luque, 2001; Garc´ıa-Fayos and Gasque, 2002; Maestre *et al.*, 2003). Maestre *et al*. (2003) described a negative effect of *Pinus* on late- successional shrub seedlings in semi-arid plantations of Spain. There are three hypotheses to explain this interaction: 1) competition between introduced seedlings and *Pinus*, 2) allelopathic effects of Pinus litter or root exudates, and 3) competition between introduced seedlings and the herbaceous species, which is facilitated by *Pinus* (Bautista and Vallejo, 2002). Community organization and plant succession are under the control of biotic processes, particularly plant-plant interactions such as competition, facilitation, and allelopathy. Allelopathy is a process by which a plant releases biochemicals that influence the growth and establishment of other plants (Inderjit, 2005). Allelopathic substances are released into the environment through foliar leachates, root exudation, leaf litter, other-residue decomposition, or volatilization and may interfere with various physiological processes (such as photosynthesis, nutrient uptake, cell division, or elongation). Though these detrimental but also valuable effects on receptor plants, allelochemicals play important roles in modifiable species diversity (Chou, 1999). However, if allelochemicals can directly affect plant neighbors, allelopathic expression may in turn be modified by soil microbial communities. This is particularly important in Mediterranean plant communities that feature species rich in secondary metabolites, and for which the ecological application of allelopathy remains unclear. The long-term aim of our research is to analyze how allelochemicals of *Pinus halepensis* (Aleppo pine) influence plant biodiversity in Mediterranean open mosaic habitats during the colonization of secondary succession. Moreover, *P. halepensis* is rich in secondary metabolites (Macchioni *et al*., 2003; Fernandez *etal*., 2009) that are thought to play a role in plant-plant interactions through allelopathic processes. The effects of secondary metabolites on the breakdown process has remained a major challenge because of the very wide-ranging diversity of secondary metabolites and the impact of these mixtures on the food chain. *P. halepensis* has been extensively planted in semi-arid areas throughout the world. This has often led to slow-growth stands that: a) suffer from insect plagues, b) nutrient depletion and c) fail to promote the recovery of native vegetation.

Plant ecologists have paid considerable attention into understanding and quantifying plant interactions in various environmental conditions and among various species. However, the role played by positive interactions (facilitation) is now widely recognized, particularly in harsh environments (Callaway, 2007; Brooker *et al*., 2008), and has been evaluated by numerous studies (Castro *et al*., 2004; Go´ mez-Aparicio *et al*., 2004; Padilla & Pugnaire, 2006; Gomez & Aparicio 2009). Facilitative interactions between two species can be direct or indirect. For occurrence, in forest communities, adult trees can limit seedling survival and growth through light interception, but can also prevent the development of competing herbaceous species. Indirect facilitation occurs when the negative effect of competing is less than the positive effect of increasing resources due to herb growth limitation. By contrast, a net competitive effect is observed if the direct negative effect on seedlings is higher than the indirect positive effect on seedlings induced by a release from herb competition (Pages & Michalet 2003). In plant communities, direct and indirect interactions co-occur, and the outcome of positive and negative interactions is difficult to predict as it depends on numerous issues. A better understanding and quantification of plant interactions has practical implications in forestry, in particular for restoration activities in the Mediterranean area, where planting actions usually involve introducing object tree species with removal of preexisting vegetation. Furthermore, positive and negative interactions occur simultaneously. Most symbiotic (mutually positive) interactions in tropical forests involve relationships between plants and animals or between plants and microbes (fungi, bacteria and algae).

1. 3. Chemical defenses in *P. halepensis*:

Allelopathy in nature and ecosystems is receiving increasing attention because allelochemicals significantly reduce the growth of other plants. Allelochemicals are found to be released to environment in appreciable quantities via root exudates, leaf leachates, roots and other degrading plant residues, which include a wide range of phenolic acids such as benzoic and cinnamic acids, alkaloids, terpenoids and others. These compounds are known to modify growth, development of plants, including germination and early seedling growth. The noticeable physiological effects from allelopathy interactions are frequently observed as inhibited or delayed seed germination or reduced seedling growth. The term allelopathy is used for describing the chemical interaction between two plants. Allelochemicals can be present in leaves, flowers, roots, bark, and fruits of plants (El-Shora and Abd El-Gawad, 2015; Saadaoui *et al*., 2015). Vast array of secondary metabolites in plants are known as allelochemicals. They belong to various chemical classes such as alkaloids, phenolics, flavonoids, terpenoids and cyanogenic glucosides (Saleh and Madany, 2013; El-Shora and Abd El – Gawad, 2014; Mishra, 2015). Plants produce a wide range of carbon-based secondary metabolites (CBSM) which have important functions such as wound healing, defense against herbivores, control of the rates of plant decomposition and mediation of interaction between plants and soil biota. Among these CBSM the polyphehols derived from the phenylpropanoid pathways such as soluble phenolics and flavonoids are quantitatively the most important, accounting for about 30% of the organic carbon cycling in the terrestrial biosphere. Moving of allelochemicals into the rhizosphere happen through leaching from leaves as well as other aerial parts of the plants by root exudation, volatile emissions, and the breakdown of leaf litter and bark (Weir *et al*., 2004; El-Shora and Abd El–Gawad, 2014). The allelochemicals action on plants is known to be a diverse action and it includes a large number of biochemical reactions resulting into their modifications and finally affecting the growth of target plants (Yu *et al*., 2003; Elisante *et al*., 2013). The influence of allelochemicals usually occurs in the early life cycle of plants. The concentration of plant secondary metabolites was found to be influenced by environmental conditions such as light intensity, carbon dioxide levels, temperature, fertilization, biotic and abiotic factors, which can change the concentration of these active constituents (El-Shora and Abdel – Gawad, 2015; Mishra, 2015). In the Northern Mediterranean basin, *P. halepensis* Miller may play an important role in plant succession through some processes, in AL-Jabal Al-Akhdar area have been served as basis of traditional medicinal systems for thousands of years (El-Barasi *et al.*, 2013). The medicinal and perfumed properties of the chemical compounds (turpentine, resins, essential oil and phenolic, etc.) of pine make it one of the most popular plants throughout all civilization. Pine is widely used in traditional therapeutic repetition in world and has economic importance. In addition, such plants produce a remarkable diverse array of over 5,00,000 low and high molecular mass natural products which are known as secondary metabolites, that can be used as an alternative form of health care as well as screening for active compounds that have significant effects against human and plant pathogens. *Cupressus sempervirens*; *Juniperus phoenicea*, *Olea europaea* and *Pinus halepensis* are tree species which grow widely in temperate areas include Al-Jabal Al-Akhdar region, in Libya and have been used in traditional medicine in many part of the world. *P. halepensis* seeds are traditionally used throughout Tunisia and other Arabic countries. Essential oils from *Pinus* species have been reported to have numerous therapeutic properties. They are also used as fragrances in cosmetics, flavoring additives for food and beverages, scenting agents in a variety of household products and intermediates in the synthesis of perfume chemicals. Some researchers work on the chemical composition of *P. halepensis* essential oil from Italy (Fekih *et al*., 2014). Diversity of secondary metabolites that are produced by plants (terpenoids and phenolic compounds), chiefly Mediterranean species. Aleppo pine is known to be a major producer of secondary metabolites with allelopathic and autotoxic capacities (Robles *et al.*, 2003; Maestre & Cortina 2004; Orme~no, *et al.*, 2007). The ecological role of these compounds includes defence against predators, pathogens and competing organisms, and tolerance towards some abiotic factors (changes in temperature, pollution and drought (Robles *et al*., 2003; Yazaki 2006; Dicke & Baldwin, 2010). The litter decomposition process involves soil decomposers (including soil fauna, fungi and bacteria) that are specialized in degrading these compounds, depending on their chemical nature. However, many secondary metabolites are difficult to degrade and can prove toxic for certain decomposers.

Allelopathic mechanisms are recognized as one of the drivers in the successional replacement of plant species. Allelopathy is a major driver of many biotic interactions owing to the huge diversity of secondary metabolites that are produced by plants (terpenoids and phenolic compounds), particularly Mediterranean species. Phytochemical screening of acetone extract of *P. halepensis* needles and bark revealed the presence of secondary metabolites like terpenoids, essential oils, terpenes, turpentine and phenolic compounds. Recently, an increase interest in natural substances extracted from such plants has been observed in literatures due to their significant impact from an environmental point of view, as well as to find effective alternatives to the industrially synthesized chemicals. *P. halepensis* species synthesizes a wide range of secondary metabolites that are partially released during needle decomposition, and which can thus affect the food chain. Litter decomposition is a key process connecting ecosystem structure and function, and involving microbial and faunal components.

1.4. Effect of seasonal variations on the production of secondary metabolites:

Fundamental metabolic processes of plants are considered to be the primary metabolic processes that occur by the same mechanism in the cells of all plants. However, plants produce a large number of compounds, secondary metabolites, which enable the biochemical communication inside the ecosystem. Biochemical aspect of the synthesis of secondary metabolites depend on the plant genetic, taxonomy, the stage of development, the season, the presence of parasites and others. The variations could also be the result of abiotic factors. The metabolic processes leading to accumulation of these active constituents in the plant are basically controlled by the physiological age of the plant and the surrounding environmental conditions, as well as, the genetic factors. Therefore, it is of great importance from the production point of view, to follow up the growth parameters and chemical composition of the plant throughout the growing season. During seasonal changes, many investigators reported that the content of phenolics and flavonoids varied with the developmental phase of the plants. Desert plants adapt themselves to the harsh environment and survive in high temperature, moisture stress, water scarcity, intense solar radiations, etc. Arid zone with such climatic variability plays an important role in secreting the secondary metabolites. Currently, most pharmaceutically important secondary metabolites are isolated from wild or cultivated plants. such plants have many natural enemies; these include viruses, fungi, worms, insects, bacteria and many herbivorous. Environmental conditions affect the plant growth as well as the formation of secondary metabolites, as they are mostly formed in young and actively growing tissues. Thus the seasonal changes have effects on the physiological parameters. Despite the existence of genetic control, gene expression, and genotypes, the total content and relative proportions of secondary metabolites in plants may vary over time and space (seasonal and daily variation as well as, interplant, and interspecies distinctions), so that they occur at different levels. Seasonality, circadian rhythms, plant development, phenology, temperature, altitude, water availability, UV radiation, nutrients, pollution, mechanical stimuli, and attacks by herbivores or pathogens are considered to be the factors that most affect the occurrence of plant metabolites. Studies about the factors influencing secondary metabolite concentration are restricted to a few commercially important species native mainly to temperate regions. This information is necessary for both evolutionary and chemotaxonomic studies and shall assist expansion of the current knowledge about the ecological interactions taking place between a certain plant and its surroundings (Chaves *et al*., 2013).

1.5. Effects of *Pinus halepensis* on plant seeds and seedlings

Seed germination represents a risky transition from the stage most tolerant to environmental conditions (i.e., resting seed) to the weakest and most vulnerable stage in plant development, the seedling. Different environmental factors may determine seed germination, such as temperature, moisture and light. In addition, the chemical environment surrounding the seed must be suitable, and the presence of allelochemical inhibitors released by the surrounding vegetation may also determine germination success. The most widely used biological assays for allelochemicals are seed germination and seedling growth studies. By its richness in secondary metabolites (Pasqua *et al*., 2002; Macchioni *et al.*, 2003; Maestre *et al*., 2003; Pasqualini et al., 2003), *P. halepensis* could play an important role, in secondary succession through several processes. For example, secondary compounds (terpenoids and/or phenolic compounds) can affect root symbionts and site quality through interference with decomposition, mineralization and humification (Kuiters, 1990; Kainulainen and Holopainen, 2002). They could also influence secondary succession by interspecific competition through allelopathy (Rice, 1984; Lambers *et al*., 1998). Allelochemicals (mostly phenolic compounds and terpenoids; Rice, 1984; Rizvi *et al*., 1999) can be released by different ways: roots exudation, decomposition of plant organs (e.g. litter) or rain leaching (Rice, 1984). Concerning *P. halepensis*, Maestre *et al*. (2003), observed an inhibitory effect of this species on seedling establishment of various species in pine stands suggesting allelopathic effects of litter or root exudates.

1. 6. Secondary metabolites of target plant species (*Ceratonia siliqua* L.):

The major sugars were identified and quantified in the pods of carob *(Ceratonia siliqua* L.). Total phenolics, gallic acid equivalents, proanthocyanidins, gallo tannins, catechins and flavonols, were isolated from pods of *Ceratonia siliqua* (Ayaz*et al*, 2007).

1. 7. Objectives:

The main objectives of this work are as follows:

1-To analyze how allelochemicals of *Pinus halepensis* (Aleppo pine) influence plant biodiversity in Mediterranean.

2- To test whether *P. halepensis* allelochemicals might drive plant biodiversity in Mediterranean open mosaic habitats in the green mountain, therefore, a lab experiment was performed which consisting of *P. halepensis* tree aqueous extract present in its needles & bark. These kinds of experiments are an important prerequisite for understanding the scale of allelopathic mechanisms in plant- plant interaction.

3- To a evaluate the effect of these allelochemical in terms of seed germination, germination inhibition percentages and germination rate and early seedling development investigations were carried out to evaluate the effect of these allelochemical in expressions of shoot length, root length, seedling length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, seedling vigour and tolerance indices, measurement of specific length shoot and root, root / shoot ratio and mean values of moisture content percentages in terms of fresh and dry weight.

4- To study the effect of secondary metabolites present in the soil rhizosphere under *P. halepensis* for the influence on the growth of *C. siliqua*, for these experiment different types of soil rhizosphere suspension were collected at different distances of different depths to investigate their allelopathic effects on *C. siliqua* in terms of germination tests and early seedling development parameters.

- Therefore, the overall objective of this research is to evaluate experimentally the allelopathic effects of the needles and bark of introduced *P. halepensis* tree and soil rhizosphere around it on seed germination and seedling growth of native *C. siliqua* plant species grown in Al-Jabal Al-Akhdar area, Libya.

II. CHAPTER TWO

Materials and methods

2. 1. Plant material and soil samples:

Fresh needles and bark parts, soil rhizosphere around a tree of introduced *P. halepensis* and seeds of native *C. siliqua* were collected from Al-Jabal Al-Akhdar near Wadi Alkoof area in East Libya during different growing seasons: autumn, winter, spring and summer. The collection of all materials was carried out during the end of the middle month of each growing seasons.

2. 2. Seed preparation:

Mature seeds of *C. siliqua* were cleaned, mechanically scarified and sterilized with 5% of sodium hypochlorite (Clorox) for 15 minutes and then thoroughly washed with tap water for several times followed by soaking in distilled water overnight for 24 hours. These seeds were usedfor the investigations in all germination bioassays under the effect of aqueous extracts air-dried needles and bark of *P. halepensis* and soil rhizosphere around the tree collected in different growing seasons (winter, summer).

2. 3. Soil rhizosphere collection:

Soil rhizosphere around the introduced *P. halepensis* tree was collected during different two growing seasons (winter, summer). Soil was collected at three different distances: 0.0 m (near the vicinity of the trunk), 2, and 4 meters away from the trunk. Each soil type at each distance was collected at two different depths (10 and 20 cm) according to plate (3. 1).The soil was used for examining the effect of different soil rhizosphere around the introduced *P. halepensis* collected in the four seasons on the growth of native *C. siliqua* plants.

2. 4. Plant organs and soil extracts preparation:

Needles, bark and soil rhizosphere were cleaned, air-dried and ground into powder using (Kandy) blinder. A hundred (100 gm) of each plant part and each type of soil rhizosphere collected in different growing seasons were dissolved in 1000 ml of distilled water and soaked for 72 hours on shaker then filtered through gauze then filtered the extract by Buchner funnel, this gives the concentration of 100 g / l which was diluted in 75, 50, 25, 0.0 g / l (distilled water was used as a control treatment).

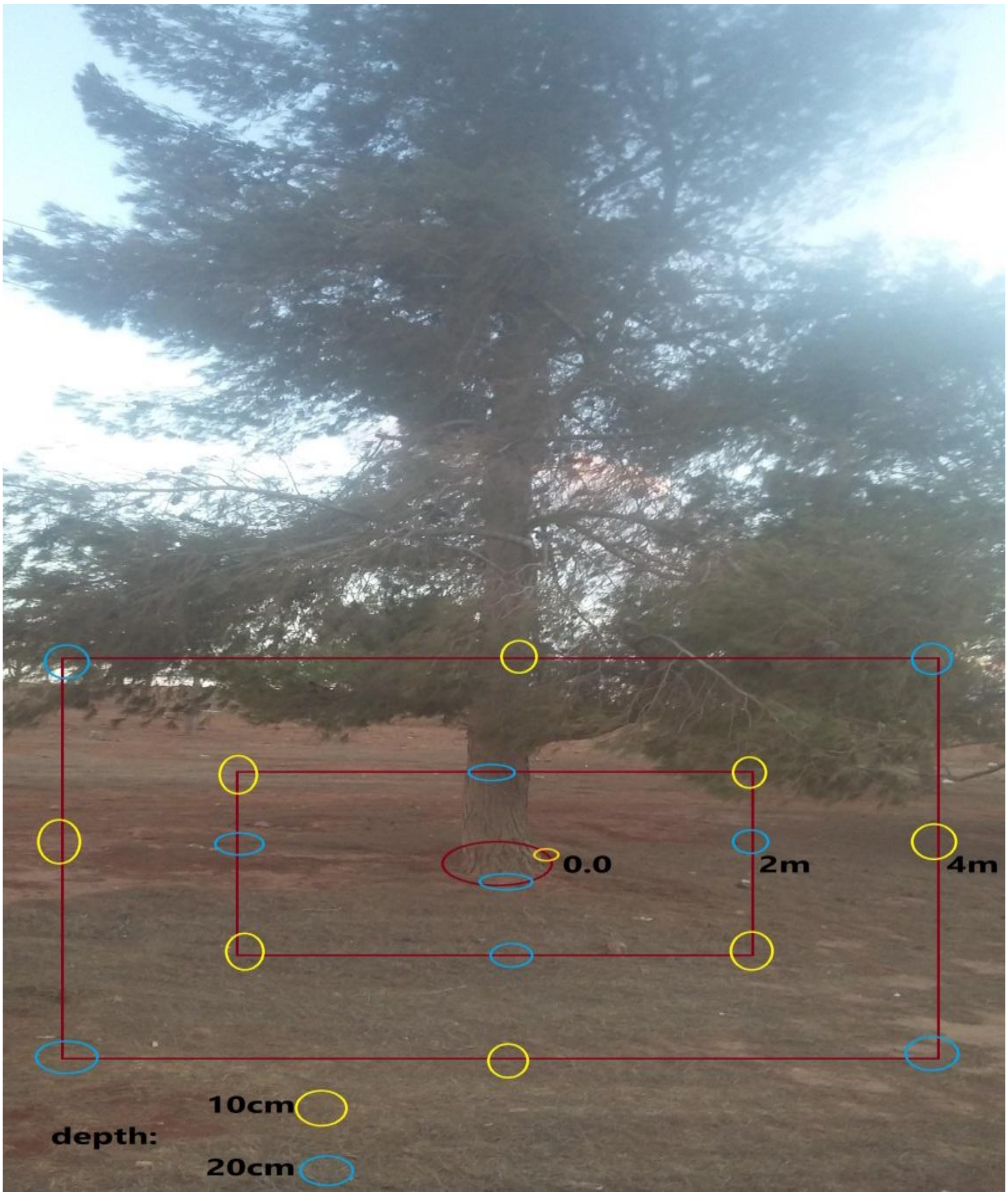

Plate 2. 1. Sample collection methods of soil rhizosphere.

Based on the results represented for the effect of needles & bark aqueous extract of *P. halepensis* collected during different growing season, it can be concluded that, the most potent season was winter and the least

potent season was Summer. Therefore, the soil rhizosphere collected during their seasons were used to test the effect soil suspension of the rhizosphere at 0, 2, 4 meter with 2 different depths (10 and 20 cm) for seed germination and seedling development of *C. siliqua*.

2. 5. Effects of different aqueous extracts and soil rhizosphere:

2. 5. 1. Seed germination test:

Ten seeds of *C. siliqua* were placed in petri-dishes lined with double layer filter papers (Whatmann number 1, 9.0 cm in diameter) and three replicates for each treatment were used for five concentrations (0.0, 25, 50, 75, and 100 g / l). Ten ml in of each extract of different concentrations of various parts of plant *P. halepensis* tree were added. Petri-dishes were kept in an incubator (TDO 66) at 20 ºC ± 2.

Seeds were considered germinated when the radical was emerged from the test and the number of germinated seeds was recorded every day for the first week.

Germination percentage (GP) and Germination speed (GR), were calculated based on the following equations:

Germination percentage (GP) $= \frac{\sum G}{N}$ X 100(Saberi *et al*., 2011)

Where GP is germination percentage, G is the number of germinated seeds and N is the number of seeds.

Germination speed (GR) $=\sum_{i=1} \frac{S1}{D1}$ (Saberi *et al*., 2011)

Where S1 is the number of germinated seed at each counting D1 is the number of day until n counting and n is the number of counting

Inhibition percentages (IG%) of seed germination were calculated by:

$IG\% = \frac{NO.of\ non\ germinaed\ seeds(D_n)}{Total\ no.of\ seeds} \times 100$ (Kumar *et al*., 2010)

Vigour index = Total germination percentage – Plant length.

Plant length = Root length + Shoot length. (Saberi *et al*., 2013)

2. 5. 2. Early seedling development:

2. 5. 2. Measurement of fresh and dry parameters:

Seedlings of *C. siliqua* were grown in the incubator for three weeks. They were observed for any contamination and solution was added when it was needed. At the end of growing period, these plants were washed then were separated into shoots and roots for the following measurements: length of shoots and roots (mm) and fresh weight (mg) of shoots and roots.

The separated shoots and roots were placed in an oven at 85 °C for 72 hours until the weight was fixed.

Dry mass (mg) was measured for the shoots and roots for every individual plant grown under different types of needles, bark and soil rhizosphere around *P. halepensis* during various growing seasons.

To calculate the Specific root and shoot length the following equation was used:

$\text{Specific length}_{(\text{root \& shoot})} = \frac{\text{length of organ(mm)}}{\text{organ mass weight(mg)}}$

$\text{Tolerance index} = \frac{\text{length of plant in tretment}}{\text{length of plant in control}}$ (El-Dengawy*et al*., 2011)

Moisture content percentages of shoot & root on the basis of fresh and dry mass.

Moisture content percentages = $\frac{\text{weight of shoot or root)}}{\text{weigth of plant(plant)}}$ X 100

(Reeb and Milota, 1999).

Root / shoot ratio = $\frac{\text{root dry weigth}}{\text{shoot dry weigth}}$ (Sanquetta *et al*., 2011)

2. 6. Statistical analysis:

Minitab (version 13) statistical software was used for the data analysis to indicate significant differences within (analysis of variance, ANOVA) and between (Turkey's pairwise comparison test) different treatments in all the bioassays used in this work.

III. CHAPTER THREE

Results

3. 1. Effects of different concentrations of aqueous extract of *P. halepensis* needles and bark (autumn collection) on *C. siliqua*:

3. 1. 1. Effects of needle extracts:

3. 1. 1. 1. Seed germination:

Germination process of *C. siliqua* seeds had started from the fourth day of germination period. Daily and final cumulative germination percentages were reduced with increasing the concentration of aqueous extract of *P. halepensis* needles collected during autumn season. The differences were significant within the means of final cumulative seed germination ($F_{(4, 29)}$ = ; *P* <0.) (Table 1). The percentages of seed germination and inhibition are shown in Figure (1). Rate of carob seed germination was greatly decreased ($F_{(4, 29)} = 6.25$; $P < 0.01$) under the concentration of 100 g / l of the needles extract (Fig. 2). Tukey's pairwise comparisons test showed significant differences between concentrations up to 75 g / l of the extract including control treatment and 100 g / l, for both cumulative seed germination and the rate of seed germination of *C. siliqua* seeds under different concentrations of needles aqueous extract of *P. halepensis* (autumn collection).

3. 1. 1. 2. Early seedling development:

Fresh parameters such as, length of shoot, root and seedling, fresh weight of

Table 1. Effect of different concentrations of needles (autumn collection) aqueous extract of P. halepensis on mean daily germination percentages of *C. siliqua* seeds:

Concentration (g / l)	D 1	D 2	D 3	D 4	D 5	D 6	D 7
	+	+	+	+	**	**	**
0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	66.6 ± 8.82	93.3 ± 6.7 [a]	96.7 ± 3.3 [a]	100 ± 0.0 [a]
25	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	46.7 ± 24.0	86.7 ± 8.8 [a]	90.0 ± 10.0 [a]	90 ± 10.0 [a]
50	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	23.3 ± 3.33	80.0 ± 5.8 [a]	86.0 ± 3.3 [a]	86.6 ± 3.3 [ac]
75	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	30.0 ± 15.3	63.3 ± 3.3 [ab]	66.0 ± 3.3 [bc]	80.0 ± 5.8 [bc]
100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	6.67 ± 3.33	43.3 ± 8.8 [b]	46.0 ± 6.7 [bc]	60.0 ± 5.8 [bc]

D = day + = Not significant ± = SEMean ** = Significant at $P < 0.01$ Different letters = Significant

Similar letters = Not significant

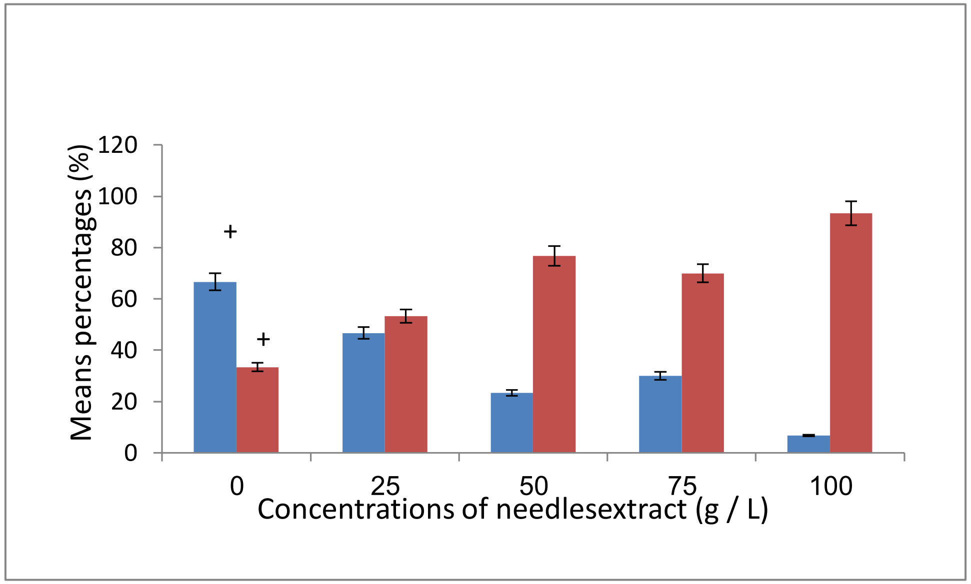

Figure 1. Effect of different concentrations of needles (autumn collection) aqueous extract of *P. halepensis* on mean germination promotion (A) and inhibition (B) percentages of *C. siliqua* seeds

Bars = SEMean + = Not significant

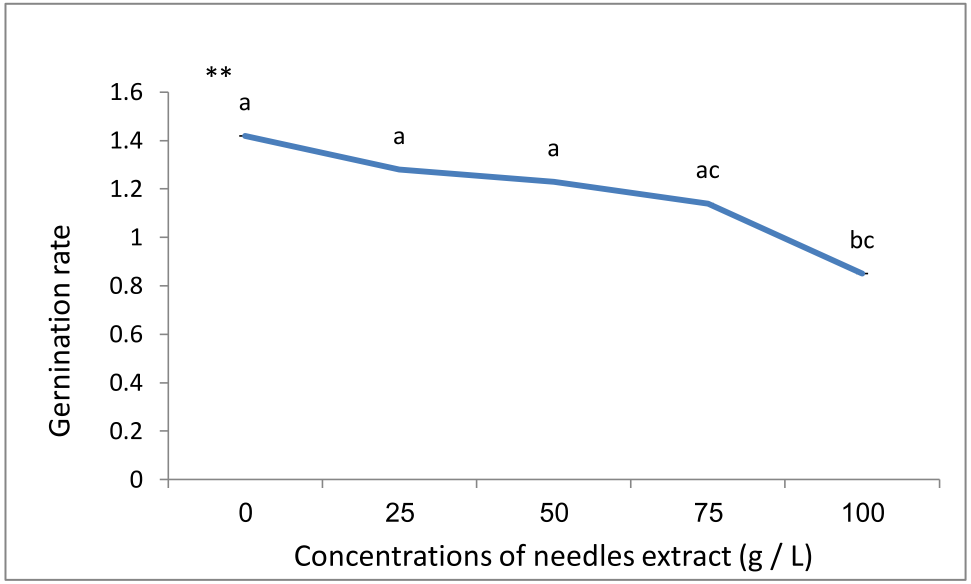

Figure 2. Effect of different concentrations of needles (autumn collection) aqueous extract of *P. halepensis* on mean germination rate of *C. siliqua* seeds

Bars = SEMean ** = Significant at *P*< 0.01 Different letters = Significant Similar letters = Not significant

shoot and root, vigor and tolerance indices were measured for *C. siliqua* seedlings grown under different concentrations of *P. halepensis* needles collected in autumn season and are represented in table (2). Results needles extract of *P. halepensis*. With increasing extract concentrations, there were great reductions in length of shoot (($F_{(4, 29)} = 22.61$; $P < 0.001$), root ($F_{(4, 29)} = 29.21$; $P < 0.001$), and seedling ($F_{(4, 29)} = 29.40$; $P < 0.001$), shoot fresh weight ($F_{(4, 29)} = 13.17$; $P < 0.001$) (Table 2), seedling vigor ($F_{(4, 29)} = 11.24$; $P < 0.01$) (Fig. 3) and tolerance ($F_{(4, 29)} = 25.83$; $P < 0.001$) (Fig. 4) indices of *C. siliqua* seedlings grown under the effect of autumn needles extract of *P. halepensis*. Tukey's pairwise comparisons test indicated significant lower values of the above measured parameters of *C. siliqua* seedlings grown under concentrations of 50, 75, and 100 g / l of aqueous extract of *P. halepensis* needles compared to that of lower concentrations including control treatment. Dry mass measurements (Table 3) include: specific shoot length, specific root length, dry weight of shoot and root, and root / shoot ratio. Significant lower values were obtained for seedlings under higher concentrations of needles aqueous extract *P. halepensis*, for example, specific shoot length ($F_{(4, 29)} = 3.12$; $P < 0.05$), dry weight of shoot ($F_{(4, 29)} = 16.80$; $P < 0.001$), and root ($F_{(4, 29)} = 9.23$; $P < 0.001$). Differences were significant between needles aqueous extract up to 75 g / l and 100 g / l of the same extract. There was no significant differences for root / shoot ratio of *C. siliqua* seedlings grown under the same media (Table 3).

Moisture content percentages on the basis of fresh and dry mass were taken for the same seedlings under different concentrations of *P. halepensis* needles

Table 2. Effect of different concentrations of needles (autumn collection) aqueous extract of *P. halepensis* on mean measurement of fresh parameters of *C. siliqua* seedlings:

Concentration (g / l)	Shoot length (mm)	Root length (mm)	Seedling length (mm)	Shoot fresh mass (mg)	Root fresh mass (mg)
0.0	*** 70.93 ± 4.72 [a]	*** 38.20 ± 4.08 [a]	*** 109 ± 7.64 [a]	*** 0.15 ± 0.013 [a]	+ 0.0016 ± 0.001
25	58.00 ± 4.51 [ac]	25.40 ± 1.80 [b]	83.4 ± 5.63 [a]	0.12 ± 0.012 [a]	0.011 ± 0.002
50	51.10 ± 6.01 [bc]	16.53 ± 2.00 [b]	67.63 ± 7.69 [ab]	0.11 ± 0.013 [ac]	0.009 ± 0.003
75	22.97 ± 3.87 [bd]	9.90 ± 2.09 [bc]	23.87 ± 5.71 [bc]	0.07 ± 0.012 [bcd]	0.006 ± 0.001
100	17.40 ± 4.85 [bd]	4.67 ± 1.29 [bc]	22.07 ± 6.03 [bc]	0.04 ± 0.011 [bd]	0.005 ± 0.01

+ = Not significant ± = SEMean *** = Significant at $P < 0.001$ Different letters = Significant

Similar letters = Not significant

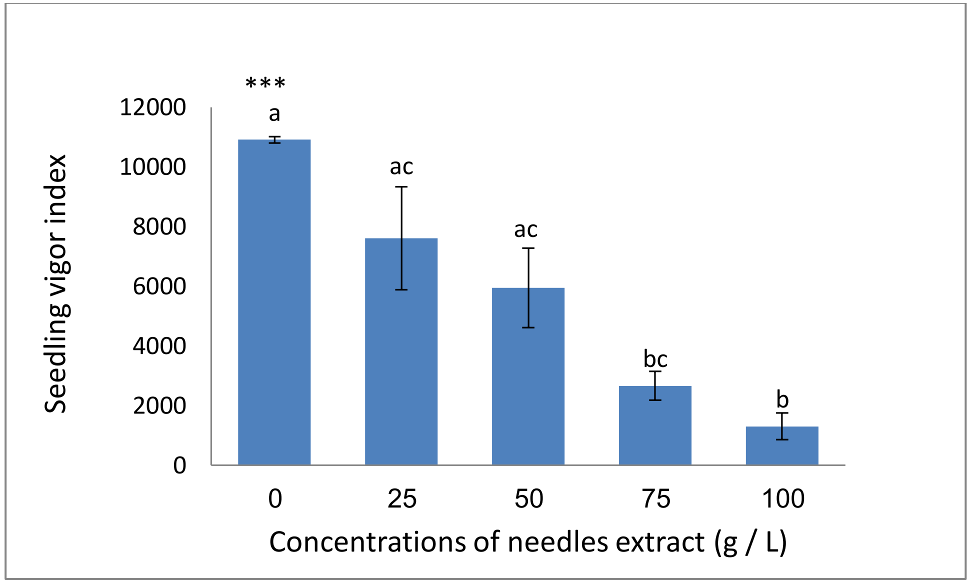

Figure 3. Effect of different concentrations of needles (autumn collection) aqueous extract of *P. halepensis* on mean vigor index of *C. siliqua* seedlings

Bars = SEMean *** = Significant at *P*< 0.01 Different letters = Significant Similar letters = Not significant

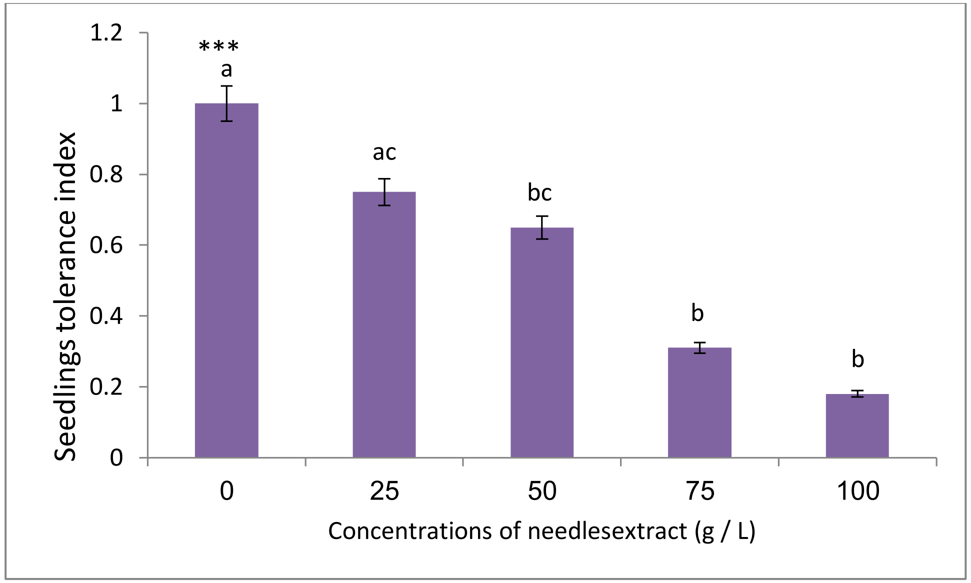

Figure 4. Effect of different concentrations of needles (autumn collection) aqueous extract of *P. halepensis* on mean tolerance index of *C. siliqua* seedlings

Bars = SEMean *** = Significant at $P < 0.001$ Different letters = Significant Similar letters = Not significan

Table 3. Effect of different concentrations of needles (autumn collection) aqueous extract of *P. halepensis* on mean measurement of specific length (shoot and root), dry mass (shoot and root), and root / shoot ratio of *C. siliqua* seedlings:

Concentration (g / l)	Specific shoot length	Specific root length	Shoot dry mass (mg)	Root dry mass (mg)	Root / Shoot ratio
0.0	* 1264.0 ± 123 [a]	+ 7079.0 ± 721	*** 0.06 ± 0.004 [a]	*** 0.006 ± 0.00051 [a]	+ 0.10 ± 0.010
25	1080.7 ± 65.9 [ac]	5087.0 ± 441	0.05 ± 0.003 [a]	0.006 ± 0.00041 [a]	0.10 ± 0.012
50	1199.0 ± 107 [a]	3481.0 ± 295	0.04 ± 0.044 [ac]	0.005 ± 0.00057 [a]	0.11 ± 0.011
75	1053.08 ± 48.6 [bc]	5422.0 ± 264	0.03 ± 0.005 [bc]	0.004 ± 0.00068 [ac]	0.11 ± 0.013
100	785.0 ± 68.6 [ac]	2686 ± 144	0.02 ± 0.004 [b]	0.002 ± 0.00048 [bc]	0.11 ± 0.010

+ = Not significant ± = SEMean * = Significant at *P*< 0.05 *** = Significant at *P*< 0.001
Different letters = Significant Similar letters = Not significant

collected in autumn season.

Although there were no significant differences in these measurements, they were decreased by increasing the concentrations of needles extract (Table 4).

3. 1. 2. Effects of bark extracts:

3. 1. 2. 1. Seed germination:

The germination of *C. siliqua* seeds was not affected by different concentrations of aqueous bark extract of *P. halepensis* during autumn season (Table 5). But there were significant reductions of seed germination ($F_{(4, 29)} = 8.57$; $P < 0.01$) and inhibition ($F_{(4, 29)} =$; $P < 0.01$).

Tukey's pairwise comparisons test reveals that the significant differences were obtained for seed under lower concentrations (0.0 and 25 g / l) in comparison with the higher concentrations of bark extract (Fig. 5). Furthermore, the same levels of *P. halepensis* bark had no significant differences within the rate of germination *C. siliqua* seeds (Fig. 6).

3. 1. 2. 2. Early seedling development:

Response patterns of *C. siliqua* seedlings under different concentrations of bark extract of *P. halepensis* in autumn season were quite different from seedlings grown under the needles treatments (Table 6).

The significant differences were found within different treatment means of bark extract in measuring the length of shoot (F (4, 29) = 4.88; P <0.01), root (F (4,

Table 4. Effect of different concentrations of needles (autumn collection) aqueous extract of *P. halepensis* on mean values of moisture content percentages in terms of fresh and dry mass of *C. siliqua* seedlings:

Concentration (g / l)	Moisture content percentages as fresh mass		Moisture content percentages as dry mass	
	Shoot	Root	Shoot	Root
0.0	+ 89.21 ± 4.12	+ 10.72 ± 0.87	+ 73.66 ± 4.25	+ 5.99 ± 0.66
25	87.68 ± 3.52	10.04 ± 0.94	70.12 ± 0.94	5.65 ± 0.57
50	74.48 ± 6.98	10.42 ± 0.81	68.55 ± 0.81	5.52 ± 1.10
75	55.01 ± 8.38	10.39 ± 1.06	51.0 ± 1.06	4.99 ± 1.14
100	48.86 ± 7.97	9.88 ± 0.82	54.39 ± 0.82	3.47 ± 1.70

+ = Not significant ± = SEMean

Table 5. Effect of different concentrations of bark (autumn collection) aqueous extract of *P. halepensis* on mean daily germination percentages of *C. siliqua* seeds:

Concentration (g / l)	D 1	D 2	D 3	D 4	D 5	D 6	D 7
0.0	+ 1.0 ± 0.0	** 46.7 ± 8.8 [a]	+ 100.0 ± 0.0	+ 100.0 ± 0.0	+ 100.0 ± 0.0	+ 100.0 ± 0.0	+ 100.0 ± 0.0
25	0.0 ± 0.0	36.0 ± 12.1 [a]	90.0 ± 5.77	96.7 ± 3.33	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
50	0.0 ± 0.0	16.7 ± 3.3 [ac]	93.0 ± 6.67	96.7 ± 3.33	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
75	0.0 ± 0.0	3.33 ± 3.3 [bc]	70.0 ± 5.77	90.0 ± 5.77	93.3 ± 3.33	100.0 ± 0.0	100.0 ± 0.0
100	0.0 ± 0.0	0.0 ± 0.0 [bc]	80.0 ± 10.0	96.7 ± 3.33	96.7 ± 3.33	96.7 ± 3.33	96.7 ± 3.33

D = day + = Not significant ± = SEMean ** = Significant at $P < 0.01$ Different letters = Significant

Similar letters = Not significant

Table 6. Effect of different concentrations of bark (autumn collection) aqueous extract of *P. halepensis* on mean measurement of fresh parameters of *C. siliqua* seeds:

Concentration (g / l)	Shoot length (mm)	Root length (mm)	Seedling length (mm)	Shoot fresh mass (mg)	Root fresh mass (mg)
0.0	** 106.7 ± 2.70 [a]	*** 68.20 ± 4.15 [a]	*** 174.5 ± 4.8 [a]	+ 0.43 ± 0.08	** 0.06 ± 0.017 [a]
25	106.5 ± 3.40 [a]	62.5 ± 4.04 [ac]	169.1 ± 6.5 [ac]	0.40 ± 0.08	0.025 ± 0.003 [b]
50	101.0 ± 4.0 [ac]	52.3 ± 3.44 [bc]	153.3 ± 6.1 [ad]	0.39 ± 0.07	0.025 ± 0.002 [b]
75	101.5 ± 3.20 [ad]	50.8 ± 2.73 [bc]	152.3 ± 4.3 [bcd]	0.38 ± 0.01	0.025 ± 0.002 [b]
100	89.20 ± 3.97 [bcd]	34.8 ± 2.86 [b]	124.1 ± 6.0 [b]	0.37 ± 0.02	0.024 ± 0.002 [b]

+ = Not significant ± = SEMean * = Significant at $P< 0.05$ ** = Significant at $P< 0.01$

*** = Significant at $P< 0.001$ Different letters = Significant Similar letters = Not significant

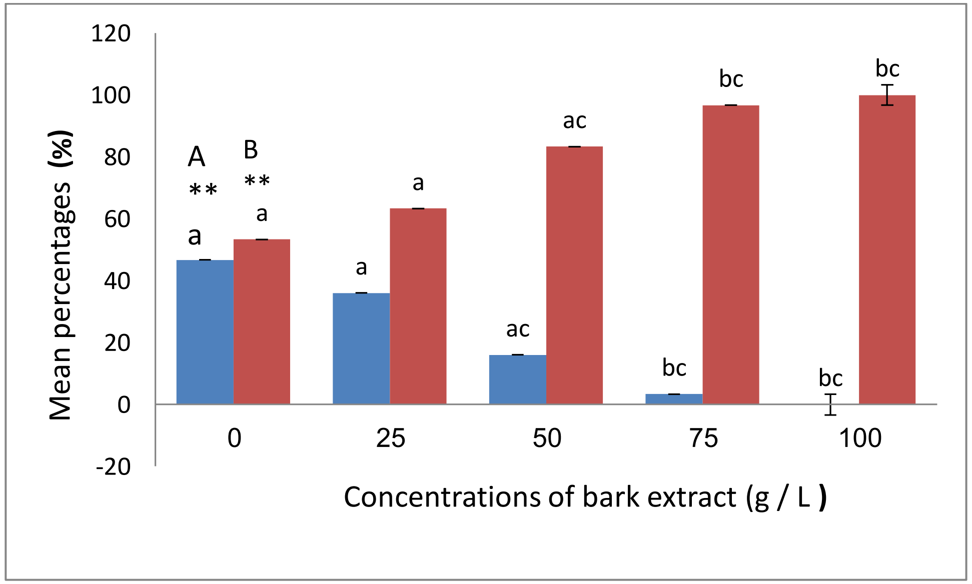

Figure 5. Effect of different concentrations of bark (autumn collection) aqueous extract of *P. halepensis* on mean germination promotion (A) and inhibition (B) percentages of *C. siliqua* seeds

Bars = SEMean ** = Significant at *P*< 0.01 Different letters = Significant Similar letters = Not significant

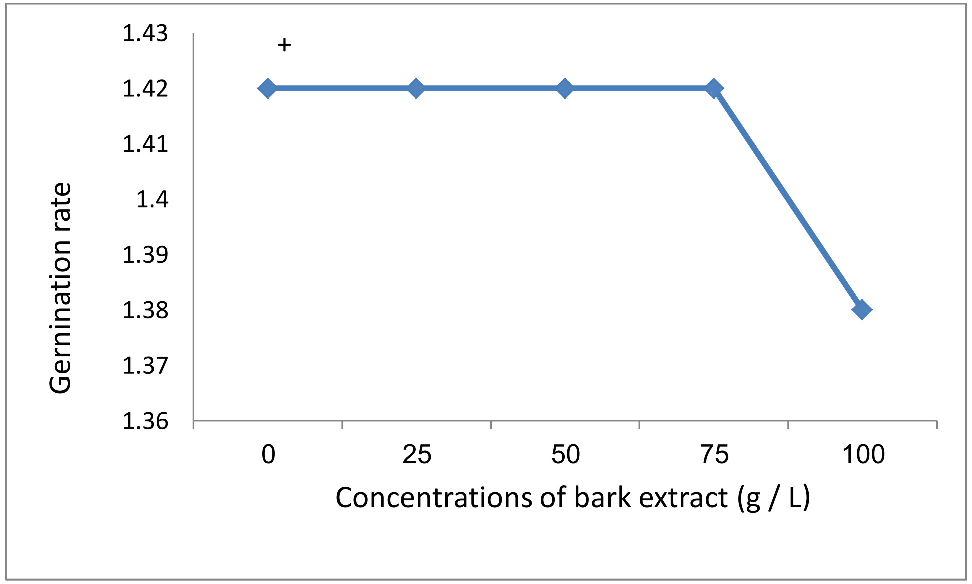

Figure 6. Effect of different concentrations of bark (autumn collection) aqueous extract of *P. halepensis* on mean germination rate of *C. siliqua* seeds

Bars = SEMean + = Not significant

29) = 13.30; P <0.001), seedling (F (4, 29) = 12.46; P <0.001), and root fresh weight (F (4, 29) = 4.45.; P <0.01).

The inhibitory effect on the above measured parameters was increased with increasing the concentration of bark extract collected in autumn. Tukey's pairwise comparisons test presented the significant differences in shoot length and seedling length between lower concentrations (0.0, 25, and 50 g / l) and higher concentrations (75 and 100 g / l). In the case of root length the differences were occurred between concentrations of (0.0 and 25 g / l) and those of 50, 75, and 100 g / l of the same extract. Whereas, for root fresh weight of *C. siliqua* seedlings, the differences were significant between control treatment and other treatment means of *P. halepensis* bark (autumn collection) extract. Both vigor and tolerance indices were significantly decreased by increasing the bark concentration within different treatment means, for vigor ($F_{(4, 29)}$ = 5.71; P <0.05) and tolerance ($F_{(4, 29)}$ = 6.86; P <0.001) indices of *C. siliqua* seedlings are shown in figures 7 and 8 respectively. Tukey's pairwise comparisons test showed significant differences between lower concentrations up to 50 g / l and 100 g / l concentration which caused reduction of both parameters of seedlings developed under bark extract obtained in autumn season.

Dry biomass measures of *C. siliqua* seedlings are shown in table (7). These measurements were of significant lower values within higher concentrations of bark extract, for specific shoot length ($F_{(4, 29)}$ = 3.64; P <0.01), specific root length ($F_{(4, 29)}$ = 9.73; P <0.001), and shoot dry mass ($F_{(4, 29)}$ = 3.19; P <0.05).

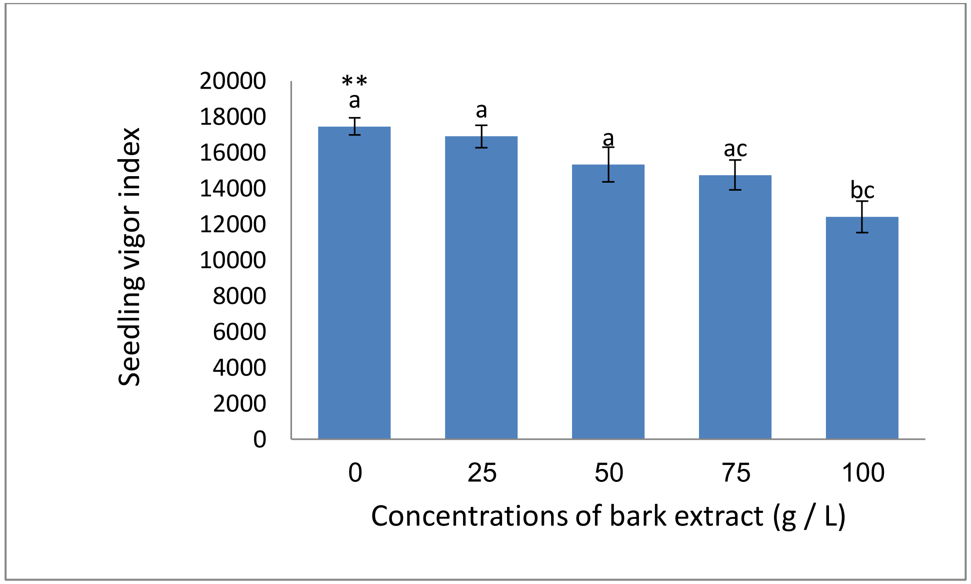

Figure 7. Effect of different concentrations of bark (autumn collection) aqueous extract of *P. halepensis* on mean vigor index of *C. siliqua* seedlings

Bars = SEMean ** = Significant at $P< 0$ Different letters = Significant Similar letters = Not significant

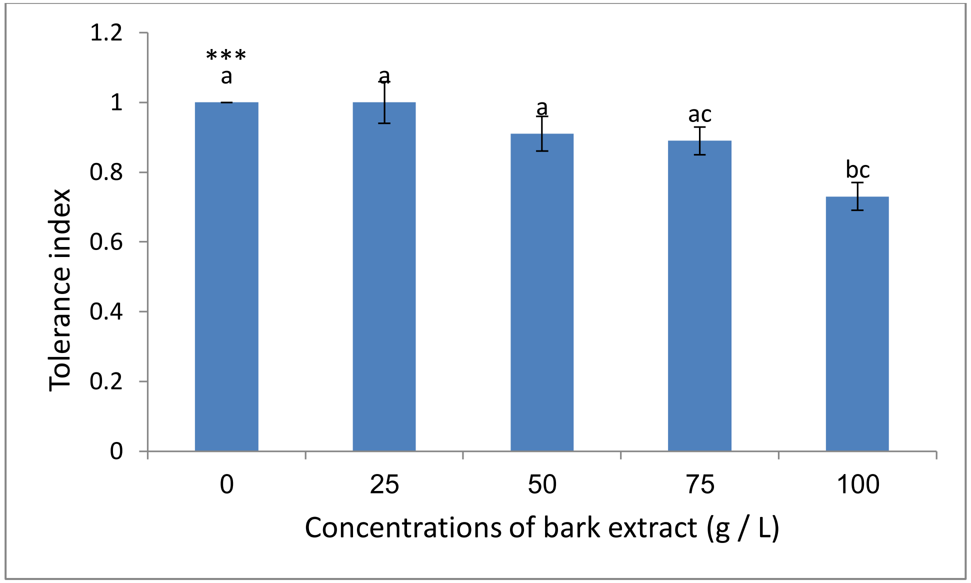

Figure 8. Effect of different concentrations of bark (autumn collection) aqueous extract of *P. halepensis* on mean tolerance index of *C. siliqua* seedlings

Bars = SEMean *** = Significant at *P*< 0.001 Different letters = Significant Similar letters = Not significant

Tukey's pairwise comparisons test showed significant differences between lower treatments including the control and higher concentrations above 50 g / l. No significant differences were found within various treatments of *P. halepensis* bark (autumn collection) extract for root / shoot ratio *C. siliqua* seedlings (Table 7).

For moisture content percentages in terms of fresh and dry mass of the target seedlings, these percentages were reduced by all levels of the extract concentration. But the differences were significant only for moisture content percentages of fresh mass of shoot ($F_{(4, 29)}$ = 12.30; *P* <0.001) and root ($F_{(4, 29)}$ = 8.41; *P* <0.001). Significant differences in these measures were existed between 0.0 g / l and all other treatment means (Table 8).

3. 2. Effects of different concentrations of aqueous extract of *P. halepensis* needles and bark (winter collection) on *C. siliqua*:

3. 2. 1. Effects of needle extracts:

3. 2. 1. 1. Seed germination:

The influence of aqueous extract of *P. halepensis* needles collected in winter season was tested with different concentrations for daily germination percentages of *C. siliqua* seeds (Table 9). Germination was begin at the third day of germination period with great significant reduction under all concentrations of needle extract ($F_{(4, 29)}$ = 110.78; *P* <0.001) with no seed germination under 100 g / l. Seed germination percentages were significantly decreased ($F_{(4, 29)}$ = ; *P* <0.001) with significant increase of germination inhibition percentages ($F_{(4, 29)}$ = ; *P* <0.001). These differences were found between seeds treated with

Table 7. Effect of different concentrations of bark (autumn collection) aqueous extract of *P. halepensis* on mean measurement of specific length (shoot and root), dry mass (shoot and root), and root / shoot ratio of *C. siliqua* seedlings:

Concentration (g / l)	Specific shoot length	Specific root length	Shoot dry mass (mg)	Root dry mass (mg)	Root / Shoot ratio
0.0	** 2201.0 ± 75.3 [a]	*** 12663.0 ± 1164 [a]	* 0.062 ± 0.01 [a]	+ 0.0064 ± 0.0004	+ 0.12 ± 0.08
25	1985.0 ± 89.4 [ac]	9940.0 ± 514 [ac]	0.058 ± 0.01 [ac]	0.0063 ± 0.0003	0.11 ± 0.008
50	1883.0 ± 152 [ad]	9906.0 ± 502 [ac]	0.058 ± 0.013 [ac]	0.0062 ± 0.0004	0.11 ± 0.009
75	1778.0 ± 53.9 [bcd]	8161.0 ± 976 [bc]	0.057 ± 0.01 [ac]	0.0059 ± 0.0004	0.10 ± 0.007
100	1753.0 ± 89.6 [bcd]	6201 ± 354 [b]	0.053 ± 0.01 [bc]	0.0058 ± 0.0003	0.10 ± 0.008

+ = Not significant ± = SEMean * = Significant at $P< 0.05$ ** = Significant at $P< 0.01$

*** = Significant at $P< 0.001$ Different letters = Significant Similar letters = Not significant

Table 8. Effect of different concentrations of bark (autumn collection) aqueous extract of *P. halepensis* on mean values of moisture content percentages in terms of fresh and dry mass of *C. siliqua* seedlings:

Concentration (g / l)	Moisture content percentages as fresh mass		Moisture content percentages as dry mass	
	Shoot	Root	Shoot	Root
0.0	*** 108.73 ± 1.41 [a]	*** 14.27 ± 1.41 [a]	+ 93.21 ± 0.69	+ 10.62 ± 0.62
25	94.32 ± 0.43 [b]	10.34 ± 0.43 [b]	90.33 ± 0.75	8.78 ± 0.61
50	93.51 ± 0.62 [b]	9.78 ± 0.62 [b]	89.78 ± 0.66	8.00 ± 0.66
75	93.98 ± 0.55 [b]	7.23 ± 0.66 [b]	89.01 ± 0.77	6.91 ± 0.58
100	92.10 ± 0.46 [b]	6.70 ± 0.46 [b]	88.51 ± 0.64	5.95 ± 0.65

+ = Not significant ± = SEMean *** = Significant at $P< 0.001$ Different letters = Significant Similar letters = Not significant

Table 9. Effect of different concentrations of needles (winter collection) aqueous extract of *P. halepensis* on mean daily germination percentages of *C. siliqua* seeds:

Concentration (g / l)	D 1	D 2	D 3	D 4	D 5	D 6	D 7
	+	+	***	***	***	***	*
0.0	2.0 ± 0.0	0.0 ± 0.0	73.33 ± 3.3 [a]	80.0 ± 3.33 [a]	96.7 ± 3.33 [a]	96.7 ± 3.3 [a]	100.0 ± 0.0 [a]
25	0.0 ± 0.0	0.0 ± 0.0	3.33 ± 3.33 [b]	20.0 ± 5.77 [b]	36.67 ± 6.7 [b]	70.0 ± 5.8 [b]	96.67 ± 3.3 [a]
50	0.0 ± 0.0	0.0 ± 0.0	3.33 ± 3.33 [b]	26.7 ± 3.33[b]	36.67 ± 6.7 [b]	50.0 ± 5.8 [b]	86.6 ± 5.8 [ac]
75	0.0 ± 0.0	0.0 ± 0.0	6.67 ± 3.33 [b]	23.3 ± 3.33 [b]	30.0 ± 5.77 [b]	66.0 ± 3.3 [b]	73.3 ± 3.3 [bc]
100	0.0 ± 0.0	0.0 ± 0.0	0.00 ± 0.00 [b]	20.0 ± 5.77 [b]	30.0 ± 5.77 [b]	60.0 ± 0.0 [b]	68.7 ± 6.7 [bc]

D = day + = Not significant ± = SEMean * = Significant at $P< 0.05$ *** = Significant at $P< 0.001$

Different letters = Significant Similar letters = Not significant

0.0 g / l and all other concentrations of the same needles extract (Fig. 9).

Final germination rate of *C. siliqua* seeds was significant ($F_{(4, 29)}$ = ; P <0.05) with lower value caused by needles extract of 75 g / l. The differences were indicated between this concentration and other treatment means including the control (Fig. 10).

Final germination rate was significantly ($F_{(4, 29)}$ = 110.8; P <0.01) reduced by 75 g / l needles extract of winter collection (Fig. 10).

3. 2. 1. 2. Early seedling development:

Fresh parameters of *C. siliqua* seedlings were investigated using various concentrations of needles aqueous extract *P. halepensis* collected during winter season (Table 10). All the parameters were significantly differ in length of shoot ($F_{(4, 29)}$ = 23.79; P <0.001), root ($F_{(4, 29)}$ = 14.19; P <0.001), seedling ($F_{(4, 29)}$ = 22.18; P <0.001), fresh mass of shoot ($F_{(4, 29)}$ = 9.86; P <0.001), and root ($F_{(4, 29)}$ = 11.53; P <0.001). Seedling vigor and tolerance indices were found to be decreased with increased needles extract concentrations. There were significant differences within different levels of the tested extract, for vigor index (Fig. 11), the analysis was ($F_{(4, 29)}$ = 5.28; P <0.05) and for tolerance index (Fig. 12) it was ($F_{(4, 29)}$ = **11.53; *P* <0.001**) with lower values at the concentration of 100 g / l the same media of winter collection used for early developed seedlings of *C. siliqua*. Tukey’s pairwise comparisons test showed significant differences between different treatment means. All dry mass parameters of the target plant seedlings developed under different concentrations of the same extract,

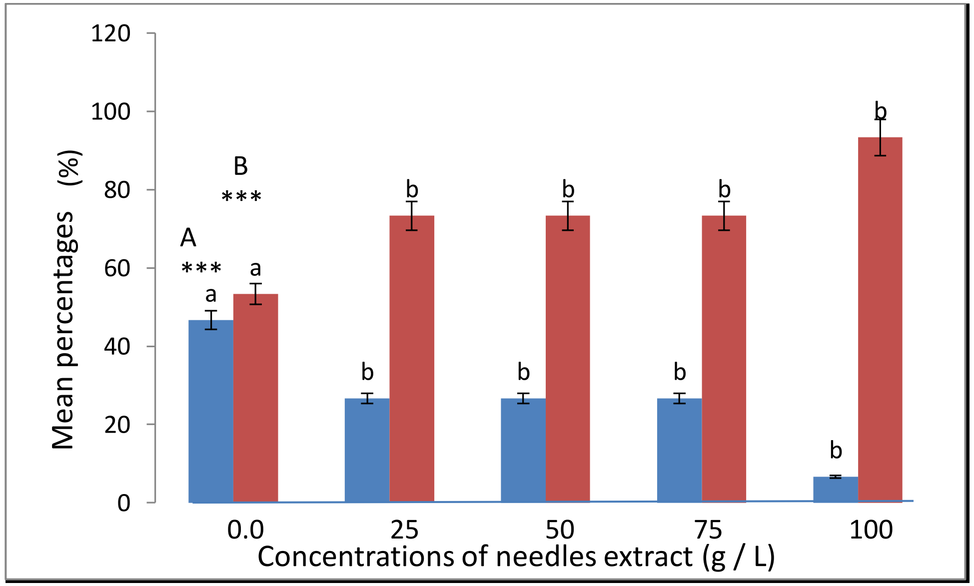

Figure 9. Effect of different concentrations of needles (winter collection) aqueous extract of *P. halepensis* on mean germination promotion (A) and inhibition (B) percentages of *C. siliqua* seeds

Bars = SEMean *** = Significant at *P*< 0.001 Different letters = Significant Similar letters = Not significant

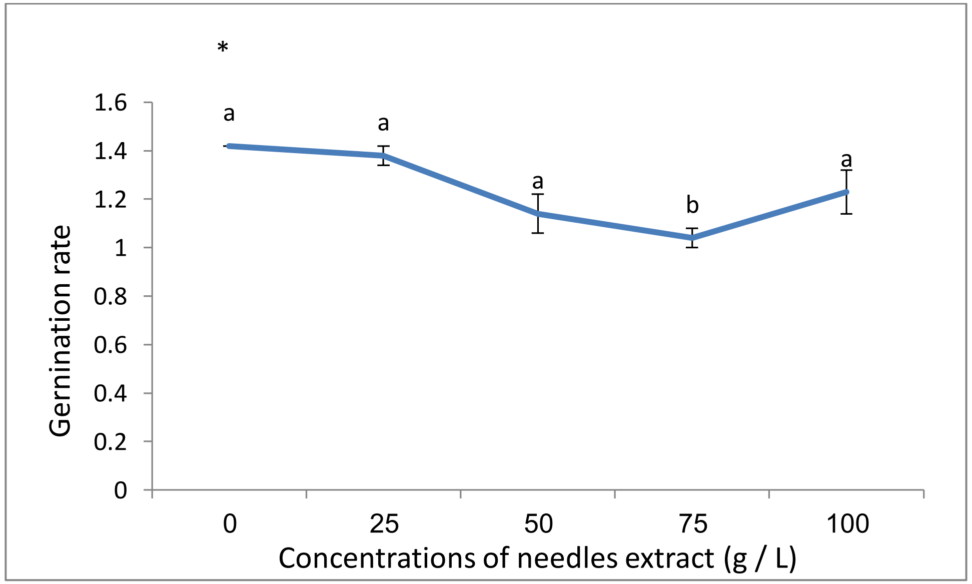

Figure 10. Effect of different concentrations of needles (winter collection) aqueous extract of *P. halepensis* on mean germination rate of *C. siliqua* seeds

Bars = SEMean * = Significant at $P< 0.05$ Different letters = Significant Similar letters = Not significant

Table 10. Effect of different concentrations of needles (winter collection) aqueous extract of *P. halepensis* on mean measurement of fresh parameters of *C. siliqua* seedlings:

Concentration (g / l)	Shoot length (mm)	Root length (mm)	Seedling length (mm)	Shoot fresh mass (mg)	Root fresh mass (mg)
0.0	*** 119.77 ± 4.15 [a]	*** 46.40 ± 1.88 [a]	*** 166.17 ± 5.61 [a]	*** 0.52 ± 0.02 [a]	*** 0.055 ± 0.022[a]
25	110.93 ± 4.66 [a]	46.33 ± 1.84 [a]	157.37 ± 6.51 [a]	0.42 ± 0.024 [ac]	0.054 ± 0.004 [a]
50	76.73 ± 7.80 [bc]	31.17 ± 3.61 [bc]	107.09 ± 11.3 [bc]	0.33 ± 0.032 [bc]	0.043 ± 0.005 [ac]
75	66.37 ± 7.13 [b]	26.97 ± 3.24 [b]	93.03 ± 10.0 [bc]	0.33 ± 0.032 [bc]	0.031 ± 0.003 [bc]
100	45.87 ± 7.09 [bd]	20.37 ± 3.57 [bd]	66.02 ± 10.05 [bd]	0.27 ± 0.039 [bd]	0.023 ± 0.003 [b]

± = SEMean *** = Significant at *P*< 0.001 Different letters = Significant Similar letters = Not significant

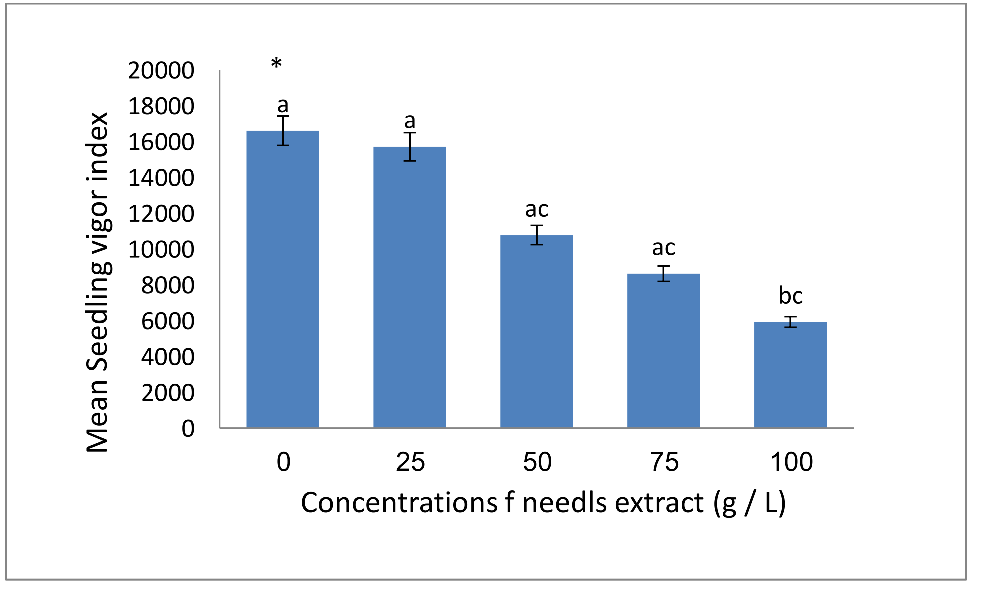

Figure 11. Effect of different concentrations of needles (winter collection) aqueous extract of *P. halepensis* on mean vigor index of *C. siliqua* seedlings

Bars = SEMean * = Significant at *P*< 0.05 Different letters = Significant Similar letters = Not significant

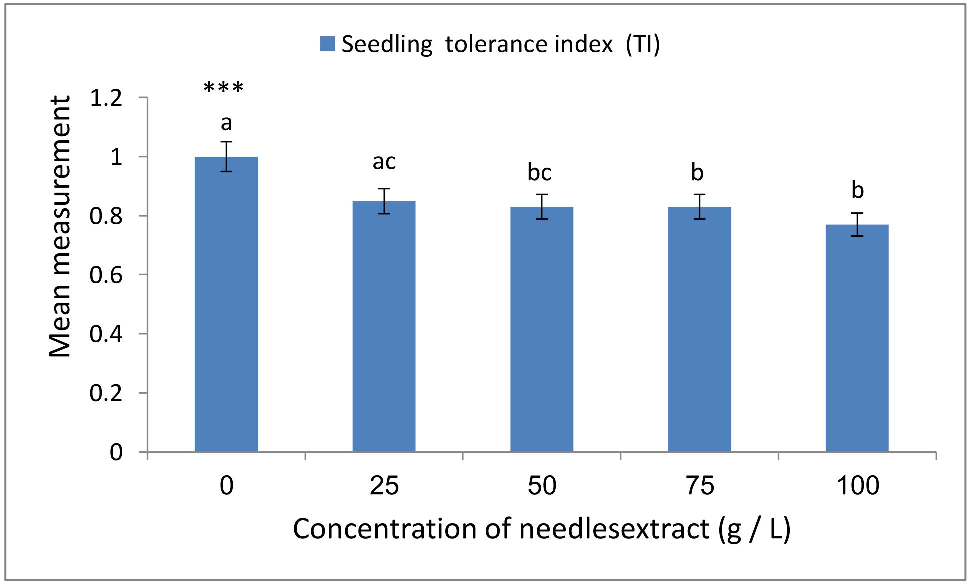

Figure 12. Effect of different concentrations of needles (winter collection) aqueous extract of *P. halepensis* on mean tolerance index of *C. siliqua* seedlings

Bars = SEMean *** = Significant at *P*< 0.001 Different letters = Significant Similar letters = Not significant

followed similar response patterns of that for the fresh mass (Table 11). They all decreased with increasing needles extract levels. There were significant differences within different concentrations used for specific shoot length ($F_{(4, 29)}$ = 24.84; *P* <0.001), specific root length ($F_{(4, 29)}$ = 5.61; *P* <0.001), dry mass of shoot ($F_{(4, 29)}$ = 4.37; *P* <0.001), root ($F_{(4, 29)}$ = 9.57; *P* <0.001), and root / shoot ratio ($F_{(4, 29)}$ = 13.4; *P* <0.001). Tukey's pairwise comparisons test revealed significant differences in specific shoot and root lengths between low concentrations (0.0, 25 g / l) and concentrations of 50, 75, and 100 g / l. For dry mass of shoot and root / shoot ratio, differences were occurred between extract concentrations up to 75 and 100 g / l, for root dry mass the differences were found between concentrations up to 50 and those of 75 and 100 g / l.

Moisture content percentages were calculated in terms of fresh and dry mass of *C. siliqua* seedlings under the effect of different levels of aqueous of *P. halepensis* needles during winter season (Table 12). There were significant differences between various levels of needles extract in these parameters which were significantly reduced in seedlings under higher concentrations (75 and 100 g / l). ANOVA showed differences in fresh mass of shoot ($F_{(4, 29)}$ = 4.63; *P* <0.01), root ($F_{(4, 29)}$ = 7.23; *P* <0.001), dry mass of shoot ($F_{(4, 29)}$ = 4.85; *P* <0.01), and root ($F_{(4, 29)}$ = 8.29; *P* <0.001).

These differences were significant between various treatment means of aqueous extract of *P. halepensis* needles collected in winter season.

3. 2. 2. Effects of bark extracts:

3. 2. 2. 1. Seed germination:

Table 11. Effect of different concentrations of needles (winter collection) aqueous extract of *P. halepensis* on mean measurement of specific length (shoot and root), dry mass (shoot and root), and root / shoot ratio of *C. siliqua* seedlings:

Concentration (g / l)	Specific shoot length	Specific root length	Shoot dry mass (mg)	Root dry mass (mg)	Root / Shoot ratio
0.0	*** 2616.0 ± 108 [a]	*** 8375.0 ± 410 [a]	*** 0.05 ± 0.002 [a]	*** 0.006 ± 0.0002 [a]	*** 0.13 ± 0.008 [a]
25	2321.0 ± 111 [ac]	8043.0 ± 644 [ac]	0.05 ± 0.002 [a]	0.006 ± 0.0003 [a]	0.12 ± 0.007 [a]
50	1980.0 ± 101 [bc]	5613.0 ± 406 [bc]	0.04 ± 0.004 [ac]	0.006 ± 0.0006 [a]	0.12 ± 0.010 [a]
75	1647.0 ± 147 [b]	4748.0 ± 476 [bc]	0.04 ± 0.004 [ac]	0.004 ± 0.0004 [b]	0.09 ± 0.010 [a]
100	999.0 ± 160 [b]	3510 ± 362 [b]	0.03 ± 0.004 [bc]	0.003 ± 0.0004 [b]	0.06 ± 0.010 [b]

± = SEMean *** = Significant at $P < 0.001$ Different letters = Significant Similar letters = Not significant

Table 12. Effect of different concentrations of needles (winter collection) aqueous extract of *P. halepensis* on mean values of moisture content percentages in terms of fresh and dry mass of *C. siliqua* seedlings:

Concentration (g / l)	Moisture content percentages as fresh mass		Moisture content percentages as dry mass	
	Shoot	Root	Shoot	Root
0.0	** 90.12 ± 0.6 [a]	*** 11.78 ± 1.42 [a]	** 88.82 ± 0.56 [a]	*** 10.90 ± 0.56 [a]
25	84.80 ± 3.20 [a]	9.87 ± 0.62 [ac]	83.09 ± 0.94 [a]	8.12 ± 0.60 [ac]
50	80.21 ± 3.90 [ac]	9.13 ± 1.06 [ac]	77.78 ± 6.53 [ac]	8.00 ± 1.10 [ac]
75	76.87 ± 2.10 [ac]	6.79 ± 0.76 [bc]	74.88 ± 6.73 [ac]	5.71 ± 0.78 [bc]
100	70.26 ± 4.60 [bc]	5.07 ± 0.82 [b]	68.90 ± 5.62 [bc]	4.98 ± 0.84 [b]

± = SEMean ** = Significant at $P< 0.01$ *** = Significant at $P< 0.001$ Different letters = Significant

Similar letters = Not significant

The results of daily cumulative germination percentages of *C. siliqua* seeds placed in media of different concentrations of aqueous extract of *P. halepensis* bark of winter season, were of significant low values ($F_{(4, 29)}$ = ; P <0.001) with the higher concentrations up to the sixth day of germination time (Table 13). There were significant differences in cumulative germination ($F_{(4, 29)} = 4.50$; P <0.05) and inhibition percentages ($F_{(4, 29)} = 5.50$; P <0.05) of *C. siliqua* seeds during the third day within different treatments of bark aqueous extract for winter collection (Fig. 13). Tukey's pairwise comparisons test showed significant differences between all bark extract concentrations up to 75 g / l and 100 g / l. Germination rate of the same target plant species was not affected by all levels *P. halepensis* bark (winter collection) solutions (Fig. 14).

3. 2. 2. 2. Early seedling development:

Early seedling development was evaluated in terms of length of shoot, root and seedling, fresh mass of shoot, root, vigor (Fig. 15) and tolerance (Fig. 16) indices of *C. siliqua* seedlings, these measures were taken under the influence of various solutions of aqueous extract of *P. halepensis* bark from winter season (Table 14). Only shoot fresh mass was significantly reduced ($F_{(4, 29)} = 6.10$; P <0.001). Additionally, significant differences were found within different concentrations only for shoot dry mass ($F_{(4, 29)} = 2.93$; P <0.05) (Table 15). Tukey's pairwise comparisons test indicated that, the significant differences were occurred between bark extract concentrations up to 75 g / l and that of 100 g / l for both measures. Root / shoot ratio of *C. siliqua*, as one of dry measurements indicator, was not affected by aqueous extract of *P. halepensis* bark (winter collection).

Table 13. Effect of different concentrations of bark (winter collection) aqueous extract of *P. halepensis* on mean daily germination percentages of *C. siliqua* seeds:

Concentration (g / l)	D 1	D 2	D 3	D 4	D 5	D 6	D 7
	+	+	*	**	***	*	+
0.0	0.0 ± 0.0	0.0 ± 0.0	47.67 ± 3.5 [a]	70.0 ± 3.93 [a]	96.7 ± 2.89 [a]	100.0 ± 0.0 [a]	100.0 ± 0.0
25	0.0 ± 0.0	0.0 ± 0.0	26.67 ± 4.2 [ac]	43.3 ± 4.3 [ac]	66.67 ± 4.3 [b]	96.7 ± 3.3 [ac]	96.67 ± 3.33
50	0.0 ± 0.0	0.0 ± 0.0	26.67 ± 3.3 [ac]	50.0 ± 5.8 [ac]	60.0 ± 5.7 [b]	83.3 ± 4.1[ac]	96.67 ± 3.33
75	0.0 ± 0.0	0.0 ± 0.0	26.67 ± 6.8 [ac]	43.3 ± 4.3 [bc]	63.3 ± 2.9 [b]	80.3 ± 3.9 [bc]	100.0 ± 0.0
100	0.0 ± 0.0	0.0 ± 0.0	6.67 ± 2.77 [bc]	30.0 ± 5.8 [bc]	63.3 ± 2.9 [b]	83.3 ± 4.1 [bc]	93.3 ± 2.99

D = day + = Not significant ± = SEMean * = Significant at $P < 0.05$ ** = Significant at $P < 0.01$

*** = Significant at $P < 0.001$ Different letters = Significant Similar letters = Not significant

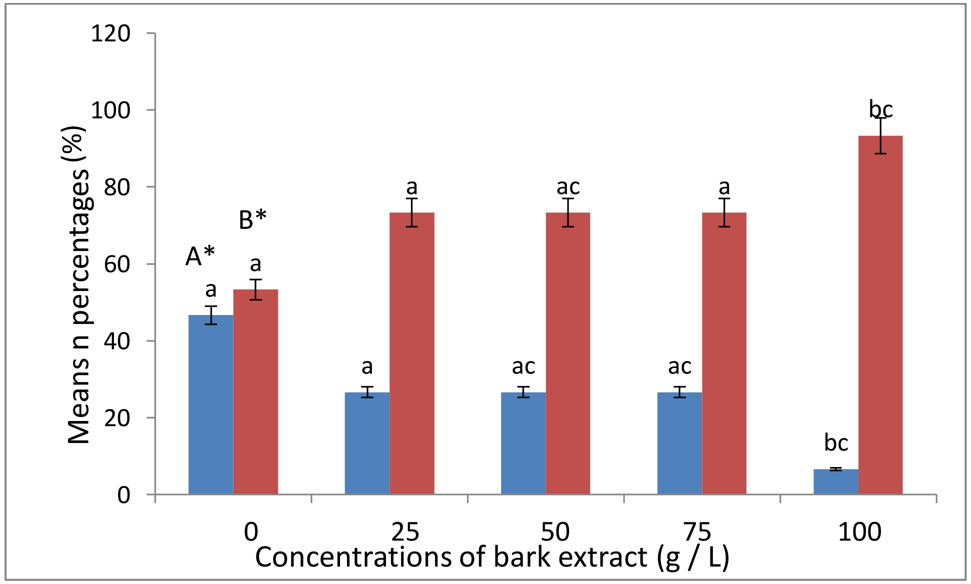

Figure 13. Effect of different concentrations of bark (winter collection) aqueous extract of *P. halepensis* on mean germination promotion (A) and inhibition (B) percentages of *C. siliqua* seeds

Bars = SEMean * = Significant at *P*< 0.05 Different letters = Significant Similar letters = Not significan

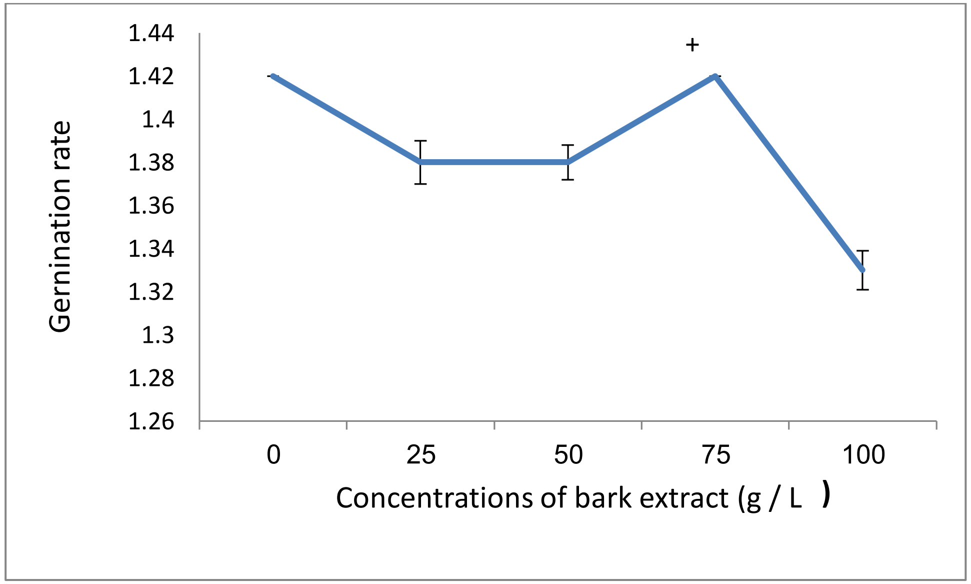

Figure 14. Effect of different concentrations of bark (winter collection) aqueous extract of *P. halepensis* on mean germination rate of *C. siliqua* seeds

Bars = SEMean + = Not significant

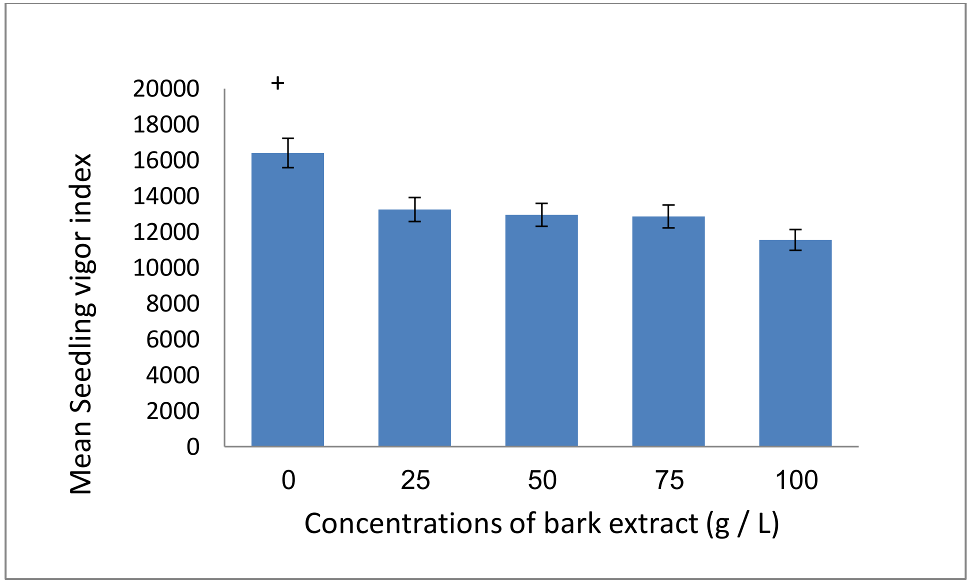

Figure 15. Effect of different concentrations of bark (winter collection) aqueous extract of *P. halepensis* on mean vigor index of *C. siliqua* seedlings

Bars = SEMean + = Not significant

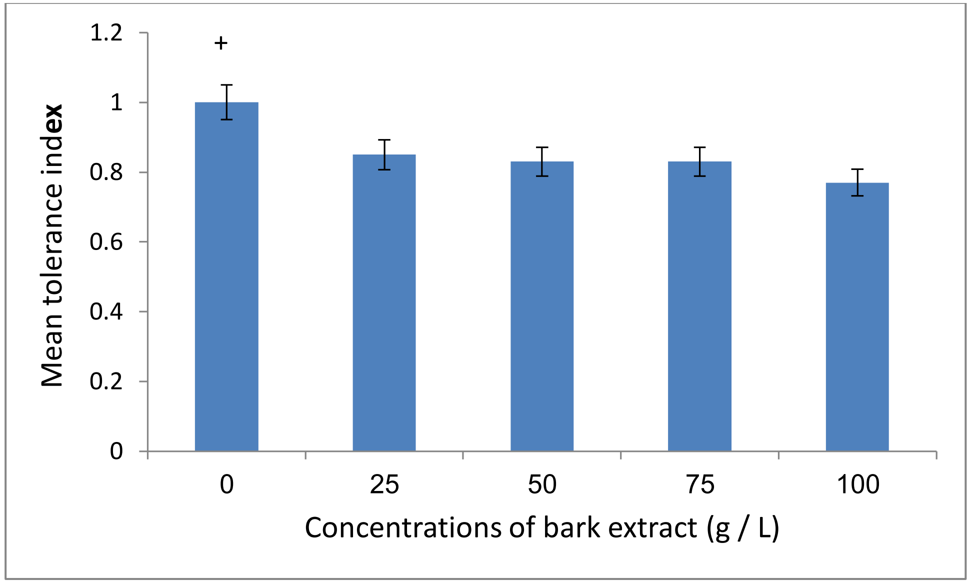

Figure 16. Effect of different concentrations of bark (winter collection) aqueous extract of *P. halepensis* on mean tolerance index of *C. siliqua* seedlings

Bars = SEMean + = Not significant

Table 14. Effect of different concentrations of bark (winter collection) aqueous extract of *P. halepensis* on mean measurement of fresh parameters of *C. siliqua* seedlings:

Concentration (g / l)	Shoot length (mm)	Root length (mm)	Seedling length (mm)	Shoot fresh mass (mg)	Root fresh mass (mg)
0.0	+ 115.0 ± 4.17	+ 48.40 ± 5.02	+ 164.20 ± 4.55	*** 0.49 ± 0.012 [a]	+ 0.054 ± 0.001
25	95.40 ± 5.62	40.67 ± 2.44	136.07 ± 5.12	0.35 ± 0.034 [b]	0.045 ± 0.002
50	94.97 ± 3.53	39.40 ± 3.61	134.37 ± 4.77	0.35 ± 0.032 [b]	0.039 ± 0.003
75	93.53 ± 5.97	39.17 ± 4.11	134.70 ± 6.33	0.33 ± 0.029 [b]	0.039 ± 0.002
100	86.53 ± 4.66	37.13 ± 3.48	123.67 ± 5.02	0.31 ± 0.016 [b]	0.037 ± 0.002

± = SEMean + = Not significant *** = Significant at $P < 0.001$ Different letters = Significant Similar letters = Not significant

Table 15. Effect of different concentrations of bark (winter collection) aqueous extract of *P. halepensis* on mean measurement of specific length (shoot and root), dry mass (shoot and root), and root / shoot ratio of *C. siliqua* seedlings:

Concentration (g / l)	Specific shoot length	Specific root length	Shoot dry mass (mg)	Root dry mass (mg)	Root / Shoot ratio
0.0	+ 2996.9 ± 98	+ 7356.0 ± 52	* 0.044 ± 0.002 [a]	+ 0.007 ± 0.0005	+ 0.17 ± 0.0062
25	2690.1 ± 10	7111.o ± 60	0.034 ± 0.009 [ac]	0.0059 ± 0.0004	0.17 ± 0.0069
50	2593.9 ± 10	6750.0 ± 33	0.034 ± 0.003 [ac]	0.0058 ± 0.0003	0.17 ± 0.0077
75	2537.2 ± 12	5855.0 ± 65	0.033 ± 0.004 [ac]	0.0058 ± 0.0002	0.16 ± 0.0079
100	2443.4 ± 11	5412.0 ± 40	0.023 ± 0.004 [bc]	0.0054 ± 0.0003	0.16 ± 0.057

± = SEMean + = Not significant * = Significant at $P< 0.05$ Different letters = Significant Similar letters = Not significant

Also, moisture content percentages on the basis of fresh and dry mass of the same tested plant seedlings was not affected by the same used media of aqueous extract of *P. halepensis* bark (Table 16).

3. 3. Effects of different concentrations of aqueous extract of *P. halepensis* needles and bark (spring collection) on *C. siliqua*:

3. 3. 1. Effects of needle extracts:

3. 3. 1. 1. Seed germination:

C. siliqua seeds had started to germinate from the second day of germination period, with reduced cumulative germination percentages of aqueous extract of *P. halepensis* needles collected in spring season (Table 17). There were significant differences within different concentrations of the extract the second day of germination process ($F_{(4, 29)} = 3.67$; $P < 0.01$). The inhibition percentages of the second day was significantly higher within different treatment means **($F_{(4, 29)} = 3.67$; $P < 0.01$)** (Fig. 17). Germination rate showed significant ($F_{(4, 29)} = 6.25$; $P < 0.01$) decreased values in seeds of *C. siliqua* treated with higher concentrations from 50 g / l of aqueous extract of *P. halepensis* needles in spring season (Fig. 18). The differences were found to be significant, for both germination and inhibition percentages and germination rate, between lower concentrations (0.0 and 25 g / l) and higher concentrations of 50, 75, and 100 g / l of the tested plant extract.

3. 3. 1. 2. Early seedling development:

Table 18 summaries the mean measurement of fresh parameters of *C. siliqua* seedlings grown in different concentrations of needles (spring collection)

Table 16. Effect of different concentrations of bark (winter collection) aqueous extract of *P. halepensis* on mean values of moisture content percentages in terms of fresh and dry mass of *C. siliqua* seedlings:

Concentration (g / l)	Moisture content percentages as fresh mass		Moisture content percentages as dry mass	
	Shoot	Root	Shoot	Root
0.0	+ 89.92 ± 0.77	+ 12.57 ± 0.44	+ 85.97 ± 0.45	+ 10.60 ± 0.89
25	88.95 ± 3.62	11.84 ± 0.36	85.30 ± 2.11	9.88 ± 0.51
50	89.00 ± 3.01	11.66 ± 0.70	85.61 ± 2.84	8.66 ± 1.02
75	89.61 ± 4.18	10.35 ± 0.66	84.03 ± 2.70	8.46 ± 0.93
100	87.42 ± 1.72 ^{bc}	10.30 ± 0.42	83.90 ± 0.12	7.12 ± 1.50

± = SEMean + = Not significant

Table 17. Effect of different concentrations of needles (spring collection) aqueous extract of *P. halepensis* on mean daily germination percentages of *C. siliqua* seeds:

Concentration (g / l)	D 1	D 2	D 3	D 4	D 5	D 6	D 7
	+	**	*	+	+	+	+
0.0	0.0 ± 0.0	46.67 ± 6.7 [a]	86.67 ± 8.8 [a]	96.67 ± 3.33	96.7 ± 3.33	100.0 ± 3.3	100.0 ± 0.0
25	0.0 ± 0.0	26.67 ± 8.8 [a]	83.33 ± 3.3 [a]	86.67 ± 3.33	96.67 ± 5.7	93.3 ± 6.7	93.33 ± 3.3
50	0.0 ± 0.0	16.67 ± 6.7 [b]	86.67 ± 6.7 [a]	86.67 ± 3.33	90.67 ± 3.3	100.0 ± 5.8	100.0 ± 0.0
75	0.0 ± 0.0	6.67 ± 3.33 [b]	56.67 ± 3.3 [bc]	83.33 ± 3.33	90.0 ± 5.77	900.0 ± 5.7	96.67 ± 3.3
100	0.0 ± 0.0	3.33 ± 3.33 [b]	76.67 ± 3.3 [bc]	83.33 ± 3.33	90.0 ± 5.77	90.0 ± 5.7	93.33 ± 3.3

D = day + = Not significant ± = SEMean * = Significant at $P < 0.05$ ** = Significant at $P < 0.01$

Different letters = Significant Similar letters = Not significant

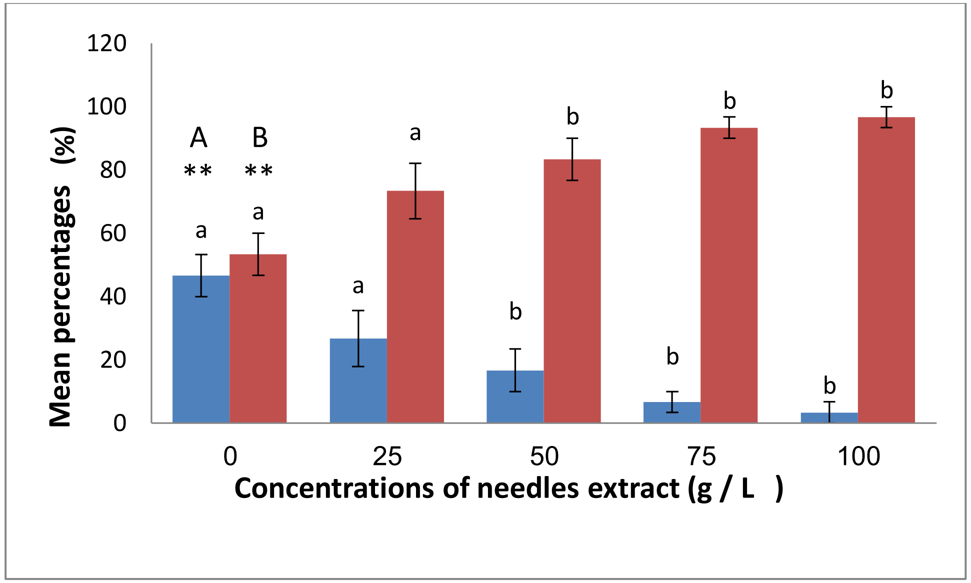

Figure 17. Effect of different concentrations of needles (spring collection) aqueous extract of *P. halepensis* on mean germination promotion (A) and inhibition (B) percentages of *C. siliqua* seeds

Bars = SEMean ** = Significant at *P*< 0.01 Different letters = Significant Similar letters = Not significant

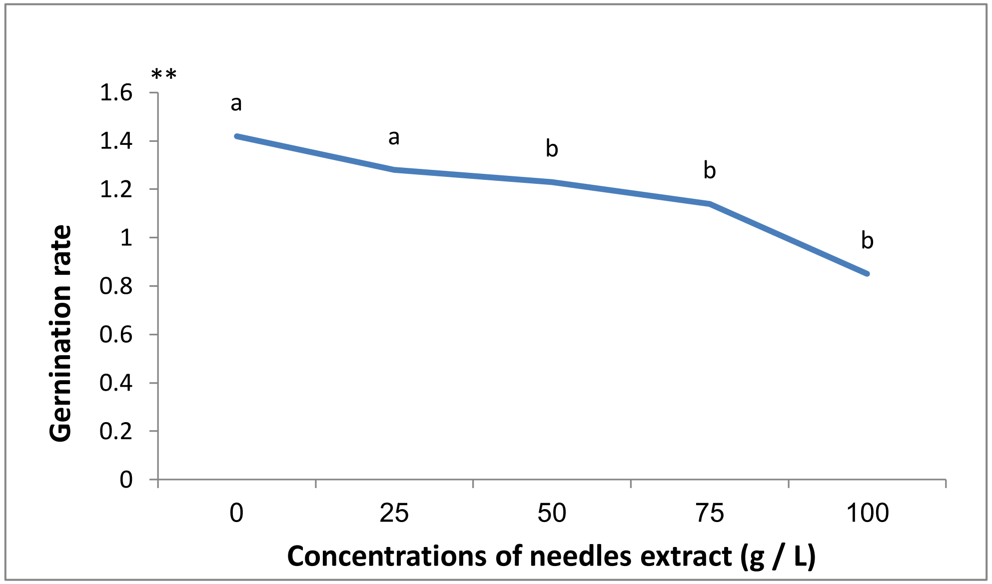

Figure 18. Effect of different concentrations of needles (spring collection) aqueous extract of *P. halepensis* on mean germination rate of *C. siliqua* seeds

Bars = SEMean ** = Significant at *P*< 0.001 Different letters = Significant Similar letters = Not significant

Table 18. Effect of different concentrations of needles (spring collection) aqueous extract of *P. halepensis* on mean measurement of fresh parameters of *C. siliqua* seedlings:

Concentration (g / l)	Shoot length (mm)	Root length (mm)	Seedling length (mm)	Shoot fresh mass (mg)	Root fresh mass (mg)
0.0	+ 113.52 ± 27.12	** 56.55 ± 4.81 [a]	** 179.07 ± 8.66 [a]	+ 0.523 ± 0.031	* 0.060 ± 0.004[a]
25	110.83 ± 22.33	55.83 ± 4.70 [ac]	166.66 ± 7.00 [ac]	0.513 ± 0.014	0.006 ± 0.004 [ac]
50	101.87 ± 19.73	51.67 ± 2.84 [ac]	153.53 ± 5.29 [bc]	0.491 ± 0.018	0.0491 ± 0.004 [ac]
75	101.0 ± 21.97	49.04 ± 3.26 [bc]	150.04 ± 6.36 [bc]	0.496 ± 0.019	0.0480 ± 0.002 [ac]
100	99.38 ± 19.02	44.93 ± 2.39 [bc]	144.31 ± 4.51 [bc]	0.483 ± 0.180	0.0460 ± 0.003 [bc]

± = SEMean + = Not significant * = Significant at $P < 0.05$ ** = Significant at $P < 0.01$
Different letters = Significant Similar letters = Not significant

aqueous extract of *P. halepensis*. ANOVA revealed significant differences within various treatments of the tested plant extract, with reduced values of root length ($F_{(4, 29)}$ = 22.61; P <0.01), seedling length ($F_{(4, 29)}$ = 19.05; P <0.01), and root fresh mass ($F_{(4, 29)}$ = 9.11; P <0.05). Furthermore, there were significant differences in seedling vigor ($F_{(4, 29)}$ = 19.05; P <0.01) and seedling tolerance ($F_{(4, 29)}$ = 19.05; P <0.01) indices *C. siliqua* seedlings developed under the effect of different concentrations of aqueous extract of *P. halepensis* needles as shown in figures 19 and 20 respectively.

Tukey's pairwise comparisons test indicated that, the significant differences for root length, were found between concentrations of 0.0, 25, 50 g / l and 75, 100 g / l, for seedling length and tolerance index, were between low concentrations include 0.0 g / l and others from 50 g / l., and for root fresh mass and vigor index, the differences were obtained between 0.0 and 100 g / l of spring needles extract.

Different concentrations of needle (spring collection) aqueous extract of *P. halepensis* had no inhibitory effects on the mean measurements of specific length (shoot and root), dry mass (shoot and root), and root / shoot ratio of *C. siliqua* seedlings (Table 19). Needles (spring collection) aqueous extract of *P. halepensis* also showed no inhibition of the mean values of moisture content percentages in terms of fresh and dry mass of *C. siliqua* seedlings (Table 20).

3. 3. 2. Effects of bark extracts:

3. 3. 2. 1. Seed germination:

The results of daily cumulative germination percentages of *C. siliqua* seeds placed in different applications of aqueous extract of *P. halepensis* bark collected in spring are shown in table 21. These measurements were with significant low

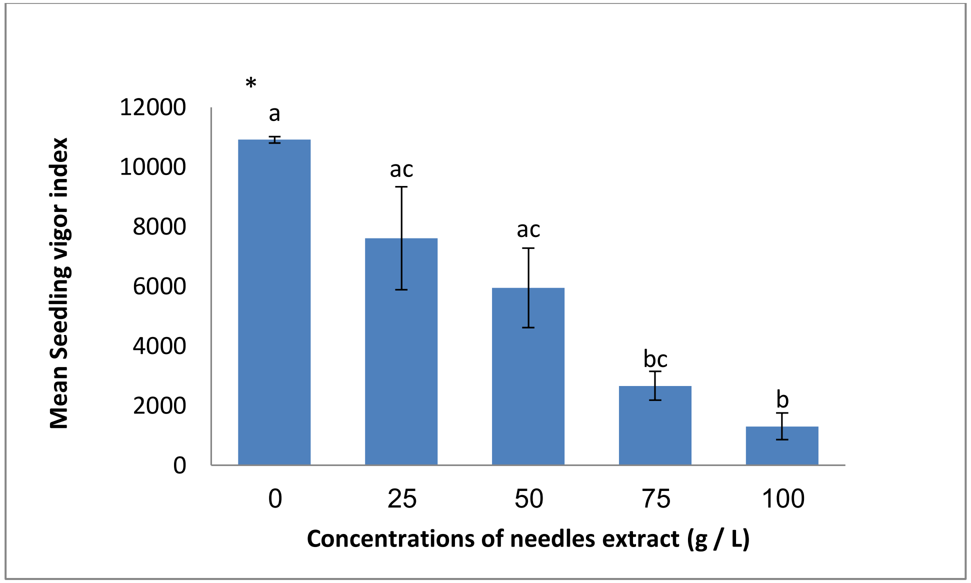

Figure 19. Effect of different concentrations of needles (spring collection) aqueous extract of *P. halepensis* on mean vigor index of *C. siliqua* seedlings

Bars = SEMean * = Significant at $P< 0.05$ Different letters = Significant Similar letters = Not significant

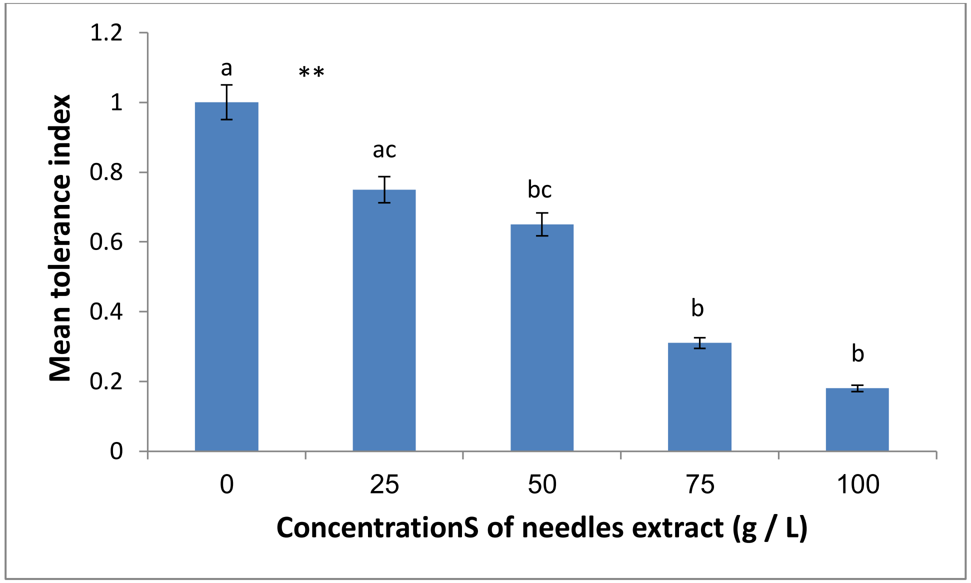

Figure 20. Effect of different concentrations of needles (winter collection) aqueous extract of *P*. *halepensis* on mean tolerance index of *C*. *siliqua* seedlings

Bars = SEMean ** = Significant at *P*< 0.01 Different letters = Significant Similar letters = Not significant

Table 19. Effect of different concentrations of needles (spring collection) aqueous extract of *P. halepensis* on mean measurement of specific length (shoot and root), dry mass (shoot and root), and root / shoot ratio of *C. siliqua* seedlings:

Concentration (g / l)	Specific shoot length	Specific root length	Shoot dry mass (mg)	Root dry mass (mg)	Root / Shoot ratio
0.0	+ 2787.7 ± 74.0	+ 11998.0 ± 490	+ 0.05 ± 0.0021	+ 0.0058 ± 0.0004	+ 0.130 ± 0.028
25	2395.8 ± 96.9	11728.0 ± 946	0.047 ± 0.0021	0.0055 ± 0.0003	0.120 ± 0.029
50	2248.0 ± 96.8	11024.0 ± 676	0.046 ± 0.014	0.0051 ± 0.0003	0.110 ± 0.041
75	2221.5 ± 86.7	10974.0 ± 2078	0.046 ± 0.0021	0.0050 ± 0.0002	0.110 ± 0.026
100	2239.1 ± 91.9	10916.0 ± 1144	0.045 ± 0.0013	0.0046 ± 0.0002	0.100 ± 0.025

± = SEMean + = Not significant

Table 20. Effect of different concentrations of needles (spring collection) aqueous extract of *P. halepensis* on mean values of moisture content percentages in terms of fresh and dry mass of *C. siliqua* seedlings:

Concentration (g / l)	Moisture content percentages as fresh mass		Moisture content percentages as dry mass	
	Shoot	Root	Shoot	Root
0.0	+ 89.14 ± 1.59	+ 11.59 ± 1.59	+ 89.69 ± 0. 86	+ 10.30 ± 0.68
25	88.48 ± 0.67	10.51 ± 0.67	89.37 ± 0.67	10.00 ± 0.67
50	87.55 ± 0.60	9.08 ± 0.60	90.15 ± 0.54	8.84 ± 0.54
75	87.09 ± 0.40	8.90 ± 0.40	90.22 ± 0.53	7.77 ± 0.53
100	85.03 ± 0.82	9.59 ± 0.82	90.59 ± 0.55	5.99 ± 0.55

± = SEMean + = Not significant

Table 21. Effect of different concentrations of bark (spring collection) aqueous extract of *P. halepensis* on mean daily germination percentages of *C. siliqua* seeds:

Concentration (g / l)	D 1	D 2	D 3	D 4	D 5	D 6	D 7
	+	+	*	**	**	+	+
0.0	2.0 ± 0.0	0.0 ± 0.0	50.00 ± 5.8 [a]	73.33 ± 3.3 [a]	96.67 ± 3.3 [a]	100.0 ± 0.0	100.0 ± 0.0
25	0.0 ± 0.0	0.0 ± 0.0	40.00 ± 5.8 [ac]	63.33 ± 3.3 [a]	86.67 ± 3.3 [a]	96.67 ± 3.3	96.67 ± 3.3
50	0.0 ± 0.0	0.0 ± 0.0	26.67 ± 3.3 [ac]	50.00 ± 5.8 [ac]	80.00 ± 5.8 [ac]	100.0 ± 0.0	100.0 ± 0.0
75	0.0 ± 0.0	0.0 ± 0.0	33.33 ± 6.8 [ac]	60.00 ± 5.8 [a]	76.67 ± 3.3 [ac]	96.67 ± 3.3	100.0 ± 0.0
100	0.0 ± 0.0	0.0 ± 0.0	20.00 ± 5.8 [bc]	26.67 ± 6. 7 [bc]	56.67 ± 3.3 [bc]	93.33 ± 3.3	96.67 ± 3.3

D = day + = Not significant ± = SEMean * = Significant at $P < 0.05$ ** = Significant at $P < 0.01$

Different letters = Significant Similar letters = Not significant

values up to the fifth day ($F_{(4, 29)}$ = ; *P* <0.01) of germination time. There were significant differences in decreased cumulative germination ($F_{(4, 29)} = 4.36$; *P* <0.05) and in increased inhibition ($F_{(4, 29)}$ = ; *P* <0.01) percentages of *C. siliqua* seeds germinated under the aqueous extract of *P. halepensis* bark (Fig. 21). Tukey's pairwise comparisons test showed significant differences, in cumulative seed germination during the fifth day, between the control treatment and that of 100 g / l spring bark concentration. For both seed germination and inhibition percentages, the differences were between seeds under control treatment and those under 75 and 100 g / l.

All various levels of *P. halepensis* bark collected in spring season showed no inhibitory effects on the germination rate of *C. siliqua* seeds (Fig. 22).

3. 3. 2. 2. Early seedling development:

The response of fresh parameters of shoot length, root length, seedling length, shoot fresh mass, root fresh mass, seedling vigor index, and seedling tolerance index were evaluated for *C. siliqua* seedlings developed under treatment levels of aqueous extract of *P. halepensis* bark collected in spring season (Table 22).

ANOVA showed significant reductions in shoot length ($F_{(4, 29)} = 22.99$; *P* <0.05), seedling length ($F_{(4, 29)} = 3.21$; *P* <0.05), shoot fresh mass ($F_{(4, 29)} = 2.81$; *P* <0.05), root fresh mass ($F_{(4, 29)} = 2.83$; *P* <0.05), seedling vigor index ($F_{(4, 29)} = 5.79$; *P* <0.05) (Fig. 23), and seedling tolerance index ($F_{(4, 29)} = 2.44$; *P* <0.05) (Fig. 24). Tukey's pairwise comparisons test revealed significant differences, in all mentioned above parameters, between control treatment (0.0 g / l) and concentrations of 75 and 100 g / l of spring bark extract of *P. halepensis*. Whereas, both dry measuresand moisture content percentages

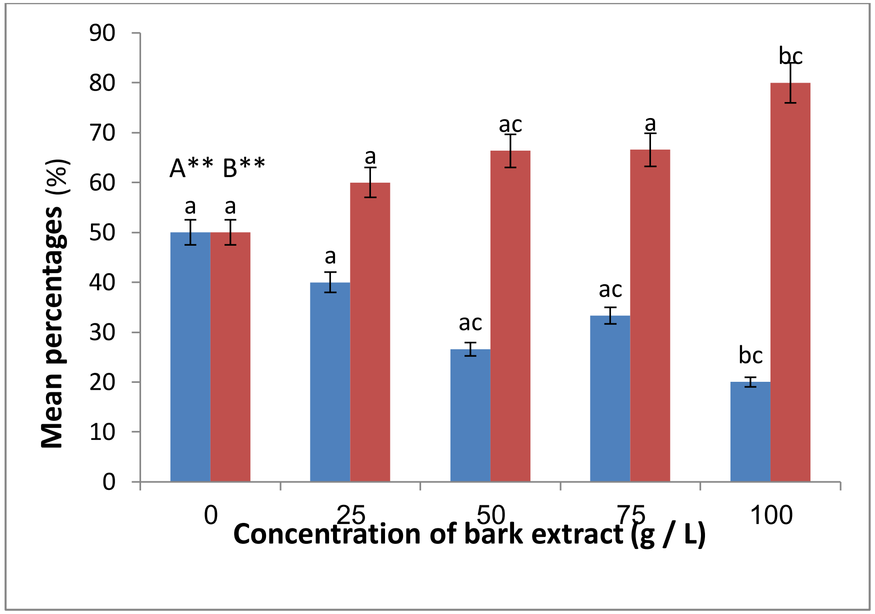

Figure 21. Effect of different concentrations of bark (spring collection) aqueous extract of *P. halepensis* on mean germination promotion (A) and inhibition (B) percentages of *C. siliqua* seeds

Bars = SEMean   ** = Significant at *P*< 0.01   Different letters = Significant   Similar letters = Not significant

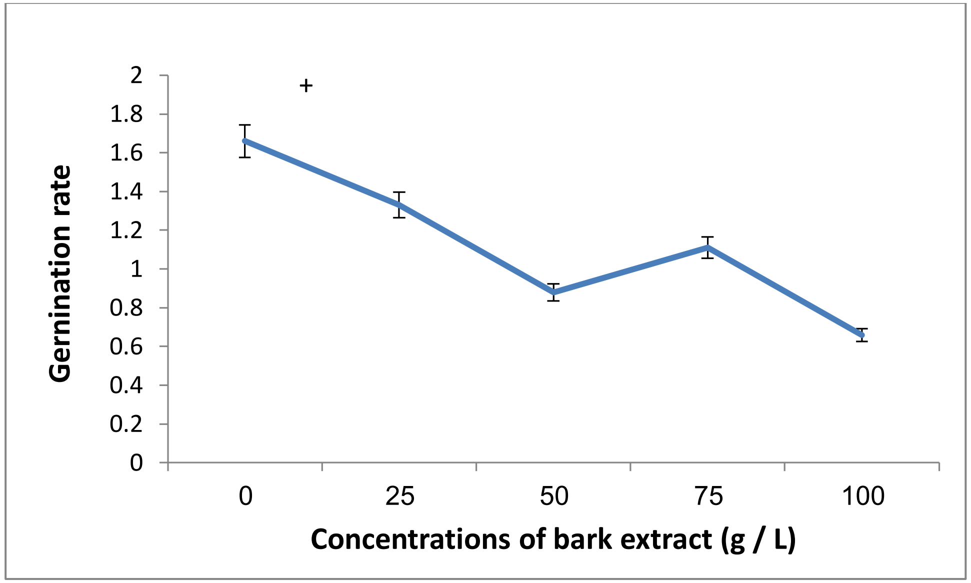

Figure 22. Effect of different concentrations of bark (spring collection) aqueous extract of *P. halepensis* on mean germination rate of *C. siliqua* seeds

Bars = SEMean + = Not significant

Table 22. Effect of different concentrations of bark (spring collection) aqueous extract of *P. halepensis* on mean measurement of fresh parameters of *C. siliqua* seedlings:

Concentration (g / l)	Shoot length (mm)	Root length (mm)	Seedling length (mm)	Shoot fresh mass (mg)	Root fresh mass (mg)
0.0	* 118.83 ± 4.77 [a]	+ 44.20 ± 3.05	* 163.03 ± 6.68 [a]	* 0.525 ± 0.022 [a]	* 0.048 ± 0.001[a]
25	104.83 ± 6.85 [ac]	40.30 ± 3.55	145.13 ± 9.66 [ac]	0.484 ± 0.028 [ac]	0.044 ± 0.003 [ac]
50	99.03 ± 6.26 [ac]	40.70 ± 3.01	139.73 ± 6.67 [ac]	0.444 ± 0.022 [ac]	0.045 ± 0.005 [ac]
75	92.90 ± 7.37 [bc]	35.30 ± 3.21	128.20 ± 9.29 [bc]	0.412 ± 0.032 [bc]	0.036 ± 0.002 [ac]
100	94.97 ± 5.40 [bc]	34.40 ± 2.38	129.37 ± 6.62 [bc]	0.435 ± 0.026 [bc]	0.034 ± 0.002 [bc]

± = SEMean + = Not significant * = Significant at $P < 0.05$ Different letters = Significant Similar letters = Not significant

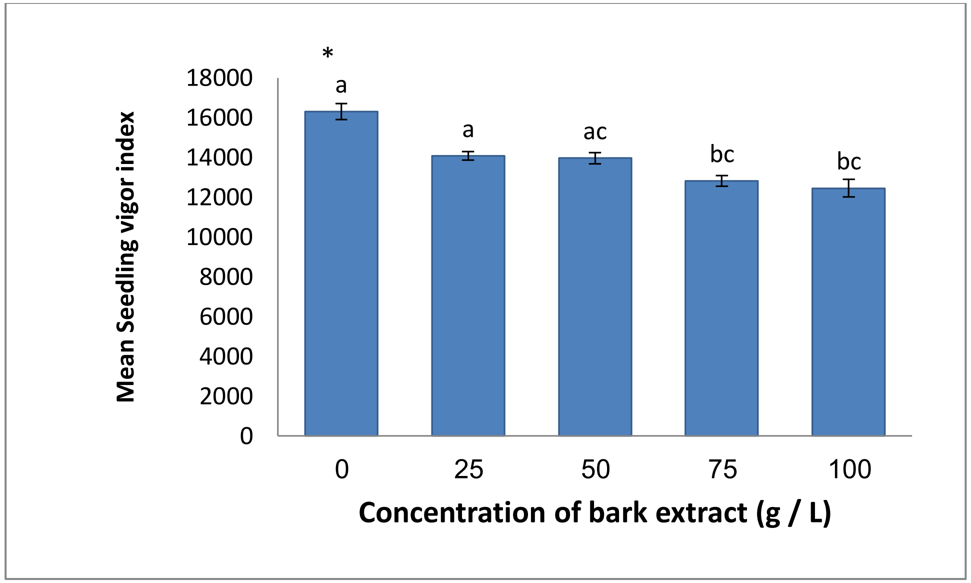

Figure 23. Effect of different concentrations of bark (spring collection) aqueous extract of *P. halepensis* on mean vigor index of *C. siliqua* seedlings

Bars = SEMean * = Significant at *P*< 0.05

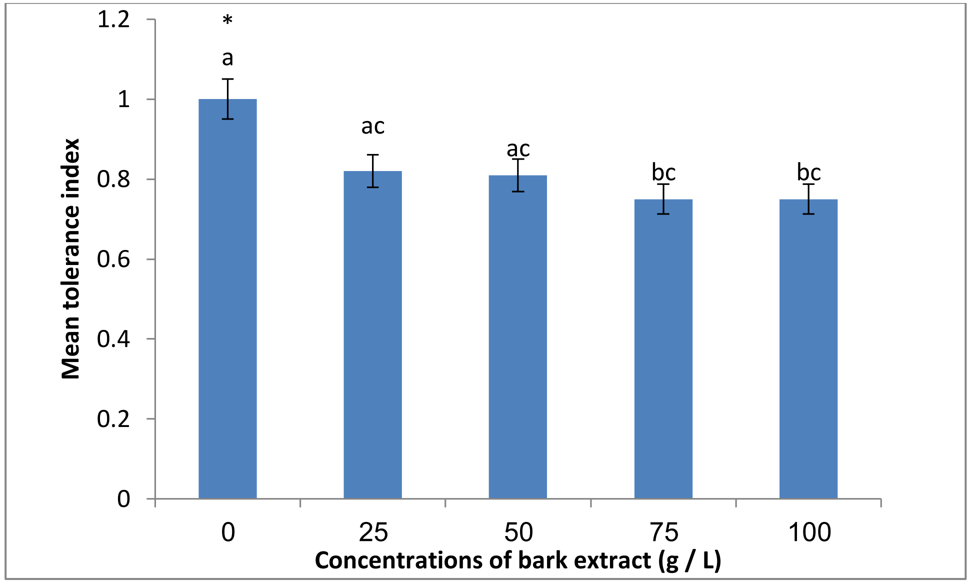

Figure 24. Effect of different concentrations of bark (spring collection) aqueous extract of *P. halepensis* on mean tolerance index of *C. siliqua* seedlings

Bars = SEMean * = Significant at $P < 0.05$

in terms of fresh and dry mass of *C. siliqua* seedlings were not affected by exogenous application of various treatments of *P. halepensis* bark collected in spring season as shown in tables 23 and 24 respectively.

3. 4. Effects of different concentrations of aqueous extract of *P. halepensis* needles and bark (summer collection) on *C. siliqua*:

3. 4. 1. Effects of needle extracts:

3. 4. 1. 1. Seed germination:

The mean of daily cumulative seed germination percentages of *C. siliqua* germinated in different concentrations of aqueous extract *P.halepensis* needles of summer collection were decreased under all levels of the tested plant extract (Table 25), with significant ($F_{(4, 29)} = 2.66$; $P < 0.05$) great reduction during the third day of germination period. Significant differences were also found within various treatment levels for germination promotion ($F_{(4, 29)} = 3.80$; $P < 0.05$) and germination inhibition ($F_{(4, 29)} = 3.80$; $P < 0.05$) percentages (Fig. 25). Tukey's pairwise comparisons test indicated significant differences, in both parameters, between seeds

under the influence of control treatment (0.0 g / l) and those under 100 g / l of the same needles of summer collection. Whereas, germination rate of the same seeds was not affected by needles extract of *P. halepensis* collected in summer season (Fig. 26).

3. 4. 1. 2. Early seedling development:

Fresh measures of *C. siliqua* seedlings include, length of root, seedling length, fresh mass of shoot, and root were not affected by different concentrations of

Table 23. Effect of different concentrations of bark (spring collection) aqueous extract of *P. halepensis* on mean measurement of specific length (shoot and root), dry mass (shoot and root), and root / shoot ratio of *C. siliqua* seedlings:

Concentration (g / l)	Specific shoot length	Specific root length	Shoot dry mass (mg)	Root dry mass (mg)	Root / Shoot ratio
0.0	+ 2207.0 ± 103	+ 6915.0 ± 559	+ 0.053 ± 0.0024	+ 0.0067 ± 0.0002	+ 0.13 ± 0.0072
25	2038.0 ± 164	6498.0 ± 602	0.050 ± 0.0029	0.0060 ± 0.0003	0.12 ± 0.0085
50	1943.0 ± 123	8850.0 ± 407	0.050 ± 0.025	0.058 ± 0.0004	0.11 ± 0.0083
75	1829.0 ± 149	5987.0 ± 794	0.049 ± 0.0031	0.0054 ± 0.0004	0.11 ± 0.0087
100	1868.0 ± 104	6138 ± 420	0.049 ± 0.0022	0.0056 ± 0.0003	0.11 ± 0.0057

± = SEMean + = Not significant

Table 24. Effect of different concentrations of bark (spring collection) aqueous extract of *P. halepensis* on mean values of moisture content percentages in terms of fresh and dry mass of *C. siliqua* seedlings:

Concentration (g / l)	Moisture content percentages as fresh mass		Moisture content percentages as dry mass	
	Shoot	Root	Shoot	Root
0.0	+ 90.99 ± 0.68	+ 11.27 ± 0.55	+ 87.01 ± 0.55	+ 9.00 ± 0. 86
25	88.77 ± 4.25	10.89 ± 0.71	82.68 ± 4.14	8.83 ± 0.57
50	86.00 ± 3.18	9.83 ± 0.67	82.78 ± 3.05	8.89 ± 1.02
75	85.59 ± 5.19	9.51 ± 0.75	81.48 ± 3.00	8.41 ± 1.31
100	82.16 ± 1.21	9.01 ± 0.22	80.63 ± 0.45	8.84 ± 1.21

± = SEMean + = Not significant

Table 25. Effect of different concentrations of needles (summer collection) aqueous extract of *P. halepensis* on mean daily germination percentages of *C. siliqua* seeds:

Concentration (g / l)	D 1	D 2	D 3	D 4	D 5	D 6	D 7
	+	+	*	+	*	**	+
0.0	0.0 ± 0.0	0.0 ± 0.0	56.67 ± 3.3 [a]	73.33 ± 3.33	96.7 ± 3.33 [a]	100.0 ± 0.0 [a]	100.0 ± 0.0
25	0.0 ± 0.0	0.0 ± 0.0	43.33 ± 3.3 [ac]	60.00 ± 5.77	90.00 ± 6.7 [a]	100.0 ± 0.0 [ac]	100.0 ± 0.0
50	0.0 ± 0.0	0.0 ± 0.0	43.33 ± 3.3 [ac]	60.00 ± 10.0	86.67 ± 8.8 [a]	96.00 ± 3.3 [ac]	86.6 ± 5.8
75	0.0 ± 0.0	0.0 ± 0.0	33.33 ± 3.3 [ac]	56.67 ± 3.33	70.0 ± 5.77 [b]	100.0 ± 0.0 [ac]	100.0 ± 0.0
100	0.0 ± 0.0	0.0 ± 0.0	20.00 ± 6.8 [bc]	50.0 ± 5.77	73.33 ± 5.8 [b]	86.67 ± 3.3 [bc]	93.33 ± 3.3

D = day + = Not significant ± = SEMean * = Significant at $P< 0.05$ ** = Significant at $P< 0.01$

Different letters = Significant Similar letters = Not significant

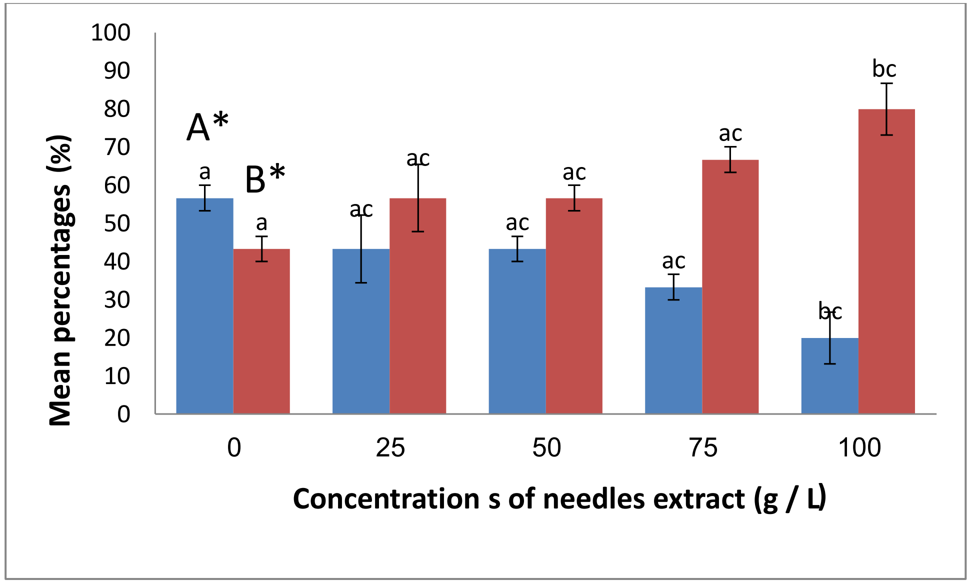

Figure 25. Effect of different concentrations of needles (summer collection) aqueous extract of *P. halepensis* on mean germination promotion (A) and inhibition (B) percentages of *C. siliqua* seeds

Bars = SEMean ** = Significant at $P< 0.01$ Different letters = Significant Similar letters = Not significant

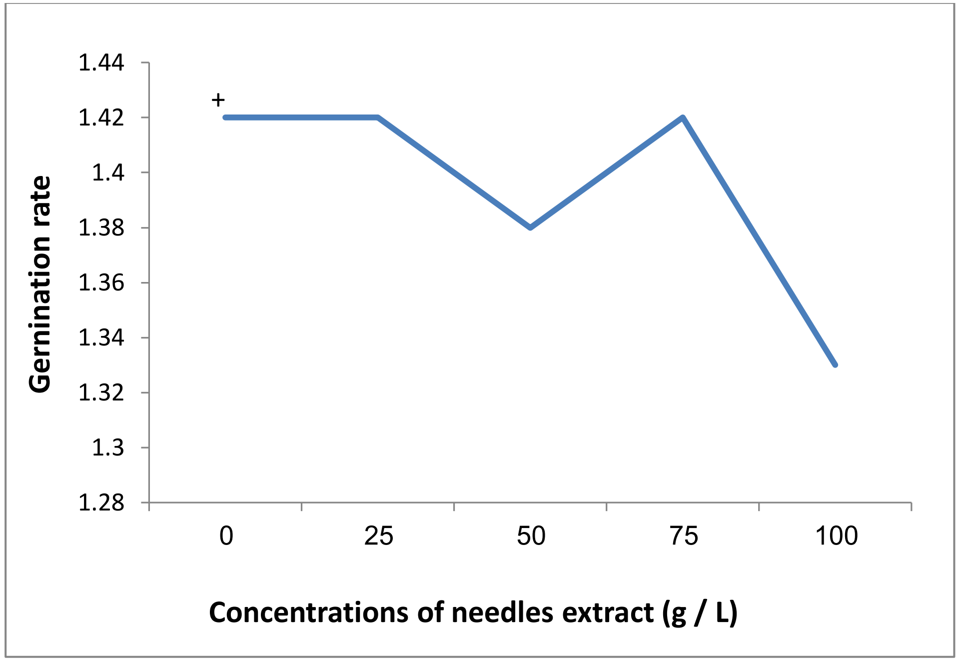

Figure 26. Effect of different concentrations of needles (summer collection) aqueous extract of *P. halepensis* on mean germination rate of *C. siliqua* seeds

Bars = SEMean ** = Significant at *P*< 0.001 Different letters = Significant Similar letters = Not significant

summer needles extract. But shoot length was of significant ($F_{(4, 29)} = 12.69$; $P < 0.05$) low value with the effect of 100 g / l concentration of *P. halepensis* needles of summer season (Table 26). The differences were found to be not significant, for this parameter, between seedling established under control level (0.0 g / l) and those under all treatments of the same media. Both seedling vigor and tolerance indices of the target plant seedling were not affected by all *P. halepensis* needles extract of summer as shown in figures 27 and 28 respectively.

Furthermore, dry measure responses of *C. siliqua* seedlings such as, specific shoot and root length, dry mass of shoot and root, and root / shoot ratio, along with moisture content percentages were not significantly affected by external application of various levels of aqueous extract of *P. halepensis* needles collected in summer season, as shown in tables 27 and 28 respectively.

3. 4. 2. Effects of bark extracts:

3. 4. 2. 1. Seed germination:

P. halepensis bark (summer collection) extract of different concentrations was tested for the germination process of *C. siliqua* seeds, which had started to germinate from the third day of germination time (Table 29). Mean daily germination percentages was significantly ($F_{(4, 29)} = 3.80$; $P < 0.05$) decreased during this day. There were significant differences within different treatments of bark extract for seed germination promotion ($F_{(4, 29)} = 3.80$; $P < 0.05$) and seed germination inhibition ($F_{(4, 29)} = 14.53$; $P < 0.05$) percentages (Fig. 29).

Tukey's pairwise comparisons test revealed significant differences in both germination promotion and inhibition percentages *C. siliqua* seeds under control treatment (0.0 g / l) and all other treatment levels of bark extract of

Table 26. Effect of different concentrations of needles (summer collection) aqueous extract of *P. halepensis* on mean measurement of fresh parameters of *C. siliqua* seedlings:

Concentration (g / l)	Shoot length (mm)	Root length (mm)	Seedling length (mm)	Shoot fresh mass (mg)	Root fresh mass (mg)
0.0	* 116.3 ± 3.43 [a]	+ 43.27 ± 2.21	+ 154.77 ± 5.22	+ 0.49 ± 0.017	+ 0.042 ± 0.004
25	109.8 ± 10.3 [ac]	40.00 ± 4.21	149.80 ± 9.10	0.47 ± 0.047	0.037 ± 0.004
50	98.33 ± 6.63 [ac]	37.23 ± 2.42	135.52 ± 8.66	0.44 ± 0.030	0.036 ± 0.003
75	94.27 ± 6.07 [ac]	37.17 ± 2.52	131.43 ± 7.66	0.42 ± 0.024	0.035 ± 0.002
100	87.07 ± 6.94 [bc]	36.00 ± 3.07	126.22 ± 10.00	0.38 ± 0.033	0.035 ± 0.003

± = SEMean + = Not significant * = Significant at $P < 0.05$ Different letters = Significant Similar letters = Not significant

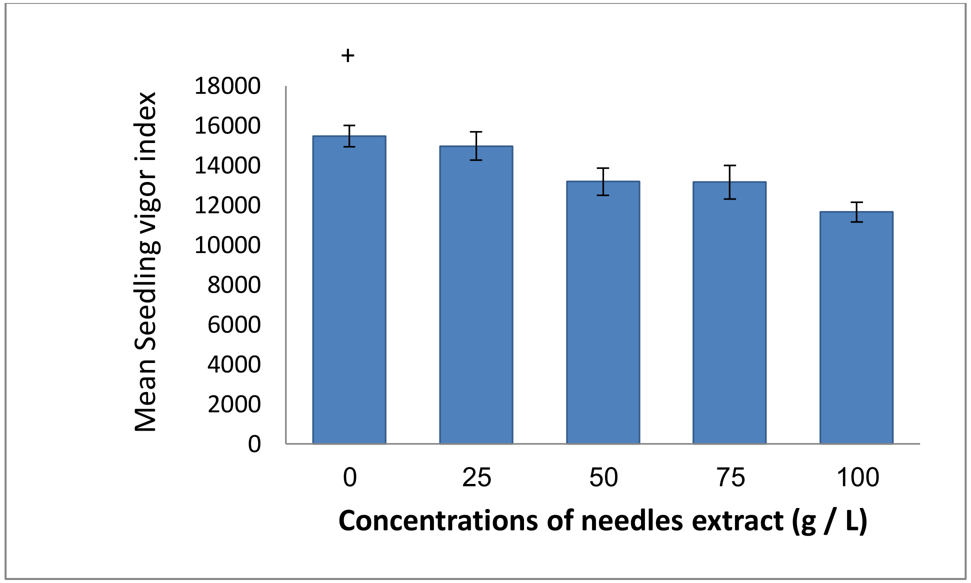

Figure 27. Effect of different concentrations of needles (summer collection) aqueous extract of *P. halepensis* on mean vigor index of *C. siliqua* seedlings

Bars = SEMean ± = SEMean + = Not significant

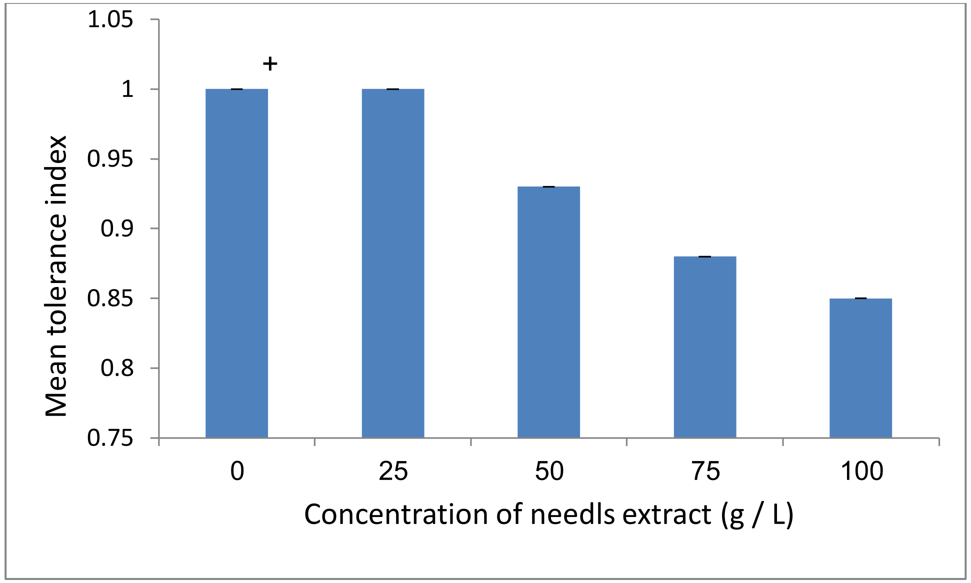

Figure 28. Effect of different concentrations of needles (summer collection) aqueous extract of *P. halepensis* on mean tolerance index of *C. siliqua* seedlings

Bars = SEMean ± = SEMean + = Not significant

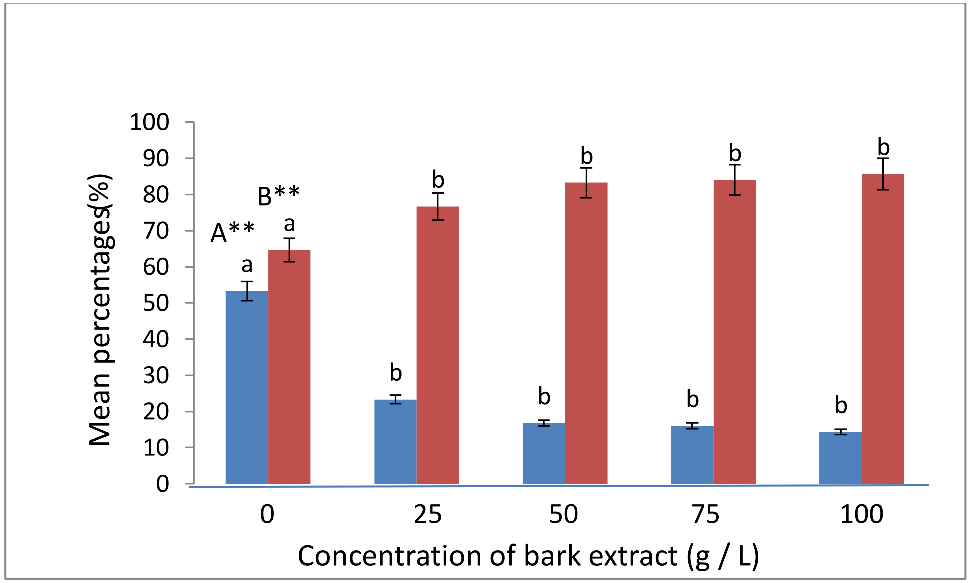

Figure 29. Effect of different concentrations of bark (summer collection) aqueous extract of *P. halepensis* on mean germination promotion (A) and inhibition (B) percentages of *C. siliqua* seeds

Bars = SEMean ** = Significant at *P*< 0.01 Different letters = Significant Similar letters = Not significa

Table 27. Effect of different concentrations of needles (summer collection) aqueous extract of *P. halepensis* on mean measurement of specific length (shoot and root), dry mass (shoot and root), and root / shoot ratio of *C. siliqua* seedlings:

Concentration (g / l)	Specific shoot length	Specific root length	Shoot dry mass (mg)	Root dry mass (mg)	Root / Shoot ratio
0.0	+ 2368.2 ± 83.0	+ 8179.0 ± 38.60	+ 0.055 ± 0.001	+ 0.0063 ± 0.0004	+ 0.11 ± 0.03
25	2302.7 ± 96.0	7194.0 ± 87.51	0.048 ± 0.0024	0.0054 ± 0.0002	0.11 ± 0.05
50	2114.0 ± 56.0	7098.0 ± 68.43	0.047 ± 0.002	0.0050 ± 0.0001	0.11 ± 0.03
75	2120.0 ± 50.0	7003.0 ± 76.00	0.043 ± 0.002	0.0048 ± 0.0006	0.11 ± 0.02
100	2040.0 ± 83.9	7089 ± 51.03	0.043 ± 0.003	0.0048 ± 0.0002	0.11 ± 0.02

± = SEMean + = Not significant

Table 28. Effect of different concentrations of needles (summer collection) aqueous extract of *P. halepensis* on mean values of moisture content percentages in terms of fresh and dry mass of *C. siliqua* seedlings:

Concentration (g / l)	Moisture content percentages as fresh mass		Moisture content percentages as dry mass	
	Shoot	Root	Shoot	Root
0.0	+ 91.90 ± 0.51	+ 11.44 ± 1.59	+ 89.82 ± 0.68	+ 8.10 ± 0.51
25	92.00 ± 0.48	10.00 ± 0.76	88.60 ± 0.67	7.90 ± 0.48
50	91.80 ± 0.44	9.90 ± 0.60	88.20 ± 0.54	8.00 ± 0.33
75	92.00 ± 0.42	10.08 ± 0.0.40	88.00 ± 0.53	7.60 ± 0.75
100	92.00 ± 0.75	10.01 ± 0.82	88.04 ± 0.55	7.60 ± 0.66

± = SEMean + = Not significant

Table 29. Effect of different concentrations of bark (summer collection) aqueous extract of *P. halepensis* on mean daily germination percentages of *C. siliqua* seeds:

Concentration (g / l)	D 1	D 2	D 3	D 4	D 5	D 6	D 7
0.0	+ 0.0 ± 0.0	+ 0.0 ± 0.0	** 53.33 ± 6.8 [a]	+ 60.00 ± 5.8	+ 96.7 ± 3.33	+ 100.0 ± 0.0	+ 100.0 ± 0.0
25	0.0 ± 0.0	0.0 ± 0.0	23.33 ± 3.3 [b]	40.00 ± 5.8	100.0 ± 0.0	100.0 ± 0.0	96.67 ± 3.3
50	0.0 ± 0.0	0.0 ± 0.0	16.76 ± 3.3 [b]	46.67 ± 6.7	93.33 ± 6.7	96.67 ± 3.3	96.67 ± 3.3
75	0.0 ± 0.0	0.0 ± 0.0	16.00 ± 5.8 [b]	50.00 ± 5.8	90.00 ± 10.0	96.67 ± 3.3	96.67 ± 3.3
100	0.0 ± 0.0	0.0 ± 0.0	14.33 ± 3.3 [b]	50.00 ± 5.8	90.00 ± 10.0	100.0 ± 0.0	100.0 ± 0.0

D = day + = Not significant ** = Significant at $P < 0.01$

P. halepensis collected in summer season. Also, different concentrations of the same extract showed no effects on the germination rate of the same target plant species (Fig. 30).

3. 4. 2. 2. Early seedling development:

C. siliqua seedlings were subjected to various treatments of bark extract for the evaluation of some fresh parameters (Table 30). All these measurements were not affected by the tested plant extract except, shoot fresh mass which was significantly ($F_{(4, 29)} = 3.61$; $P < 0.05$) reduced under all concentrations of the same extract, and the differences were found to be significant between seedlings developed under control (0.0 g / l) treatment and those under the concentration of 100 g / l bark extract of *P. halepensis* collected in summer season. No significant differences were found within treatment means of bark extract collected in summer season for seedling vigor index and seedling tolerance index of *C. siliqua* seedlings (Figs.31 and 32) respectively. Results of dry mass measures of *C. siliqua* seedlings under the effect of aqueous extract of *P. halepensis* bark of summer collection are shown in table 31. These measures were, specific shoot length, specific root length, shoot dry mass, root dry mass, and root / shoot ratio. There were significant differences only in shoot dry mass ($F_{(4, 29)} = 2.81$; $P < 0.05$), which was decreased under bark extract concentrations from 50 g / l and above. Tukey's pairwise comparisons test indicate significant differences, of this parameter, between seedlings subjected to 0.0 g / l treatment and those under the concentrations of 50, 75, and 100 g / l of the same used plant extract of *P. halepensis* collected in summer time. There were no significant differences in the root / shoot and moisture content percentages of the tested

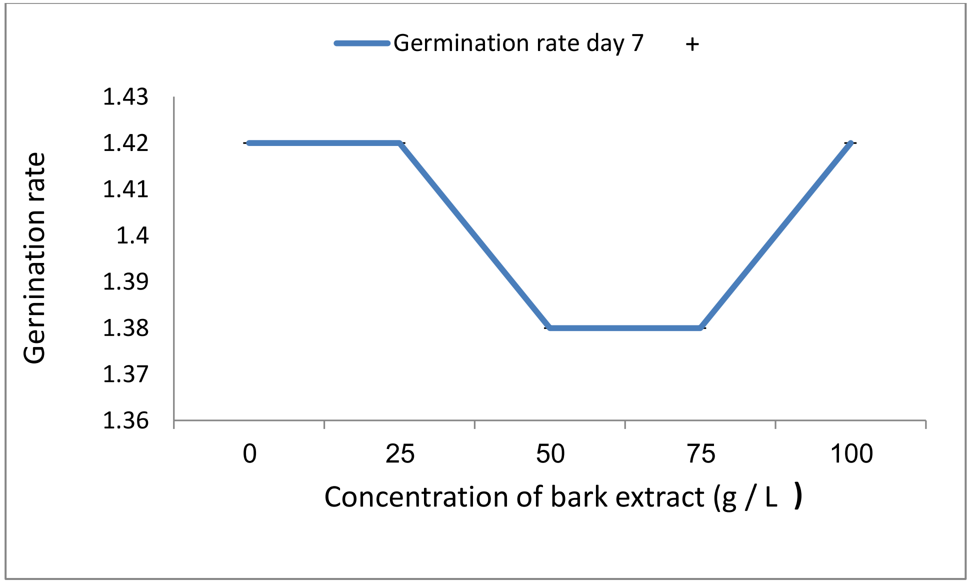

Figure 30. Effect of different concentrations of bark (summer collection) aqueous extract of *P. halepensis* on mean germination rate of *C. siliqua* seeds

Bars = SEMean + = Not significant

Table 30. Effect of different concentrations of bark (summer collection) aqueous extract of *P. halepensis* on mean measurement of fresh parameters of *C. siliqua* seedlings:

Concentration (g / l)	Shoot length (mm)	Root length (mm)	Seedling length (mm)	Shoot fresh mass (mg)	Root fresh mass (mg)
	+	+	+	*	+
0.0	105.77 ± 2.18	51.03 ± 2.38	156.80 ± 3.43	0.49 ± 0.014 [a]	0.041 ± 0.014
25	105.40 ± 4.52	49.17 ± 2.62	154.57 ± 5.95	0.44 ± 0.027 [ac]	0.044 ± 0.003
50	103.50 ± 5.62	47.70 ± 3.76	151.20 ± 8.48	0.43 ± 0.025 [ac]	0.039 ± 0.002
75	99.03 ± 4.56	46.03 ± 3.21	145.05 ± 6.49	0.42 ± 0.023 [ac]	0.036 ± 0.001
100	96.73 ± 5.67	45.87 ± 2.84	142.60 ± 8.10	0.37 ± 0.001 [bc]	0.034 ± 0.002

± = SEMean + = Not significant * = Significant at $P < 0.05$ Different letters = Significant Similar letters = Not significant

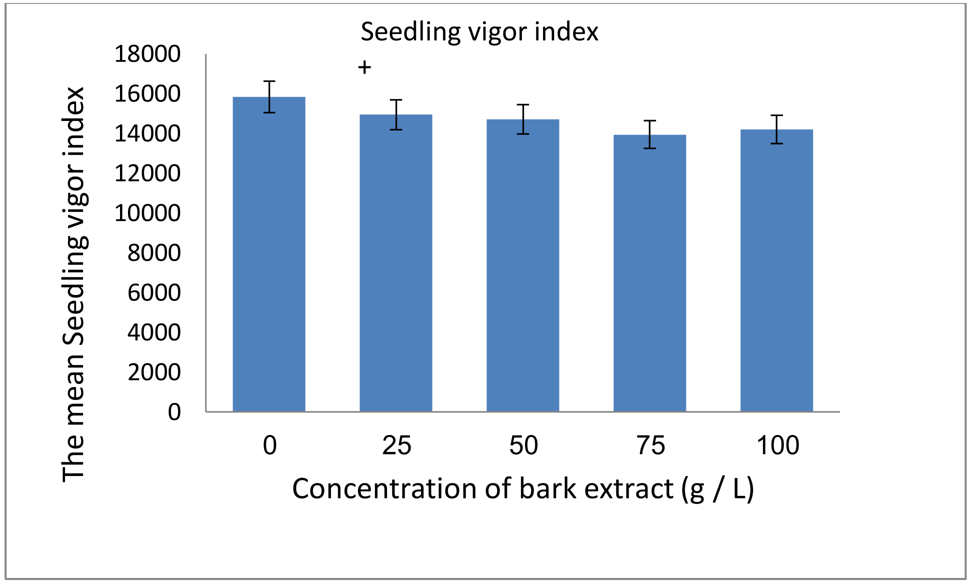

Figure 31. Effect of different concentrations of bark (summer collection) aqueous extract of *P. halepensis* on mean vigor index of *C. siliqua* seedlings

Bars = SEMean + = Not significant

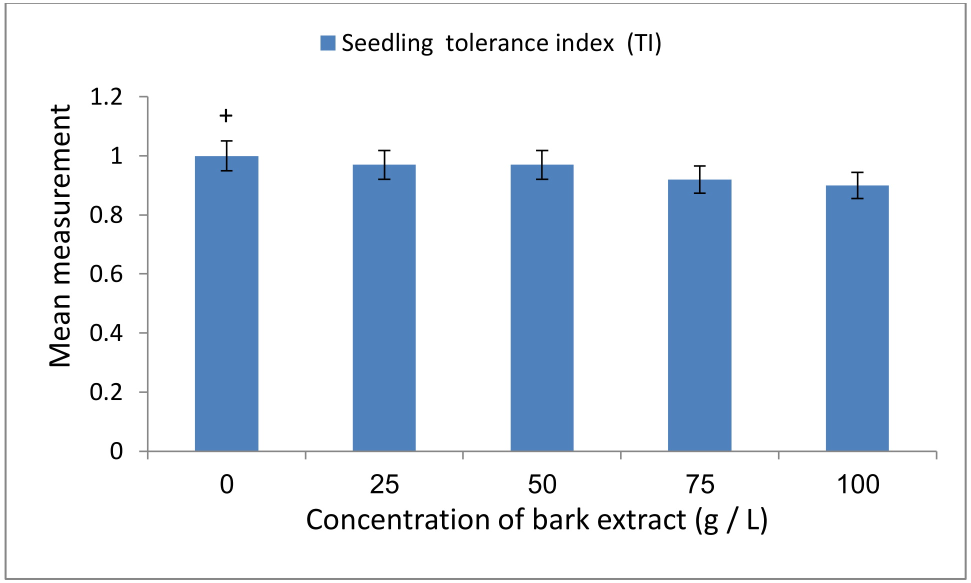

Figure 32. Effect of different concentrations of bark (summer collection) aqueous extract of *P. halepensis* on mean tolerance index of *C. siliqua* seedlings

Bars = SEMean + = Not significant

Table 31. Effect of different concentrations of bark (summer collection) aqueous extract of *P. halepensis* on mean measurement of specific length (shoot and root), dry mass (shoot and root), and root / shoot ratio of *C. siliqua* seedlings:

Concentration (g / l)	Specific shoot length	Specific root length	Shoot dry mass (mg)	Root dry mass (mg)	Root / Shoot ratio
0.0	+ 2028.8 ± 63.2	+ 1122.0 ± 433	** 0.060 ± 0.001 [a]	+ 0.0057 ± 0.0004	+ 0.12 ± 0.007
25	1994.0 ± 118	1050.0 ± 602	0.056 ± 0.002 [ac]	0.0054 ± 0.0004	0.12 ± 0.009
50	2003.0 ± 194	8850.0 ± 467	0.049 ± 0.003 [bc]	0.0054 ± 0.0003	0.11 ± 0.008
75	1986.0 ± 102	5987.0 ± 794	0.050 ± 0.003 [bc]	0.0055 ± 0.0003	0.11 ± 0.008
100	1863.0 ± 131	6138 ± 420	0.050 ± 0.002 [bc]	0.0053 ± 0.0003	0.10 ± 0.007

± = SEMean + = Not significant ** = Significant at $P< 0.0 1$ Different letters = Significant Similar letters = Not significant seedlings under the media of the extract (Table 32).

3. 5. Effects of different concentrations (suspensions) of soil rhizosphere (winter and summer collections) around *P. halepensis* tree on *C. siliqua*:

Soil rhizosphere suspension with different concentrations (0.0, 25, 50, 75, and 100 g / l) was prepared using soil of various distances (0.0, 2.0, and 4.0 m) at different depths (10, and 20 cm) around *P. halepensis* tree species. Soil rhizosphere of different distances and depths were collected in winter and summer seasons. These soil rhizosphere suspensions were tested for their effects on seed germination and early seedling development of *C. siliqua* using similar experiments which were used for the effects of needle and bark extracts with the same measures taken for seed and seedling development. Results of these experiments suggested no significant effects of different suspensions soil rhizosphere types on *C. siliqua*, and therefore, some results were selected, for example:

3. 5. 1. Seed germination:

The following parameters were measured for *C. siliqua* seeds under various concentrations of different distances and depths of soil rhizosphere. There was no effect on mean final germination percentages of seeds germinated under soil rhizosphere around *P. halepensis* tree collected in winter (Table 33) and summer (Table 34). Furthermore, different concentrations (suspensions) of soil rhizosphere of 4.0 m at 20 cm had no effect on mean germination promotion and inhibition percentages of the target seeds under both

Table 32. Effect of different concentrations of bark (summer collection) aqueous extract of *P. halepensis* on mean values of moisture content percentages in terms of fresh and dry mass of *C. siliqua* seedlings:

Concentration (g / l)	Moisture content percentages as fresh mass		Moisture content percentages as dry mass	
	Shoot	Root	Shoot	Root
0.0	+ 90.11 ± 4.42	+ 9.86 ± 0.40	+ 87.01 ± 0.55	+ 8.00 ± 0.68
25	88.99 ± 3.12	8.49 ± 0.33	84.06 ± 4.14	7.90 ± 0.57
50	87.87 ± 4.43	8.25 ± 0.30	83.30 ± 3.05	7.33 ± 0.97
75	88.08 ± 3.01	8.77 ± 0.43	83.27 ± 3.00	7.31 ± 1.31
100	87.68 ± 3.10	8.15 ± 0.71	82.50 ± 0.45	7.09 ± 1.21

± = SEMean + = Not significant

Table 33. Mean final germination percentages of *C. siliqua* seeds under various concentrations of different distances and depths of soil rhizosphere (winter collection) around *P. halepensis* tree:

	Distance (m)					
	0.0		2.0		4.0	
Concentration (g / l)	Depth (cm)					
	10	20	10	20	10	20
0.0	+ 100.0 ± 0.0	+ 100.0 ± 0.0	+ 100.0 ± 0.0	+ 100.0 ± 0.0	+ 100.0 ± 0.0	+ 100.0 ± 0.0
25	60.0 ± 3.0	100.0 ± 0.0	96.67 ± 3.33	100.0 ± 0.0	96.67 ± 3.33	100.0 ± 0.0
50	100.0 ± 0.0	100.0 ± 0.0	66.70 ± 13.80	100.0 ± 0.0	100.0 ± 0.0	96.67 ± 3.33
75	66.0 ± 3.33	96.67 ± 3.33	66.70 ± ±3.33	100.0 ± 0.0	96.67 ± 3.33	96.67 ± 3.33
100	100.0 ± 0.0	96.67 ± 3.33	100.0 ± 0.0	100.0 ± 0.0	96.67 ± 3.33	93.0 ± 8.82

+ = Not significant ± = SEMean

Table 34. Mean final germination percentages of *C. siliqua* seeds under various concentrations of different distances and depths of soil rhizosphere (summer collection) around *P. halepensis* tree:

	Distance (m)					
	0.0		2.0		4.0	
Concentration (g / l)	Depth (cm)					
	10	20	10	20	10	20
0.0	+ 100.0 ± 0.0	+ 100.0 ± 0.0	+ 100.0 ± 0.0	+ 100.0 ± 0.0	+ 100.0 ± 0.0	+ 100.0 ± 0.0
25	96.67 ± 3.33	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
50	96.67 ± 3.33	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	90.0 ± 3.33	96.67 ± 3.33
75	93.33 ± 3.33	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	96.67 ± 3.33
100	96.67 ± 3.33	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	90.0 ± 3.33	100.0 ± 0.0

+ = Not significant ± = SEMean

winter and summer collections (Figs.33 and 34) respectively. The above type of soil rhizosphere collected in winter (Fig. 35) and summer seasons (Fig. 36) showed no significant effect on the mean germination rate of *C. siliqua* seeds.

3. 5. 2. Early seedling development:

Parameters for early seedling development were measured for *C. siliqua* seedlings under the effect of all types of soil rhizosphere. There were no significant differences within different treatment means in fresh and dry measures of the seedlings, and the following are selected for example, mean tolerance index of *C. siliqua* seedlings under various concentrations of different distances and depths of soil rhizosphere of winter collection (Table 35) and summer collection (Table 36) around *P. halepensis* tree was not affect by various concentrations of all soil types.

Also, all different soil types collected in winter (Table 37) and in summer (Table 38) seasons with their various concentrations showed no influence on mean root / shoot ratio of *C. siliqua* seedlings under various concentrations of different distances and depths of soil rhizosphere around *P. halepensis* tree.

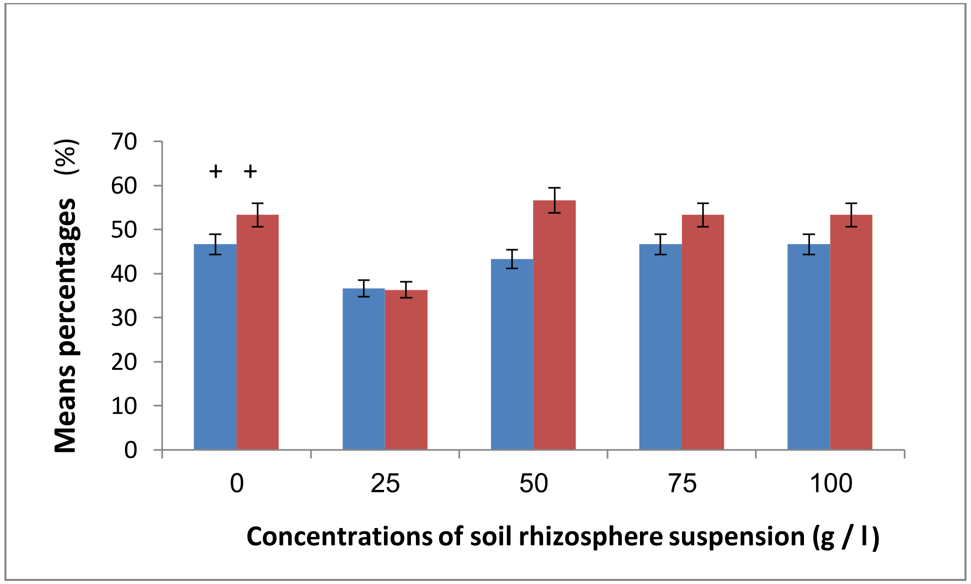

Figure 33. Effect of different concentrations of soil rhizosphere (winter collection) suspension of 4.0 m at 20 cm on mean germination promotion (A) and inhibition (B) percentages of *C. siliqua* seeds

Bars = SEMean + = Not significant

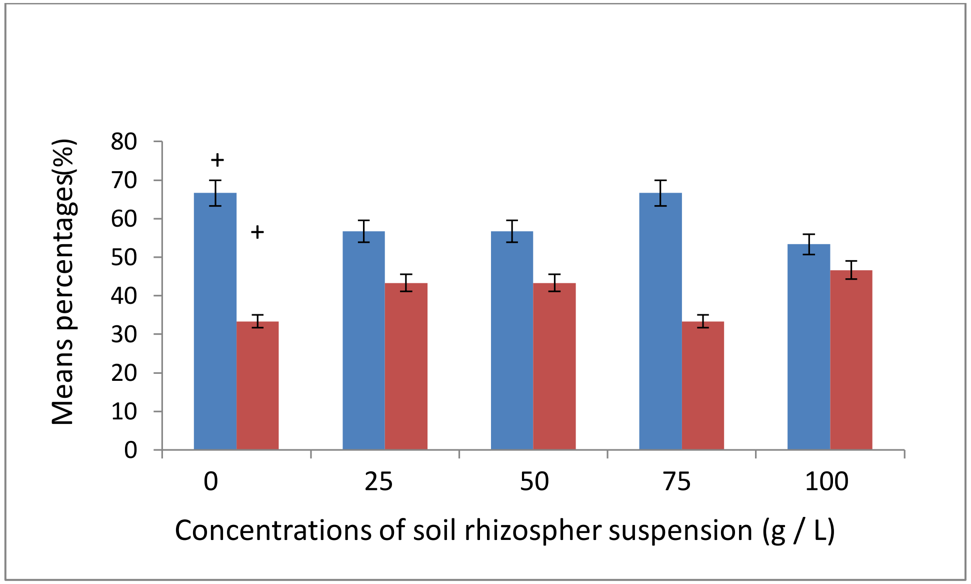

Figure 34. Effect of different concentrations of soil rhizosphere (summer collection) suspension of 4.0 m at 20 cm on mean germination promotion (A) and inhibition (B) percentages of *C. siliqua* seeds

Bars = SEMean + = Not significant

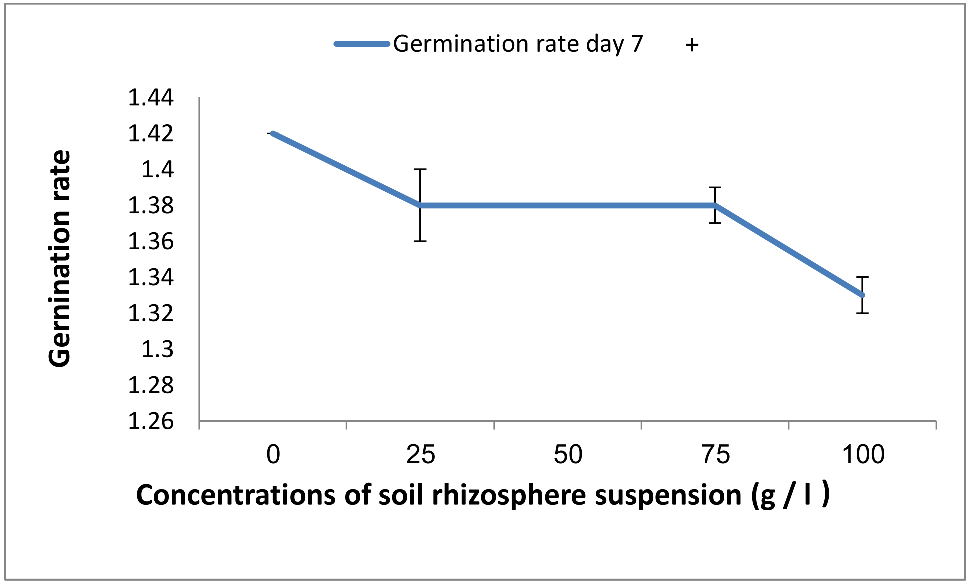

Figure35. Effect of different concentrations of soil rhizosphere (winter collection) suspension of 4.0 m at 20 cm on mean germination rate of *C. siliqua* seeds

Bars = SEMean + = Not significant

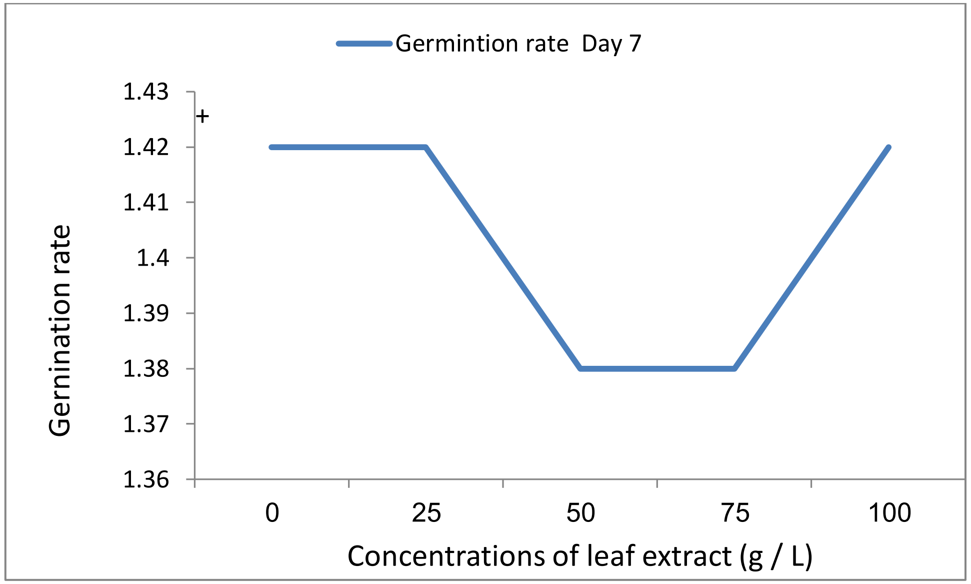

Figure 36. Effect of different concentrations of soil rhizosphere (summer collection) suspension of 4.0 m at 20 cm on mean germination rate of *C. siliqua* seeds

Bars = SEMean + = Not significant

Table 35. Mean tolerance index of *C. siliqua* seedlings under various concentrations of different distances and depths of soil rhizosphere (winter collection) around *P. halepensis* tree:

Distance (m)						
	0.0		2.0		4.0	
Concentration (g / l)	Depth (cm)					
	10	20	10	20	10	20
0.0	+ 1.00 ± 0.0	+ 1.00 ± 0.0	+ 1.00 ± 0.0	+ 1.00 ± 0.0	+ 1.00 ± 0.0	+ 1.00 ± 0.0
25	0.96 ± 0.14	0.96 ± 0.03	0.97 ± 0.03	0.96 ± 0.03	0.97 ± 0.07	0.97 ± 0.06
50	0.90 ± 0.24	0.94 ± 0.02	0.96 ± 0.06	0.96 ± 0.04	0.95 ± 0.05	0.94 ± 0.05
75	0.85 ± 0.35	0.93 ± 0.02	0.92 ± 0.04	0.95 ± 0.06	0.87 ± 0.08	0.90 ± 0.08
100	0.85 ± 0.23	0.90 ± 0.03	0.89 ± 0.01	0.93 ± 0.02	0.85 ± 0.66	0.88 ± 0.06

+ = Not significant ± = SEMean

Table 36. Mean tolerance index of *C. siliqua* seedlings under various concentrations of different distances and depths of soil rhizosphere (summer collection) around *P. halepensis* tree:

Distance (m)						
0.0			2.0		4.0	
Concentration (g / l)	Depth (cm)					
	10	20	10	20	10	20
0.0	+ 1.00 ± 0.0	+ 1.00 ± 0.0	+ 1.00 ± 0.0	+ 1.00 ± 0.0	+ 1.00 ± 0.0	+ 1.00 ± 0.0
25	0.98 ± 0.05	0.97 ± 0.18	0.99 ± 0.03	0.96 ± 0.23	0.97 ± 0.03	0.97 ±0.06
50	0.96 ± 0.02	0.94 ± 0.23	0.99 ± 0.05	0.96 ± 0.20	0.95 ± 0.04	0.96 ± 0.05
75	0.94 ± 0.04	0.92 ± 0.22	0.98 ±0.04	0.92 ± 0.16	0.91 ± 0.04	0.96 ± 0.08
100	0.91 ± 0.05	0.89 ± 0.20	0.91 ± 0.06	0.88 ± 0.20	0.91 ± 0.04	0.88 ± 0.06

+ = Not significant ± = SEMean

Table 37. Mean root / shoot ratio of *C. siliqua* seedlings under various concentrations of different distances and depths of soil rhizosphere (winter collection) around *P. halepensis* tree:

Distance (m)						
	0.0		2.0		4.0	
Concentration (g / l)	Depth (cm)					
	10	20	10	20	10	20
0.0	+ 0.11 ± 0.007	+ 0.12 ± 0.011	+ 0.12 ± 0.005	+ 0.11 ± 0.009	+ 0.12 ± 0.008	+ 0.12 ± 0.008
25	0.10 ±0.008	0.12 ± 0.007	0.12 ± 0.001	0.11 ± 0.012	0.12 ± 0.008	0.12 ± 0.008
50	0.10 ± 0.008	0.12 ± 0.008	0.12 ± 0.001	0.11 ± 0.06	0.12 ± 0.008	0.12 ± 0.010
75	0.90 ± 0.008	0.12 ± 0.003	0.12 ± 0.0031	0.11 ± 0.05	0.12 ± 0.008	0.12 ± 0.008
100	0.90 ± 0.008	0.11 ± 0.009	0.11 ± 0.008	0.11 ± 0.008	0.12 ± 0.007	0.12 ± 0.007

+ = Not significant ± = SEMean

Table 38. Mean root / shoot ratio of *C. siliqua* seedlings under various concentrations of different distances and depths of soil rhizosphere (summer collection) around *P. halepensis* tree:

Distance (m)						
0.0			2.0		4.0	
Concentration (g / l)	Depth (cm)					
	10	20	10	20	10	20
0.0	+ 0.12 ± 0.006	+ 0.12 ± 0.011	+ 0.12 ± 0.008	+ 0.12 ± 0.031	+ 0.11 ± 0.006	+ 0.12 ± 0.006
25	0.12 ± 0.006	0.12 ± 0.007	0.12 ± 0.007	0.012 ± 0.41	0.11 ± 0.008	0.10 ± 0.005
50	0.11 ± 0.007	0.12 ± 0.008	0.12 ± 0.007	0.13 ± 0.059	0.12 ± 0.009	0.10 ± 0.003
75	0.12 ± 0.010	0.10 ± 0.003	0.12 ± 0.007	0.13 ± 0.075	0.13 ± 0.014	0.11 ± 0.010
100	0.12 ± 0.006	0.11 ± 0.009	0.12 ± 0.007	0.13 ± 0.043	0.13 ± 0.009	0.10 ± 0.007

+=Not significant ± = SEMea

IV. CHAPTER FOUR

4. Discussion

4. 1. *P. halepensis* needles, bark, and soil rhizosphere effects on seed germination:

The response of the native *C. siliqua* (carob) to the effects of various organs (needles and bark) of introduced *P. halepensis* (Aleppo pine) and different types of soil rhizosphere around it of AL-Jabel AL- Akhdar, was evaluated by different parameters of its seed germination and early seedling development.

It was demonstrated that, aerial parts especially the needles *P. halepensis* contain inhibitory compounds and were more phytotoxic on seed germination of *C. siliqua*. Results indicated that, allelochemicals of aqueous extract of the needles seems to be stronger than those contained in the bark tissues. It has been shown by Godgate and Sawant (2014) that, aqueous extract of *E. globlus* bark contains more quantities of phytochemical compounds than ethyl acetate extract. Increasing the concentrations of the tested extracts caused significant reductions of the studied parameters of tested plant species. This coincides with the data reported, on volatile emissions in some pine species, by Mumm (2004) and Pureswaram (2004). Regarding the effects on seed germination, results showed significant reduction of daily and final cumulative germination percentages under aqueous extract of *P. halepensis* needles sampled in autumn, winter collections whereas, this measure of *C. siliqua* was significantly lower during 2^{nd} and 5^{th} days of germination time by the application of needles extract sampled in spring and summer seasons respectively. Daily cumulative germination percentages greatly of lower values during 2^{nd}, 6^{th}, 5^{th}, 3^{rd} days of germination period under the effect of bark extracts collected in autumn, winter, spring, and summer time respectively. But Final cumulative germination percentages of *C. siliqua* seeds were not inhibited by different concentrations of *P. halepensis* of both needles (spring and summer) and bark extracts collected in autumn, winter, spring, and summer seasons.

Reduced rate of carob seed germination under all concentrations of needles and bark extracts winter, spring. But, seed germination rate of the same seeds was not affected by needles and bark extracts of *P. halepensis* collected in summer season. Decrease of seed germination process can be stimulated by the inhibition effects of allelochemicals in the plant tissues on growth hormones, especially gibberellins (germination enhancer) and preventing seed germination process. These reduced cumulative germination percentages under different concentrations of needle and bark extracts are in correspondence with the findings of allelopathic effects of *Eucalyptus camaldulensis* on seed germination of four range species (Saberi *et al*. 2013). From the point of view of organ type, needles are more effective than the bark this is probably due to the active photosynthesis in needles, that lead to more synthesis of secondary metabolites. Additionally, seasonal variations may possess effects on the formation of these secondary metabolites. In the present study, needle extracts of *P. halepensis* collected in autumn and winter showed more inhibitory effects on the germination process of *C. siliqua* seeds than that of spring and summer needles. This might be contributed to that, high temperature in summer may cause partial evaporation of the active secondary substances. The effect of seasonal variations on the essential oil of *Mentha Canadensis* had more menthol contents in winter and lowest in summer. Furthermore, it has been postulated that genetic expression of secondary metabolites production is also influenced by the environmental factors higher temperatures (Daniels *et al*., 2019) and seasonal changes (Santos *et al*., 2012; Carmen *et al*., 2013). In another study, essential oils from the aerial parts of *Ocimum basillicum* were of highest percentages in winter and minimum in summer (Al Hussain *et al*., 2008). In contrast, thymol, the main constituents of *Origanum syriacum* essential oil showed maximum components in summer season (Fischer *et al*., 2011). In a review by Soni *et al*., (2015) revealed that, there is no generalities should be taken in consideration in sampling the plant parts. As the activity of these parts depends on the chemical composition present in them.

4. 2. *P. halepensis* needles, bark, and soil rhizosphere effects on early seedling development:

To evaluate the effects of allelochemicals contained in needles and bark of *P. halepensis* tree on early growth of *C. siliqua* seedlings, some fresh and dry measurements were carried out under different concentrations of both organ extracts. The results revealed that, length of shoot, root, and seedlings, fresh mass of shoot and root, seedling vigor and tolerance indices showed great reductions with increasing the concentrations of autumn needles extract. Whereas, the phytotoxicity of bark extract collected in the same season was less inhibitory. For example, reductions in length of shoot and seedling occurred under the concentration of 100 g / l. Length and fresh mass of roots showed the same values by application of all bark concentration. Vigor and tolerance indices were decreased by concentrations of 75 and 100 g / l of bark autumn collection. For dry mass parameters of the seedling established under the effect of both organ extracts sampled in the same season the results showed that, specific shoot length, dry mass of shoot and root were decreased by higher concentrations (75 and 100 g / l) of *P. halepensis* needles extract, with no significant differences for specific root length. However, the response of *C. siliqua* seedlings placed under bark extract in terms of dry measures is quite different for example, specific lengths of shoot and root, dry mass of root were slightly reduced by 75 and 100 g / l concentrations. Moreover, root / shoot ratio of the same seedlings under the growth media of both organs collected in autumn season showed no significant reductions. Furthermore, moisture content percentages on the basis of fresh mass of seedling grown under all concentrations of bark extract were significantly reduced, but these percentages were of no significant values of seedlings under *P. halepensis* needles extract collected in autumn season. Decrease of early establishment of *C. siliqua* seedlings exposed to allelochemicals of *P. halepensis* extracts could be contributed to the inhibition of cell division and enlargement as reported by Farajollahi *et al*., (2012), as a result of reduction of growth regulators such as cytokinin and indole acetic acid. Consequently, these may cause reduction of the early growth of seedlings. These findings come in parallel with the results stated by Nektarios *et al*., (2005) for growth reduction parameters of turf grasses plant species.

Needles extract of *P. halepensis* sampled in winter season was more inhibitory for the fresh measures of *C. siliqua* seedlings where, it shows great reductions in all these parameters of seedlings grown under concentrations of needles extract above 25 g / l. Whereas, the same measurements were not affected by different concentration of bark extract of the same tree, only shoot fresh mass was reduced by the same values under all bark concentrations. *C. siliqua* of dry responses such as specific length of shoot and root were decreased by needles extract concentrations from 50 g / l and above, dry mass of shoot, root, and root / shoot ratio were greatly reduced under the concentration 100 g / l of the same extract. For moisture content percentages, there were significant reductions on the basis of shoot fresh and dry mass under 100 g / l needles extract of winter season. Roots are more sensitive to the effect of allelochemicals and hence the moisture content percentages of root fresh and dry mass were decreased under concentrations of 75 and 100 g / l. Root / shoot ratio was significantly reduced in *C. siliqua* seedlings grown under winter needles extract. This suggests that, the inhibitory effect of allelochemicals from *P. halepensis* needles on root growth is greater than shoot growth. This coincides with the potential of allelopathic effects on seedling growth of ryegrass and Kentucky bluegrass (Aliloo *et al*., 2012).

The same parameters (fresh and dry) were under taken for the target plant species (*C. siliqua*) seedlings placed under various levels of needle and bark extracts of *P. halepensis* collected in spring season. Results of seedling fresh measures indicated reduced root length by exposure to 75 and 100 g / l, and decreased of seedling length and tolerance index occurred in seedlings under 50, 75, and 100 g / l of the same extract, while seedling vigor index was found to be reduced under 100 g / l of spring needles extract. However, these fresh measures of *C. siliqua* seedlings under aqueous extract of bark tissues of the same season, they all decreased on exposure to 75 and 100 g / l, while root length was not affected by this extract. Furthermore, all dry mass measures of root / shoot ratio and moisture content percentages in terms of fresh and dry mass of the tested seedlings exposed to different concentrations of needles and bark aqueous extract solutions were not affected.

Under summer collections of needles and bark extracts, there were no significant effects of these allelochemicals on the fresh and dry parameters including root / shoot ratio and moisture content percentages of *C. siliqua* except for shoot length which was reduced by exposure to 100 g / l of spring needles extract.

Results of this research indicated that, the needle fresh tissues extract of introduced *P. halepensis* sampled during winter season could exert more inhibitory effects on seed germination and early seedling growth of native *C. siliqua* plant species, which was found with a bare zone distance from *P. halepensis* tree in wadi Al-khoof area in AL-Jabel AL-Akhdar mountain. The impact of influences allelochemical compounds of the needles on the parameters of seed germination and seedling growth, can be considered by two ways. First, they restrict the process of cell division and second, they inhibit elongation of cells by hindering hormones production that are required for both physiological processes within the seed. Furthermore, these phytotoxic substances could disordered the physiological vital mechanisms such as, respiration, inhibition of nutrients uptake, photosynthesis (Bogatek *et al*., 2005).

According to this research soil rhizosphere suspensions were tested for their effects on seed germination and early seedling development of *C. siliqua* using similar experiments which were used for the effects of needle and bark extracts with the same measures taken for seed and seedling development. For different types of soil rhizosphere, results of these experiments postulated no toxic effects of different suspensions of soil rhizosphere types on *C. siliqua*, i.e. there were no inhibition of seed germination and seedling elongation exposed to all soil types. This suggests that selected depths were.

This could be explained by the fact that, the selected different depths of soil rhizosphere were not enough for the accumulation of these substances away from the roots of *P. halepensis* tree.

Summary and conclusions

P. halepensis (needles and bark) and soil rhizosphere were seasonally collected. Needles and bark were collected in the middle of each season (autumn, winter, spring, and summer. Based on the results obtained from the effects of needles and bark extracts, it has been decided to use the soil rhizosphere around *P. halepensis* with different distances (0.0, 2.0, and 4.0) with two depths (10 and 20 cm) of each soil type. The above mentioned soil types were collected in the middle of winter and summer seasons. Different concentrations (0.0, 25, 50, 75, and 100 g / l) were prepared from the extract of each plant organ (needles and bark) and from each soil type, to evaluate their effects on seed germination and early seedling development of native *C. siliqua*. Some parameters were carried out for seed germination and for seedling development. For seed germination tests, cumulative germination percentages, promotion and inhibition percentages of seed germination, and rate of seed germination were calculated. For early seedling development experiment, fresh parameters with seedling vigor and tolerance indices, and dry parameters with root / shoot ratio, and moisture content percentages in terms of fresh and dry mass were evaluated. Data of all experiments were statistically analyzed.

From the results obtained by the experiments related to the effects of various organs of introduced *P. halepensis* (Aleppo pine) and different types of soil rhizosphere around it, on the native *C. siliqua* (carob) of AL-Jabel AL-Akhdar, it could concluded that, Daily and final cumulative germination percentages were reduced with increasing the concentration of aqueous extract of *P. halepensis* needles collected during autumn season. Rate of carob seed germination was greatly decreased. The germination of *C. siliqua* seeds was not affected by different concentrations of aqueous bark extract of *P. halepensis* during autumn season But there were significant reductions of seed germination. Furthermore, the same levels of *P. halepensis* bark had no significant differences within the rate of germination *C. siliqua* seeds.

The influence of aqueous extract of *P. halepensis* needles collected in winter season was tested with different concentrations for daily germination percentages of *C. siliqua* seeds. Germination was begin at the third day of germination period with great reduction under all concentrations of needle extract with no seed germination under 100 g / l. Daily cumulative germination percentages of *C. siliqua* seeds placed in media of different concentrations of aqueous extract of *P. halepensis* bark of winter season, were of low values. Rate of germination the same target plant species was not affected by all levels *P. halepensis* bark (winter collection) solutions.

Cumulative germination percentages were decreased under aqueous extract of *P. halepensis* needles collected in spring season. Germination rate of *C. siliqua* showed decreased values. The results of daily cumulative germination percentages of *C. siliqua* seeds placed in different applications of aqueous extract of *P. halepensis* bark collected in spring were of reduced values. All various levels of *P. halepensis* bark collected in spring season showed no inhibitory effects on the germination rate of *C. siliqua* seeds.

The mean of daily cumulative seed germination percentages of *C. siliqua* germinated in different concentrations of aqueous extract *P. halepensis* needles of summer collection were decreased under all levels of the tested plant extract. Whereas, germination rate of the same seeds was not affected by the same collection. *P. halepensis* bark (summer collection) extract of different concentrations was tested for the germination process of *C. siliqua* seeds and showed decreased cumulative daily germination percentages with no effects on the germination rate of the same target plant species.

For early seedling development, fresh parameters of *C. siliqua* seedlings grown under different concentrations of *P. halepensis* needles collected in autumn season were of great reductions. For dry mass measurements of the same seedlings, lower values were obtained for, specific shoot length, dry weight of shoot, and root of seedlings under the same aqueous extract *P. halepensis*,

There was no significant differences for root / shoot ratio of *C. siliqua* seedlings grown under the same growth media. Moisture content percentages were decreased by increasing the concentrations of needles extract with no significant differences. Response patterns of *C. siliqua* seedlings under different concentrations of bark extract of *P. halepensis* in autumn season were quite different from seedlings grown under the needles treatments. The inhibitory effect on length of shoot, seedling, and root fresh weight parameters was increased with increasing the concentration of bark extract collected in autumn..

Both vigor and tolerance indices were decreased by increasing the bark concentration within different treatment means. No significant differences were found within various treatments of *P. halepensis* bark (autumn collection) extract for root / shoot ratio *C. siliqua* seedlings. For moisture content percentages reduced by all levels of the extract concentration, with the significant differences only for moisture content percentages of fresh mass of shoot.

Fresh measures of *C. siliqua* seedlings were investigated using various concentrations of needles aqueous extract *P. halepensis* collected during winter season. All the parameters including seedling vigor and tolerance indices were found to be decreased with increased needles extract concentrations. All dry mass measurements along with root / shoot ratio and moisture content percentages of the target plant seedlings developed under different concentrations of the same extract, were all reduced with increasing the levels of needle extract.

Early seedling development was measured for *C. siliqua* seedlings under the influence of various solutions of aqueous extract of *P. halepensis* bark from winter season. Only shoot fresh and dry mass were reduced. Both root / shoot ratio and moisture content percentages of *C. siliqua* were not affected by this extract of winter collection.

The mean measurement of root length, seedling length, and root fresh mass, seedling vigor, and seedling tolerance indices of *C. siliqua* seedlings grown in different concentrations of spring needles aqueous extract of *P. halepensis*, were reduced. Different concentrations of needle (spring collection) aqueous extract of *P. halepensis* had no inhibitory effects on the mean measurements of specific length (shoot and root), dry mass (shoot and root), root / shoot ratio and mean values of moisture content percentages of *C. siliqua* seedlings.

The response of fresh parameters of shoot length, root length, seedling length, shoot fresh mass, root fresh mass, seedling vigor index, and seedling tolerance index were evaluated for *C. siliqua* seedlings developed under treatment levels of aqueous extract of *P. halepensis* bark collected in spring season, showed reductions in shoot length, seedling length, shoot fresh mass, root fresh mass, seedling vigor index, and seedling tolerance index were evaluated for *C. siliqua* seedlings developed under different levels of aqueous extract of *P. halepensis* bark collected in spring season. Both root / shoot ratio and moisture content percentages of *C. siliqua* seedlings were not affected by exogenous application of various treatments of *P. halepensis* bark collected in spring season.

Fresh parameters of *C. siliqua* seedlings were not affected by different concentrations of summer needles extract. Only shoot length was of low value with the effect of 100 g / l. Both seedling vigor and tolerance indices of the target plant seedling were not affected by all *P. halepensis* needles extract of summer. Furthermore, dry measure responses of *C. siliqua* seedlings along with both root / shoot ratio, and moisture content percentages were not affected by the application of different concentrations of aqueous extract of *P. halepensis* needles collected in summer season.

C. siliqua seedlings were subjected to various treatments of bark extract and all fresh measurements were not affected by the tested plant extract except, shoot fresh mass which was reduced under all concentrations of the same extract of *P. halepensis* bark collected in summer season. No toxic effects of bark extract collected in summer season for seedling vigor index and seedling tolerance index of *C. siliqua* seedlings. Results of dry mass measures of *C. siliqua* seedlings under the effect of aqueous extract of *P. halepensis* bark of summer showed no detrimental effects, only shoot dry mass was decreased under the effect extract concentrations. No effects in the root / shoot and moisture content percentages of the tested seedlings under the media of the extract.

Soil rhizosphere suspension with different concentrations (0.0, 25, 50, 75, and 100 g / l) was prepared using soil of various distances (0.0, 2.0, and 4.0 m) at different depths (10, and 20 cm) around *P. halepensis* tree species. Soil rhizosphere of different distances and depths were collected in winter and summer seasons. These soil rhizosphere suspensions were tested for their effects on seed germination and early seedling development of *C. siliqua* using similar experiments which were used for the effects of needle and bark extracts with the same measures taken for seed and seedling development. Results of these experiments postulated no toxic effects of different suspensions soil rhizosphere types on *C. siliqua*, and therefore, some results were selected, for example:

Some parameters were measured for *C. siliqua* seeds under various concentrations of different distances and depths of soil rhizosphere. There was no effect on mean final germination percentages of seeds germinated under soil rhizosphere around *P. halepensis* tree collected in winter and summer seasons.

Furthermore, different concentrations (suspensions) of soil rhizosphere of 4.0 m at 20 cm had no effect on mean germination promotion and inhibition percentages of the target seeds under both winter and summer collections. The above type of soil rhizosphere collected in both seasons winter and summer showed no significant effect on the mean germination rate of *C. siliqua* seeds.

Parameters for early seedling development were measured for *C. siliqua* seedlings under the effect of all types of soil rhizosphere. There were no detrimental influence in fresh and dry measures of the seedlings, for example, mean tolerance index of *C. siliqua* seedlings under various concentrations of different distances and depths of soil rhizosphere of winter collection and summer collection around *P. halepensis* tree were not affect by various concentrations of all soil types.

Also, all different soil types collected in winter and in summer seasons with their various concentrations showed no influence on mean root / shoot ratio of *C. siliqua* seedlings under various concentrations of different distances and depths of soil rhizosphere around *P. halepensis* tree.

Therefore, it can be concluded that for the effects of extracts from different plant organs, needles extract from autumn, winter, and spring showed more inhibitory effects on seed germination and seedling parameters than that of bark extract.

For seasonal collection, the phytotoxicity exerted by organs collected in winter seasons was more potent compared with other seasons with low potency of summer season.

Future work:

However, further studies are required to evaluate the efficacy of *Piuns halepensis* needles, bark extract and soil rhizosphere against *Ceratonia siliqua* under field conditions. Therefore, future studies are needed to determine allelopathic potential of the individual compounds that are responsible for these effects. Determination of absorbed amount of secondary metabolites by species usig gas-chromatography/mass spectrometry is needed. Application of the electron microscope for anatomical studies of tested plants under different concentration of different parts of *Piuns halepensis* is also required. Where preliminary screening shown that needles extract had the strongest allelopathic effect on seed germination and early seedling developement, thus it is selected for detailed experimnts. Examination increase concentrations adove the use in the studies so that gave results more clearly.

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السمية النباتية لأشجار الصنوبر المستجلبة علي الاستجابات الفسيولوجية لأشجار الخروب المستوطنة في منطقة الجبل الاخضر

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الملخص العربـي

التضاد الحيوي (Allelopathi) : هو الآلية التي يتم من خلالها إنتاج مركبات كيميائية يطلق عليها Allelopathi compounds أو Allelochemicals والتي تعد نواتج أيضية ثانوية ويمكن أن تنتج من الأجزاء النباتية المختلفة سواء كانت اوراقاً، سيقاناً، جذوراً ، أزهاراً وثماراً وبإمكان هذه المركبات أن تتحرر إلى البيئة بعدة طرائق هي الغسيل Leaching ، التطاير Volatilization ، إفرازات الجذور Root exudation . معظم النباتات لها المقدرة علي إنتاج وإفر او مركبات كيميائية ثانويه, وهي نواتج أيض ثانويه ليس لها دور رئيسي في النمو والعمليات الفسيولوجية في النبات, وتقتصر مهمتها فقط في الدفاع علي النبات ضد العوامل الحيه مثل الكائنات الدقيقة بأنواعها, العاشبات, الحشرات والعوامل الغير حيه مثل درجة الحرارة , الأشعة, الماء, الملوحة وغيرها. تستخدم النباتات هذه

المركبات في منع إنبات بذور النباتات الأخرى, أو حتى إنبات بذور نفس النبات فيما يعرف بظاهرة التضاد السالفة الذكر.

كما عُرفت هذه الظاهرة من قبل (IAS) سنة 1996 بأنها العمليات التي تتضمن النواتج الثانوية التي تنتج من قبل النباتات في مقاومة الكائنات الدقيقة مثل الفيروسات أو الفطريات وتأثيرها علي نمو النباتات سواء من الناحية الإيجابية أو السلبية . تعتبر ظاهرة التضاد عباره عن استراتيجيات تقوم بها النباتات لمواجهة بعضها البعض في البيئه التي تتواجد بها. إن كشف الكيفية التي تعمل بها هذه الاستراتيجيات تمثل صعوبة بالغه علي صعيد الاختبارات الحيوية في المعامل بسبب دقة وتعقيد هذه الاستراتيجيات داخل الأنسجة الداخلية.

قسمت هذه المركبات علي حسب تخليقها الحيوي إلى مجاميع مختلفة مثل مجموعة التربينات ,مجموعة المركبات الفينوليه, ومجموعة المركبات التي تحتوي في تركيبها علي النيتروجين مثل القلويدات. ويعتبر نبات الصنوبر من ضمن النباتات التي تنتج هذه المركبات الايضية حيث ينتشر هذا النبات طبيعيا في الشمال الشرقي في ليبيا وبالأصح بمنطقة الجبل الاخضر. تم تصميم هذه الدراسة علي اساس معرفة تأثير اشجار الصنوبر المستزرع علي انبات ونمو بادرات اشجار الجروب البري بحيث تهدف هذه الدراسة إلى تقييم تأثير المستخلصات المائية للأعضاء المختلفة من نبات الصنوبر على نبات الخروب البري وقد تم جمعه من منطقة وادي الكوف شرق مدينة بنغازي حيث كان متوفر بكثره وايضا جمعت بذور نبات الخروب البري من نفس المنطقة بحيث أظهرت التجارب المنجزة في معامل قسم النبات كلية العلوم جامعة بنغازي اختلاف وتدرج في الجهد الأليلوباتي في

اجزاء النبات المختلفة الاوراق, القلف ومعلق التربة للنبات المستخدم في هذا البحث, حيث أن المركبات التي تحتويها هذه الأجزاء أدت في العموم الي تثبيط الإنبات في نبات الخروب البري. حيث تم إجراء تجارب مختبريه لدراسة تأثير المستخلصات المائية للأوراق والقلف ومعلق التربة لنبات الصنوبر *Pinus halepensis*, و تراكيز مختلفة من المستخلص النباتي لهذه الاجزاء سالفة الذكر وتأثيرها في إنبات البذور ونمو بادرات من نبات الخروب حيث استخدمه تراكيز مختلفة من مستخلص هذه الاجزاء 0.0, 25, 50, 75, 100 جم / لتر من اجزاء نبات الصنوبر والتي جمعت خلال مواسم السنه المختلفة وفي العموم كان اقل تأثير للمعاملات المختلفة من مستخلص الاوراق والقلف هو فصل الصيف حيث انه اظهرت المعاملات عدم تأثير المعاملات علي انبات البذور ونمو البادرات نبات الخروب. بينما اظهرت النتائج لمستخلص الاوراق الاكثر تثبيطا وانخفاض نمو البذور ونمو البادرات كان في فصل الشتاء والثبيط سجل في التراكيز الأعلى من الكنترول 25, 50, 75, 100 جم / لتر مقارنة بالكنترول بينما مستخلص القلف والذي جمع في فصل الشتاء سجل اقل تثبيط من مستخلص الاوراق الذي جمع في فصل الشتاء. اما عن المستخلصات الاوراق والقلف التي جمعت في فصلي الخريف والربيع فأظهرت اقل تاثير علي انبات البذور ونمو البادرات لنبات الخروب. واظهرت تجارب معلق التربة والذي جمعت في فصلين فقط هما فصل الشتاء وفصل الصيف حيث اظهرت التجارب انه لا يوجد تأثير من التراكيز المختلفة مع اختلاف المسافات والاعماق علي انبات البذور ونمو البادرات في فصل الصيف. بينما تجارب معلق التربة الذي جمعت في فصل الشتاء بتراكيزها ومسافاتها واعماقها المختلفة لم تؤثر التراكيز المختلفة علي انبات البذور نبات

الخروب بينما ادت الي تأثير تثبيطي علي نمو البادرات وخاصتا علي قياس الوزن الطري لساق وذلك عند تجربة معلق التربة عند مسافة 4.0 متر وعمق 10 سم.

الدراسات المستقبلية:

فحص كفاءة المستخلصات تحت الظروف الحقلية مقارنة بالظروف المعملية. تحديد الكميات الممتصة من المركبات من قبل نبات الدراسة باستخدام جهاز:

(gas–chromatography/mass spectrometry). اجراء دراسة تشريحية علي بادرات نبات الخروب المستخدم في الدراسة لمعرفة التغيرات التي تطرأ عليها نتيجة لاستخدام المستخلصات المائية ومعلق التربة لنبات الصنوبر خصوصا تحت تأثير مستخلص الاوراق الجافة لأنها اظهرت نتائج المعاملات اكثر تأثيرا وسمية عالية من باقي مستخلص القلف ومعلق التربة. القيام بدراسة زيادة فارق التركيزات المستخدمة في الدراسة فمثلا استخدام 0.0, 50, 100, 150, 200 جم/لتر فاحتمالية انها تعطي دلالات اكثر وضوحا.

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قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في علم النبات.

جامعة بنغازي

كلية العلوم

يوليو 2020