Physiological Ecology of *ceratonia siliqua*

at Al-gabal Al-akhdar area

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A thesis presented to the department of botany faculty of science Benghazi University in partial fulfillment of the requirement for the degree of master in Botany

University of Benghazi

Faculty of science

Aug 2022

Benghazi University **Faculty of Science**

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بِسْمِ اللهِ الرَّحْمَنِ الرَّحِيمِ

﴿ أَتَأْمُرُونَ النَّاسَ بِالْبِرِّ وَتَنْسَوْنَ أَنفُسَكُمْ وَأَنتُمْ تَتْلُونَ الْكِتَابَ أَفَلَا تَعْقِلُونَ ﴾

سورة البقرة، الآية (44)

Dedication

To the fountain of patience and optimism and hope

To each of the following in the presence of God and His

Messenger, my mother dear

To the big heart my dear father

To my dear husband

To those who have demonstrated to me what is the most beautiful

of my brother's life

To the people who paved our way of science and knowledge

All our teachers Distinguished

To the taste of the most beautiful moments with my friends

I guide this research

Acknowledgments

First praise and thanks to ALLAH to spire me to work on this topic and giving me strength and patient to complete this work successfully. I would like to express my deepest and sincere gratitude and thanks full to my Great Supervisor:

for his support, kindness, and a wonderful care in directing and supervising

work.

Thanks also to all whom help me in my research.

Finally thanks to my mother for take care and help and hope her health and happiness forever

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Abstract

Carob tree (*Ceratonia siliqu*a L.) is environmentally and economically important tree and is among the most difficult to propagate fruit and slow-growing an species. It is an evergreen endemic wild species found naturally in El-Jabal El-Akhdar region which is located immediately south of the coastal belt in the northeastern region of Libya.

Aim and methodology:

The first part of the study was to examine the morphological characteristics of carob tress in 4 different locations in El-Jabal El-Akhdar region (Albyadah, Tukrah, Wadi El-Kouf and Al Himadah), five characters on discriminative pods measured to know (pod size, number of seeds per pod, weight of 100 seeds, seeds size, leaf size) , another two characters measured directly in the study location which are stem diameter and whole tree height, The second part of the study was to examine the effect of different pre-sowing treatments found in previous literature on the growth of carob seeds, which are (boiling water, tab water, mechanical scarification and sulfuric acid). Germination percentage, mean germination time, seedling viogros index and seedling development individually evaluated and compared with control for each pretreatment, procedures performed according to a fully developed protocol of *in vitro* seed germination for carob found in previous literature.

Results:

For the morphological characters study generally no significant morphological differences found among theses population except in leaf size which was highly significant, Tukhra area showed smaller leaf size compared with other locations. No significant correlations between pod size and other morphological characters of carob in all locations except in Albyadah in which a significant negative correlation was found between pods sizes and seeds sizes. Compared to control, generally all pretreatments significantly enhanced the germination of carob seeds, relatively higher germination percentage was noticed in the seeds soaked in boiling water 96%, followed by carob seeds treated with sulfuric acid for 15 minutes which showed 60% germination, the mechanical scarification and sulfuric acid for 10 minutes showed germination percentages 40%, the seeds treated with sulfuric acid for 5 minutes showed relatively the germination percentage 32%, while the treatment with regular tap water showed zero germination. Faster germination was recorded by seeds pre-treated with sulfuric acid for 15 minutes, followed by germination recorded by carob seeds pre-treated with boiling water, then seeds pre-treated with sulfuric acid 10 minute, seeds

pre-treated with sulfuric acid 5 minutes and mechanical scarification showed somewhat delay in germination. Carob seeds pretreated with soaking in boiling water showed significant differences in their fresh weight, root and shoot lengths compared with untreated control seeds, while results of dry weight was not significant compared to control treatment**.**

Chapter One

1. Introduction

Carob tree (*Ceratonia siliqua* L.), St. John's bread, or locust is small to medium sized broadleaf, a slowly growing, woody evergreen, sclerophyll, and widespread species occurring as a native plant in the Mediterranean Basin (Ramón-Laca and Mabberley, 2004). Ceratonia species belong to the Caesalpiniodeae sub family of the family Leguminoseae. This species is normally dioecious and it is sclerophyllous evergreen tree that may grow up to 20 m height under best environmental circumstances (Catarino, 1993), but typically attains heights of 8 to 15 m (Goor and Barney, 1968; Ortiz *et al*., 1995). It has been grown since antiquity in most countries of the Mediterranean basin, usually in mild and dry places with poor soils. Carob is widely cultivated in Mediterranean regions (Cyprus, Greece, Portugal, Italy, Spain, Lebanon, Southern Jordan, Syria, Turkey, Egypt, Libya, Tunisia, Morocco,) and in areas of North America (Manso *et al*., 2010). Carob is considered a tropical plant that has modified well to Mediterranean climates. Its deep rooting habit and xerophilous leaf used to evade water stress (Catarino, 1993). Carob tolerates drought explaining its large distribution in the arid and semi-arid Mediterranean climate (Correia and Martins-Loucao, 1994; Lo Gullo and Salleo, 1988). Its origin seems to be the eastern Mediterranean has been domesticated since 4000 BC, and its extensive dates from at least 2000 BC culture, its longevity is considerable (up to 200 years). It is formerly operated in particular through its feed and food qualities. Thus the tree is useful in human and animal food (El Kahkahi *et al*., 2014). Morphological characters of carob pods and seeds widely used as quantitative markers to identify the variation of carob according to certain criteria in several wild populations and collections (Barracosa *et al*., 2007; Sidina *et al*., 2009).

Carob is an economically significant tree and it can be used in many trees – planting activities like charcoal, wood production, soil erosion management, land recovery, ornamental evergreen tree (Pérez-García,, 2009). Carob seeds are difficult to germinate they are tremendously hard and not willingly absorb water (Coit, 1951). Under natural conditions, only a reduced percentage of carob seeds are able to germinate. A number of factors (mechanical friction with soil particles, microbial

action, passage through the digestive tract of mammals that feed on them, etc) can change seed coat (Pérez-García,, 2009).

In Libya, Carob considered as one of the most important indigenous species that constitute the plant cover of AlJabal Al-Akhdar area (Al-Wasita, Agfentta, WadiKouf, Al-Hania, Al-Hamama, Omar Al-Mukthar, Messa, Alghariqa and North of Labraq). Carob trees distribute naturally in all regions of Al-Jabal Al-Akhdar, it grows as pure carob populations or form mixed woodland together with *Juniperus phoenicea*, *Olea europaea* L. var. Olea ster, *Quercusc occifera*, *Cupressus sempervirens* and *Pinus halepensis* El-Jabal El-Akhdar extends on the coast belt to about 300 km and rises to about 881 m above sea level. It is generally rocky and stony, and intersected frequently by many valleys. The average rainfall ranges between 250-600 mm, and the soils are terrarossa or heavy clay (Johnson, 1973; Sharaf, 1971; El-Zwaam, 1995). The carob trees are scattered in the area of El-Jabal El-Akhdar in association with many wild species such as olive (*Olea europaea*), mastic (*Pistacia lentiscus*) and juniper (*Junipurus phoenicea).*

Similar to other legume seeds, carob reveals seed coat impermeability to water therefore the seed will not germinate without seed coat scarification (Piotto and Piccini, 1996; Piotto and Ciccarese, 1999; Piotto and Di Noi, 2003). The impermeable seed coat is the cause of the physical dormancy of carob seeds. Germination can be improved by treating seeds with tap water, boiling water, sulfuric acid (H_2SO_4) or gibberellic acid (GA_3) (Batlle and Tous, 1997; Güneş *et al*., 2009; Güneş *et al*., 2013).

Mechanical or acid scarification is commonly employed to break hard seed coat and enhance seeds germination (Bonner *et al*., 1994). The dormancy-breaking treatments used in these instances must raise moisture uptake and gas exchange and should not produce alterations in the embryo and endosperm (Baskin and Baskin, 1998), in the intention to prevent seed damage, taking into account that several physiological reactions are occurred during the germination process. Based on available knowledge, only few studies have dealt with seed germination of the *C. siliqua* (Tsakaldimi and Ganatsas, 2001). In addition, information regarding the pre-treatment to be used to break the dormancy of carob seeds is often contradictory. The husk of carob seeds is very hard, therefore, its germination percentage is very low and the seeds need a long time to germinate. To get a good germination percentage of carob seeds, the seeds of carob should be scarified or treated by chemicals to improve their germination (Coit, 1951; Goor and Barney, 1968).

The aim of study:

The aim of this study is to evaluate the capability of propagation of *Ceratonia siliqu* under local Libyan environmental conditions and provide the authorities with the data to use this plant in El-Gabal El-Akhdar area in replacement process of some threatened plants. In addition, to use it in gardening and forestation programs**.**

Chapter Two

2. Literature Review

2.1. Names of the species and taxonomy of carob plant:

The scientific name of carob tree (*Ceratonia siliqua* L.) derives from Greek *keras*, horn, and Latin *siliqua*, alluding to the hardness and shape of the pod. The common name originates from the Hebrew *kharuv*, from which are derived the Arabic *kharrub* (Hammer *et al*., 1992).

The genus *Ceratonia* belongs to the family Leguminosae (syn. Fabaceae) of the order Rosales. Legumes are important members of tropical, subtropical and temperate vegetation throughout the world. This is one of the largest families of flowering plants and includes 650 genera and over 18 000 species (Polhill *et al.,* 1981) and is extremely variable in morphology and ecology. The carob tree is generally placed in the tribe Cassieae of the subfamily Caesalpinioideae; however, several authors doubt *Ceratonia*'s position in the Cassieae (Irwin and Barneby 1981; Tucker 1992a, 1992b). The diploid chromosome number for *Ceratonia* is 2*n*=24 whereas many members of the Cassieae complex have 2*n*=48 (Goldblatt, 1981). The genus *Ceratonia* is regarded as one of the most old of the legume genera (Tucker, 1992a). Taxonomically, *Ceratonia* is completely isolated from all other genera of its family (Zohary, 1973). Hillcoat *et al.,* (1980) and Tucker (1992a) considered the carob as a very isolated remnant of a part of the family Leguminosae now largely extinct. A second species of *Ceratonia – C. oreothauma* Hillcoat, Lewis and Verdc. – was only described in 1980. Two subspecies distinguished: subsp. *oreothauma*, native to Arabia (Oman), and subsp. s*omalensis*, native to the north of Somalia. *Ceratonia oreothauma* is very distinct morphologically from *C. siliqua*. In addition, *C. oreothauma* has slightly smaller pollen grains than *C. siliqua* and they are tricolporate rather than tetracolporate (Ferguson, 1980). As pollen grains are more evolved than tricolporate grains, *C. oreothauma* was suggested as the wild olver of the cultivated *C. siliqua* by (Hillcoat *et al.,* 1980).

2.2. Origin of *Ceratonia siliqua:*

The centre of origin of *C. siliqua* is not clear. It was placed by De Candolle (1883) and Vavilov (1951) in the eastern Mediterranean region (Turkey and Syria). However, Schweinfurth (1894) regarded carob as native to the highlands of southern

Arabia (Yemen). More recently it has been considered by Zohary (1973) as originating from a xerotropical Indo-Malesian flora, grouping it with *Olea*, *Laurus*, *Myrtus*, *Chamaerops* and others and placing the origin of its genus also on the Arabian peninsula. *Ceratonia oreothauma*, the only known carob-related species, is considered to have its centre of origin in southeast Arabia (Oman) and around the African horn (north of Somalia) (Hillcoat *et al.* 1980).

Climatically the centers of origin of the subfamily Caesalpinoideae warm and moist initially, but after the Cretaceous period vast drying and elevation of the lands occurred so that cooler, much drier, even desert, conditions evolved. Other caesalpinioid legumes are mainly tropical and subtropical (Cowan, 1981). In addition, Mitrakos (1988) suggested that the carob tree seems to have evolved under a climate other than Mediterranean.

2.3. Distribution of *Ceratonia siliqua:*

The original distribution of *C. siliqua* is not clear as it has undergone extensive cultivation since ancient times. Hillcoat *et al.* (1980) suggested its range in the wild included Turkey, Cyprus, Syria, Lebanon, Israel, southern Jordan, Egypt, Arabia, Tunisia and Libya and that it moved westward at an early stage. Carob is believed to have been spread by the Greeks to Greece and Italy and then by the Arabs along the coast of northern Africa into the south and east of Spain, from where it migrated to the south of Portugal and the southeast of France. Its wild occurrence in the western Mediterranean is doubtful according to Zohary (1973). Spontaneous carobs occur in many places around the western Mediterranean basin but they are regarded as feral derivatives of the fruit crop which probably evolved under domestication.

2.4. Domestication of *Ceratonia siliqua:*

Scant information is available on the origin and domestication of the carob tree. Liphschitz (1987) reported that early archaeobotanical findings (charred wood and seeds) in Israel showed that the carob existed in the eastern Mediterranean long before the start of Neolithic agriculture (4000 BC), although it is not among the prehistoric species listed by Renfrew (1973). Zohary (1973) suggested that the Mediterranean region has been at least one of its domestication centers. Zohary (1996), on the basis of literature sources and archeological evidence, reported that the carob was brought into cultivation relatively late with the ‘seconed wave’ of fruit crops domesticated in the

Old World. He attributed this lateness of domestication to the difficulty of propagating carob vegetatively. Remains of carbonized pods have been found in archeological excavations near the Vesuvio volcano in Campania, Italy, post-dating its eruption in AD 79 (Meyer, 1980). Zohary and Spiegel-Roy (1975) analyzed two kinds of information – evaluation of fossil evidence and examination of wild relatives of the cultivated crops – and concluded that olive, grapevine, date palm and fig the first important horticultural crops added to the Mediterranean grain agriculture. These 'first wave' fruit trees most likely domesticated in the Near East in prehistoric times (4th and 3rd millenia BC); they very important crops in the Early Bronze Age. Zohary (1996) suggested that similarly to most Old World fruit crops, domestication of *C. siliqua* was based on shifting from sexual reproduction (in the wild) to vegetative propagation (under cultivation). In carob, as in other fruit and nut trees, the shift to vegetative propagation is the cultivator's solution to the problem of wide variability which is characteristic of sexual reproduction in cross pollinated plants. In addition, as a predominantly dioecious tree, carob includes about 50% males and 1% hermaphrodites (Condit, 1919). Thus spontaneous promising seedlings showing superior features have been empirically selected by growers and then clonally propagated. As a consequence, wild carob trees currently growing in Mediterranean countries are not identical to the species type (Mitrakos, 1988). Hillcoat *et al.*, (1980) reported that its cultivation in ancient times would have been unnecessary since wild trees common in the eastern Mediterranean.

Wild and escaped carobs reproduce by seed while cultivated varieties are propagated vegetatively as clones. The carob does not root easily by cuttings and is only easily multiplied by budding. The propagation predominantly of female clones can change the sex ratio in a carob-production area. The three main fruit traits that distinguish domesticated carobs from their wild relatives are larger bean size, more pulp and greater sugar content. Increase in the size and number of seeds is less evident. These pod features together with productivity and environmental adaptation seem to have been the most important selection criteria for growers. The small difference in size between the pollen of the two species of this genus seems unlikely to be associated with polyploidy but is more likely to be a result of cultivation (Ferguson, 1980; Graham and Barker, 1981). Ferguson (1980) reported that similar differences in pollen size between specimens of *Olea europaea* (cultivated olive) and *Olea laperrinei* (wild olive) have been observed.

2.5. Botanical description of *Ceratonia siliqua:*

The carob tree grows as a sclerophyllous evergreen shrub or tree up to 10 m high, with a broad semispherical crown and a thick trunk with brown rough bark and sturdy branches. Leaf are 10-20 cm long, alternate, pinnate, with or without a terminal leaflet. Leaflets are 3-7 cm long, ovate to elliptic, in 4-10 normally opposite pairs, coriaceous, dark green and shiny above, pale green beneath and finely veined with margins slightly ondulate, and tiny stipules. The leaf are sclerophyllous and have a very thick single-layered upper epidermis, the cells of which contain phenolic compounds in the large vacuoles, and stomata are present only in the lower epidermis and arranged in clusters (Mitrakos 1988). Relevant parts of the plant are shown in the figure (2-1). Carob does not shed its leaf in the autumn but only in July every second year, and it only partially renews leaf in spring (April and May) (Diamantoglou and Mitrakos, 1981). The carob is a dioecious species with some hermaphroditic forms; thus male, female and hermaphrodite flowers are generally borne on different trees. Unisexual and bisexual flowers are rare in the inflorescence. The flowers are initially bisexual, but usually one sex is suppressed during late development of functionally male or female flowers (Tucker, 1992a); dioecy is not common among Leguminosae. In evolutionary terms, unisexuality is generally regarded as a derived character from bisexual ancestral state. Flowers are small and numerous, 6-12 mm long, spirally arranged along the inflorescence axis in catkin-like racemes borne on spurs from old wood and even on the trunk (cauliflory). Flowers are green tinted red. Flowers show pentamerous symmetry with calyx but not corolla placed on a short pedicel. The calyx is discs shaped, reddish-green and bears nectaries. Female flowers consist of a pistil (6-8.5 mm) on a disk and rudimentary stamens, surrounded by 5 hairy sepals. The ovary is bent, consisting of two carpels 5-7 mm long and containing several ovules. The stigma has 2 lobes. Male flowers consist of a nectarial disk with 5 stamens with delicate filaments surrounded by hairy sepals. In the centre of the disk there is a rudimentary pistil. Hermaphrodite flowers are a combination of both types, containing a pistil and a complement of 5 stamens. Pollen grains released from the anthers are of spheroidal shape and are tetracolpate (Ferguson 1980). Pollen diameter is 28-29 μm at the poles and 25-28 μm at the equator (Ferguson, 1980; Linskens and Scholten, 1980).

The fruit is an indehiscent pod, elongated, compressed, straight or curved, thickened at the sutures, 10-30 cm long, 1.5-3.5 cm wide and about 1 cm thick with

blunt or subacute apex. Pods are brown with a wrinkled surface and are leathery when ripe. The pulp comprises an outer leathery layer (pericarp) and softer inner region (mesocarp). Seeds occur in the pod transversally, separated by mesocarp They are very hard and numerous, compressed ovate-oblong, 8-10 mm long, 7-8 mm wide and 3-5 mm thick; the testa is hard and smooth, glossy brown, the hilum minute.

Fig. (2-1): Botanical description of *Ceratonia siliqua.*

2.6. Properties of *Ceratonia siliqua*:

The two main carob pod constituents are (by weight): pulp (90%) and seed (10%). Chemical composition of the pulp depends on cultivar, origin and harvesting time (Orphanos and Papaconstantinou,1969; Davies *et al.,* 1971; Vardar *et al.,* 1972; Calixto and Cañellas, 1982; Albanell *et al.,* 1991). Carob pulp is high (48-56%) in total sugar content (mainly sucrose, glucose, fructose and maltose). In addition it contains about 18% cellulose and hemicellulose. The mineral composition (in mg/ 100 g of

pulp) is: K=1100, Ca=307, Mg=42, Na=13, Cu=0.23, Fe=104, Mn=0.4, Zn=0.59 according to Puhan and Wielinga (1996). Rendina *et al.* (1969) found the lipids to consist of approximately equal proportions of saturated and unsaturated acids. Vardar *et al.* (1972) found five amino acids in pod extracts (alanine, glycine, leucine, proline and valine) and Charalambous and Papaconstantinou (1966) also reported tyrosine and phenylalanine. Ripe carob pods contain a large amount of condensed tannins (16-20% of dry weight) (Würsch *et al.*, 1984). Feeding trials showed that carob pulp contains only 1-2% digestible protein and is relatively low in metabolizable energy (Vohra and Kratzer, 1964). In food value, carob pods are similar to most cereal grains (NAS, 1979). The protein has a low digestibility because it is bound with tannins and fiber (Loo, 1969). Some researchers have suggested that condensed tannins account for observed growth-depressing effects on animals fed with a diet high in carob meal (Kamarinou *et al.*, 1979) while others believe that this effect is due to its low energy content for which animals can compensate by increasing consumption (Louca and Papas, 1973).

2.7. Uses of *Ceratonia siliqua:*

The carob is one of the most useful native Mediterranean trees. In producing countries, carob pods have traditionally been used as animal and human food and currently the main use is the seed for gum extraction. Carob pods provide fodder for ruminants (Louca and Papas, 1973) and non ruminants (Sahle *et al.*, 1992). In the wild, carob shelter, foliage and beans attract browsing animals. The pods contain indigestible and valuable seeds. Carob timber is hard and close-grained, and has been used to make utensils as well as fuel. Carob wood also was traditionally used to make slow-burning charcoal. *Ceratonia oreothauma* is extensively used for goat fodder in its native ranges (Hillcoat *et al.*, 1980). The pods are used after crushing to separate seed and pulp. The pulp can be ground into a fine powder for use in human nutrition. Carob powder consists of 46% sugar, 7% protein and small amounts of numerous minerals and vitamins and is thus quite nutritious (Whiteside, 1981). After oven-drying, the powder can be added to cakes, bread, sweets, ice creams or drinks as a flavoring (NAS, 1979). Carob powder ‘cocoa’ has advantages over chocolate in that has fewer calories and neither caffeine nor theobromine (Whiteside, 1981; Craig and Nguyen, 1984). Its flavor is not as rich as dark chocolate but resembles milk chocolate. Owing to the high sugar content of the pod and its relatively low cost, carob pulp was among the first

horticultural crops used for the production of industrial alcohol by fermentation in several Mediterranean countries (Merwin, 1981). In some countries, e.g. Egypt, carob syrup is a popular drink obtained by extracting carob kibbles with water. Single-cell organisms have been used to convert carob pulp into a high-protein feed; sugar solutions extracted from carob pods are an excellent substrate for culturing fungi such as *Aspergillus niger* and *Fusarium moniliforme* and the dried mycelium is a palatable and nutritious feed containing up to 38% crude protein by weight (Imrie, 1973; Sekeri-Pataryas *et al.,* 1973). Milled and chopped carob pomace, which are two by-products of the carob molasses industry, tested in Lebanon as a potting medium for plants and have shown good promise as substitutes for peat-based mixtures in nurseries (Rishani and Rice, 1988). The possible use by the food industry of natural antioxidants contained on the carob seed coat as a by-product of the CBG industry recently has raised some interest (Batista *et al.,* 1996). The carob product most widely used, especially for the food industry, is the carob bean gum (CBG), or locust bean gum (LBG). This gum comes from the endosperm of the seed and chemically is a polysaccharide, a galactomannan. By weight, about a third of the seed consists of gum and it is obtained from the kernel after removal of the coat and grinding. One hundred kg of seeds yields an average of 20 kg of pure dry gum (Jones, 1953). Carob gum is produced in various degrees of purity depending on how well the endosperm is separated from the embryo and seed coat. Specks of cotyledons and testa are usually present in commercial CBG preparations. For use as a natural food additive, known as E 410, only high grade is admitted; for pet food more residues are allowed. This mucilaginous gum, also known as 'tragasol', is used in a wide range of commercial products as a thickener, stabilizer, binder and gelling or dispersing agent. The food industry uses CBG for the production of a large number of different commodities: ice creams, soups, sauces, cheese, fruit pies, canned meats, confectionery, bakery products and pet foods. Technical applications of CBG include cosmetics, pharmaceuticals, film emulsions, paints, polishes, ceramics and adhesives (Johnsen *et al.*, 1988; Tous and Batlle, 1990) . In the 1980s, CBG applications : food industry (about 75%) and technical (about 25%); however, this has changed in the 1990s (because of a CBG price increase) to about 90 and 10%, respectively (Batlle, 1997). Carob is widely planted as an ornamental and shade tree on the streets of California, Australia and elsewhere; male trees are preferred as they do not provide litter from pod fall. However, the carob's value as a drought tolerant, air pollution tolerant, low-

maintenance tree for street and landscape planting could be limited by the large mature size and strong, invasive roots (Coit, 1951; NAS, 1979). Carob is now being used in xerogardening in Mediterranean countries. And since it requires little if any cultivation, tolerates poor soils and is long-lived, carob tree is often recommended for reforestation of degraded coastal zones threatened by soil erosion and desertification. It also has been recommended for planting as a windbreak around orchards (NAS, 1979; Esbenshade and Wilson 1986) and could even have some use for buffering noise from factories, roads and railways because of its dense foliage.

2.8. Climate requirements:

Areas suitable for carob should have a subtropical Mediterranean climate with cool, not cold, winters, mild to warm springs, and warm to hot dry summers. These Mediterranean-like areas range from approximately 30° to 45° in northern latitudes (Mediterranean basin, California and Arizona) and between 30° and 40° in southern latitudes (Australia, South Africa and Chile). Adult trees require no winter chilling; they can be damaged when temperatures fall below -4°C and can only withstand winter temperatures of not lower than -7°C. However, trees can withstand summer temperatures of 40°C and hot dry winds. From 5000 to 6000 hours above 9°C are needed for pods to ripen. Strong winds can break adult tree branches and detach pods. Wind can also damage young trees (Tous and Batlle, 1990). Autumn rains can interfere with pollination and affect fruit set. High humidity in spring promotes *Oidium* infection on both leaf and pods.

2.9. Soil requirements:

Carob trees can adapt to a wide range of soil types from poor sandy soils and rocky hillsides to deep soils, but they cannot withstand water logging although the root system is usually deep. In areas with shallow rocky soils, tree size and productivity are reduced. The best soils are sandy well-drained loams but calcareous soils with high lime content are also suitable. Carob also appears to tolerate salinity well (Rebour, 1971). Winer (1980) reported tolerance to a soil salt content of up to 3% NaCl.

2.10. Water requirements:

Carob, as a xerophyte, can survive dry climates without irrigation and is well adapted to dry environments with annual average rainfall between 250 and 500 mm per year (Tous and Batlle, 1990). It has developed some drought-resistance mechanisms

(Nunes *et al.*, 1989; Salleo and Lo Gullo, 1989) as mentioned in the Agronomy section. Although drought resistant, carob trees do not bear commercial crops unless they receive at least 500-550 mm per year (NAS, 1979), but 350 mm of annual rainfall are considered enough for fruit set (Coit, 1949; Ticho, 1958).

2.11. Reviews of previous studies:

Martins-Loução *et al*., (1996) found that Carob *Ceratonia siliqua* cv. Mulata seeds which subjected to 7 different pre-sowing treatments including hot and warm water treatments, scarification with acid or sand, and immersion in ethanol or potassium nitrate. The best treatments acid scarification (immersion in 90% sulfuric acid for 20 min) or treatment with warm water (40°C) for 48 h. The fresh and dry weight of seedlings showed a steady increase.

Tsakaldimi and Ganatsas, (2001), found that seeds of *Ceratonia siliqua* and Pistacia lentiscus are characterized by a hard seed coat physical dormancy. Several dormancy-breaking treatments applied for improving their germination. Acid scarification was tried for the seeds of both species as well as hot water soaking for Ceratonia siliqua and mechanical scarification for *Pistacia lentiscus* seeds. Treatments improved the germination percentages of the seeds in both species. In *Ceratonia siliqua* seeds acid scarification treatment significantly increased the germination percentage (86.7%) and reduced the mean time to complete germination (MTG). For the same species hot water soaking was less effective even though it improved the germination percentage. The treatment application in Pistacia lentiscus seeds slightly affected the amount and speed of germination in relation to control; the best results gave the acid scarification for 10 minutes exhibited a mean germination percentage 78.9% and MTG 8,34 days.

Pérez-García, (2009) conducted a study, aimed of this to study the germination characteristics (under controlled conditions of light and temperature and using different pretreatments for promoting germination) and variability of *Ceratonia siliqua* seeds. Seed collected from different individual trees tested. Constant (10°C, 15°C, 20°C, 25°C) and alternating 25/15°C temperature regimes and 16/8 h light/dark photoperiod conditions used. Mechanical scarification, dry heat, boiling water, sulfuric acid, soaking in distilled water and soaking in gibberellic acid solution used as pre-sowing treatments applied for enhancing germination. The untreated seeds showed a deep dormancy at all temperature regimes assayed (final germination

percentages ranged from 23 to 28%). Mechanical scarification, sulfuric acid and boiling water drastically improved final germination percentages (99, 88 and 80%, respectively). Therefore, the impermeability to water of the seed coat (physical dormancy) seems to be the most important causes of the seed dormancy present in this species. Great variability in seed weight, seed water content and germination parameters found among seeds belonging to different individual trees. Significant differences between different individual trees under the same incubation temperature detected for seed germination (final germination percentage ranged from 7 to 50%). However, germination rate (as expressed by mean germination time) was relatively similar among seeds from different trees. A negative significant relationship between seed weight and final germination percentage was found: the lightest seeds reached the highest germination percentages. Moreover, seed weight showed a positive significant correlation with seed water content.

Gunes *et al*., (2013) conducted a study in Turkey where *Ceratonia siliqua L*. carob seeds harvested from both wild and cultivated genotypes in Turkey subjected to mechanical scarification, soaking in hot water and dipping in sulfuric acid. All treatments hastened seed germination and seedling growth of carob compared to control. The germination percentage of control seeds similar for both wild and cultivated genotypes (13%) and it was increased up to 95% and 93% in wild and cultivated genotypes, respectively, following sulfuric acid treatment. They found that seeds germination percentage of wild and cultivated carobs was similar in all treatments indicating that domestication does not appear to have influenced germination behavior in both genotype.

Bostan and Kiliç, (2014) conducted a study which carried out to determine the effects of different treatments on seed germination on a wild carob genotype grown in Silifke province (Mersin, Turkey). In this study, the seeds stratified in 2014 year. After the stratification, sulfuric acid and gibberellic acid applied to the seeds. Experimental design was planned with three replicates, and 30 seeds per each replicate used. Carob seeds treated with different diluted sulfuric acid concentrations (Control, 80, 85, 90 and 95%) for 30 minutes in petri dishes, and then soaked in water for two days. In gibberellic acid treatments, seeds treated with 500 ppm, 1000 ppm and 1500 ppm concentrations for 24 hours. All treated seeds sowed to perlite. The results showed that the seeds didn't germinate in control group, the highest germination rate for sulfuric acid treatment was observed in 95 % sulfuric acid as 88,90 %, and the

highest germination rate for gibberellic acid treatments was observed in 1000 ppm dose as 28,90 %.

El Deen *et al*., (2014) conducted a study in which efforts made to propagate the plant by using three different methods; seeds germination, cuttings and micropropagation. Seeds and cuttings scarified and disinfected under aseptic conditions to improve the germination percentage and the percentage of success of cuttings. Results of the study showed that the highest values of seed germination percentage, the fastest germination, the greatest plant length, number of leaf/plant, root length and dry weight obtained by soaking seeds in 60% H_2SO_4.

Kruger *et al*., (2018) conducted a study about enhancing seed germination of *Ceratonia siliqua* L. for large scale production in southern Africa, the study included four treatments: the first seeds treated with boiling water (100°C) for 30 min, the second group was chemically scarified by sulfuric acid for 30 min, the third treatment mechanically scarified until the green endocarp was visible, and last was the untreated control group. All treatments then subjected to a 24 h imbibitions period in distilled water and incubated at 25°C, with three different photoperiods: 8 h light, 12 h light or complete darkness. the results of germination percentages achieved showed that mechanical scarification had the highest germination percentage (90%), followed by the sulfuric acid (36%) and boiled water (24%) treatments. Photoperiod did not affect germination.

Abdullah *et al.*, (2019) conducted a study in the Malta Forest Nursery to evaluate the effect of several pre-sowing treatments on *Ceratonia siliqua* seeds on enhancing seed germination. The experiment was laid out in randomized complete design with four replications. *Ceratonia siliqua* seeds soaked in hot water at (80^o C) for 30, 60 and 90 min, and mechanical scarification; the second trait seeds treated with factorial experiment which seeds of both two species soaked in sulfuric acid for 30, 60 and 90 min, and then immersed in tap water for 24, 48 and 72h. The results showed that the seeds of *Ceratonia siliqua* species treated with hot water for 60 min increased germination percentage (61%), and the same result obtained of *Ceratonia siliqua* seeds treated with hot water for 60 min (61%). In the factorial experiment, the seeds of *Ceratonia siliqu* species treated with Sulfuric acid scarification at 90 min, then immersed with tap water for 24h the best result of germination percentage (65%).

Ali *et al*., (2019) Carob tree *Ceratonia siliqua* L., is an evergreen endemic species found naturally in El-Jabal El-Akhdar region which is located immediately

south of the coastal belt in the northeastern region of Libya. Morphological characteristics have been the main descriptive tool to characterize a given collection or germplasm, or to identify and differentiate wild type populations. Eighteen carob population collected from six different sites in El-Jabal El-Akhdar area. Seven characters on discriminative pods measured: the length, width, thickness, number of seeds, weight of pulp, and yield as well as one character to seeds: the weight seeds. The present study showed that the choice of pod characters to assess Libyan carob diversity is a useful and powerful tool. The means and standard deviations of morphometric characters measured in Libyan carob showed highly significant differences among the studied populations for all the examined characteristics. Differences in morphometric traits of carob pods and seeds among Libyan carob populations are primarily caused by genetic factors. The pod size of Libyan carob is considered to be the medium size (10.89 – 17.55cm).

Khalid and Younis, (2019) conducted a randomized complete block design study in Iraq which carried out to determine the effects of acetic acid, hot water, and cold sand stratification scarification at different periods on seed germination of carob (*Ceratonia siliqua* L.). Carob seeds treated by soaking in glacial acetic acid for 20, 40, 60, and 120 min. at lab temperature (25oC). Hot water (100^{o}C) also used for soaking the seeds for 20, 40, 60, and 120 min, with 3 replicates, and 10 seeds per each treatment per each replicate used and the results compared to non-treated seeds. After soaking the seeds in acetic acid and hot water, they washed thoroughly and sowed in black polyethylene grow bags. The soil used was loamy with added organic matter. At the end of the experiment, data recorded on the germination percent and growth features including plant height, leaf area, root length, fresh and dry weight of vegetative parts. The results showed that the treatment with hot water for 60 minutes achieved the highest percent of germination (96.67%), highest leaf area 12.86 cm^2 , highest root dry weight (0.14 gm.). The treatment for 40 minutes with hot water gave the highest fresh weight of vegetative parts was 18.6 gm. and root fresh weight (7.05 gm.).

Chapter Three

3. Materials and method

3.1. Study location and time:

The Al-Jabal Al-Akhdar mountainous region is in north east Libya, It extends for about 300 km along the coast and climbs reaching 881m above sea level (a.s.l.) and characterized by a Mediterranean climate with cool rainy winter and hot dry summer (Domroes and El-Tantawi, 2005). The study of morphological characters was carried out during spring 2020 as in figure(3-1) and table(3-1) while the study of germination conducted in summer 2021, both growth characters and germination study carried out at the sciences faculty laboratory of Benghazi university.

Table (3-1): Sites of the examined carob populations.

Locations	Longitude	Latitude	Altitude m (a.s.l)
Albyadah	32°46'20.123"N	21°45'44"	316
Tokhra	32°32'19.252"N	21° 46′ 33″	26
Wadi El-Kouf	32° 43'16.513"N	21° 30' 10"	432
Al Himadah	32° 24' 37.149" N	20° 36' 38"	335

Shutt Rador topography mission (SRTM) dgital elevation model(DEM).

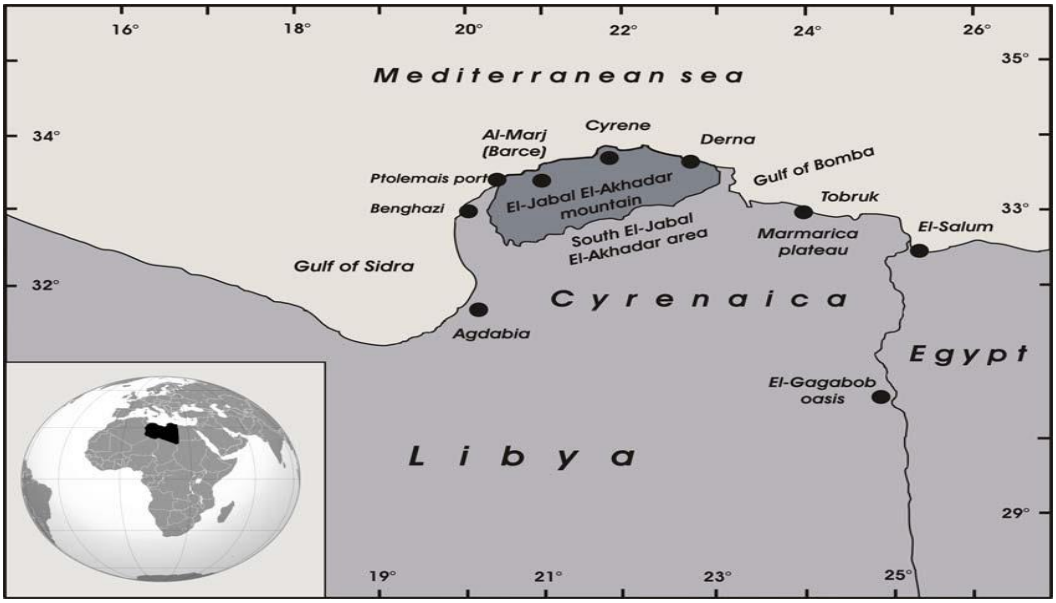

Fig. (3-1): The study locations at Al-Jabal Al-Akhdar mountainous

3.2. Growth characteristics study

3.2.1. Collection of plant material:

The plant material consists of pods of carob tree as in the figure(3-2). It was collected from four different locations in El-Jabal El-Akhdar at different height above sea level. So we brought in the pods samples to the laboratory, ten pods taken randomly from each site, and five characters on discriminative pods measured to know (pod size, number of seeds per pod, weight of pulp (100 seeds), seeds size, leaf size), Seeds will extracted manually another two characters measured directly in the study location which are stem diameter and whole tree height.

Fig. (3-2): Carob (*Ceratonia siliqua*) tree.

3.2.2. Measurements of morphological characters:

Ten pods measured for their size using ruler as in figure(3-3) (multiplied length and diameter) and the mean of each location was calculated. Number of seeds in 10 pods was also estimated and the mean of the seeds number was calculated in each area as in figure(3-4), the weight of 100 seeds collected from the same tree performed three times for each location and the mean of the three weights was calculated, seeds size calculated by multiplying the length and width of the 10 seeds and the mean was also calculated for each location, finally the sizes of 10 leaf from the same trees as in figure(3-5) also estimated for each location, the tree diameter as in figure(3-5) and height using the application to measure the lenghth of trees(Tree)

directly measured three times for each location, the diameter was measured by measuring tap.

Pod size = length x diameter x 0.78.

Leaf area = lenght x diameter x 0.45.

Fig. (3-3): Measuring the size of carob pods

Fig (3-4): Counting and measuring the size of the carob seeds

Fig. (3-5): Measuring carob leaf size

Fig (3-6): Measuring carob tree diameter.

3.3. Germination experiment of carob:

3.3.1. Carob seed Material:

Ripe fruits (pods) containing mature seeds of *Ceratonia siliqua* collected in from a wild growing population in different four locations at Al-Jabal Al-Akhdar area. Seeds belonging to individual trees chosen at random from the study site collected.

Seeds cleaned manually, placed in paper bags and stored dry under laboratory conditions (20 - 25°C) until the start of the experiment.

3.3.2. Seed viability test:

To test seed viability (is a measure of the percentage of seeds that are alive after storage), seeds placed in a beaker then soaked in water. The seeds that float up discarded.

3.3.3. Control of pathogens:

Aseptic technique is an important way to reduce pathogenic contamination by fungi, molds and bacteria by killing and minimizing their presence. All work surfaces cleaned and disinfected with 95% ethanol. The easiest way to prevent contamination is by surface sterilization of seeds to be used in the research. Seeds soaked in 70% ethanol for 1 minute then thoroughly rinsed 4 to 5 times in sterilized distilled water, to minimize microorganism development at the early stages of germination.

3.3.4. pre-sowing treatments:

A. Soaking In tap Water:

Seeds soaked in distilled water 3 volumes of water for each volume of seeds), at room temperature (approximately 25˚C) for 72 h. The water was renewed daily.

B. Boiling Water (100˚C):

Seeds immersed in boiling distilled water (3 volumes of water for each volume of seeds) and then left to cool at room temperature (approximately 25˚C) for 24 h (Pérez-García, 2009).

C. Acid scarification with Sulfuric acid (H_2SO_4):

Concentrated sulfuric acid (96% H_2SO_4) was used to soak seed individually for 5 minutes, 10 minutes and 15 minutes (3 volumes of acid for each volume of seeds). The seeds then washed in running water for 1 hour to remove any trace of acid, before being tested for germination (Pérez-García, 2009).

D. Mechanical scarification:

Chipping was achieved using a sharp blade or rough paper to carefully remove the seed coat, without damaging the radical, at the radical end of the seed ((Karaguzel *et al*., 2002 ;Pérez-García, 2009).

E. Control:

After these pre-sowing treatments, seeds set to germinate according to a fully developed protocol of *in vitro* seed germination for carob found in previous literature and watering with distilled water.

3.3.5. Germination Procedure:

1. In sterile 11 cm Petri dishes lined with double layer whatmann filter paper moisten with 10 ml of water; Seeds platted on Petri dishes under aseptic conditions.
2. Each Petri dish contained 5 seeds of one inbred-line, Petri dishes randomized in a precision incubator and maintained in the dark at 22±0.5°C, this process was in 5 replicates for each pre-sowing treatment as in figure(3-7), total number of seeds should be 25 seeds.
3. Plates watered as needed with 10 ml of water and allowed to germinate, since carob seeds takes long time to germinate seeds allowed to germinate for 21 days.
4. Every day from the beginning of germination, the number of germinated seeds was determined.
5. Germinated seeds counted daily for the calculations of daily and final germination percentages (g%) and mean germination time (MGT) seeds considered germinated when the radical had protruded 5 mm as in figure(3-7).

3.3.6. Measurements of germination experiment:

The germination percentage (G%) and mean germination time (MGT in days) recorded for all treatments.

The Germination percentage was calculated according to (ISTA, 1999) using the following formula:

$$\textbf{Number of germinated seeds/Number of total seeds} \times \textbf{100}$$

The mean germination time (MGT) was calculated according to (Ranal and Santana, 2006) by the expression of:

$$\mathbf{MGT = \Sigma niti/\Sigma ni}$$

Where *ti*: time from the start of the experiment to the *i*th observation (day for the example; *ni*: number of seeds germinated in the *i*th time (not the accumulated number, but the number correspondent to the *i*th observation)

Fig. (3-7): Germination experiment.

3.4. Seedling development study:

Germinated seeds of carob allowed to develop and grow the seedlings under the same conditions. Seedlings daily monitored, shoot and root lengths measured by the end of the experiment.

3.4.1. Measurements of seedling developing study:

At the end of the growth period in this study, root length, shoot length, fresh and dry weight of the grown plant measured as in figure(3-8). Fresh weight measured directly by sensitive balance, dry weight taken after drying of the plant in an oven at 65° C for 24 hours. The seedling vigor index was calculated according to (Abdul-Baki and Anderson, 1973) formulae as following:

SVI = (Shoot length+ Root length) × Germination percentage.

Fig (3-8): Measurements of seedling development.

3.5. Statistical Analysis :

Variables displayed as means and standard deviation. The statistical analysis was performed using SPSS (Statistical Package for Social Sciences, version 26). The first part of the study was analyzed by One-way analysis of variance (ANOVA) to find out if there was statistical differences in the mean of individual morphological parameters of carob plant in different four locations, Pearson correlation was used to correlate pod size and other morphological parameters of carob. the second part of the study evaluated by independent sample test to find out the differences in the means of germination percentage for each pre-sowing treatment and control, one way ANOVA was used to compare these pretreatments with each other LSD was performed for further statistical analysis

Chapter Four

4. Results

The goal of the present study was the assessment of several dormancy-breaking treatments applied to enhance the germination of carob seeds from Al-Jabal Al-Akhdar area and to offer a contribution to the improved information of seed biology of carob, mainly for the intention of utilization in the nursery practice. Treatments enhancing seed germination presented in bibliography investigated. five different pres-owing treatments applied and examined for their effectiveness to stimulate carob seed germination.

4.1. Evaluation of morphological characters of Libyan carob:

Different locations in El-Jabal El-Akhdar region evaluated five different plant characters on discriminative pods measured to know the length, width, thickness, number of seeds, seeds weight, leaf dimensions length and width of the whole trees.

4.1.1. Carob Pods sizes (cm^2):

As shown in the table (4-1) and figure (4-1) the length and the width of 10 pods multiplied to get the whole size of the pods, The pod size of the Libyan carob is ranged between (11.26 – 23.12 cm^2). The pods sizes compared in the four locations in second location larger volume of the pod found (mean16.9 cm^2) while the smallest volumes noticed in the fourth study location (mean 14.25 cm^2), with no significant differences in the mean of the volume of carob pods in all the study locations (p-value > 0.05).

4.1.2. Weight of 100 carob seeds(g):

Five groups of 100 viable seeds weighted from each location, seeds weights found to range between (12-18.47g), increased weight of carob seeds found in the first location, lighter carob seeds noticed in the fourth location (mean = 15.164g), as described in the table (4-2) and figure (4-2). one way ANOVA test of variance means showed no significant differences in the mean of the four locations of study (p-value > 0.05)

4.1.3. Seeds number per pod:

Seeds number per pod calculated in each study locations the range of seeds numbers ranged between (5-13 seeds per pod), the mean values measured and compared, higher number of seeds was noticed in the first and second location (mean = 9.6 and 9.8) respectively, while the third and fourth locations showed relatively lower

number of seeds (mean = 7.5 and 7.8) respectively, table (4-3 and figure 4-3) representing the number and mean of the seeds per pods. but generally no significant differences in the mean of the seeds numbers according to one way ANOVA test (p-value > 0.05).

4.1.4. Leaf area:

A number Carob plant leaf underwent measurements for their sizes (width and length) in four study location in order to compare their means the range of leaf size measurements was (3-10.26 cm^2), Carob leaf in Wadi El-Kouf location showed increased size (mean = 8.87 cm^2) compared with other locations, followed by Al Himadah (mean = 8.24 cm^2), then Albyadah (mean=7.44 cm^2), but small leaf found in Tukhra (mean = 4.56 cm^2), as shown in the table (4-4) and figure (4-4). The differences in the mean of the leaf size in the different four location was significant according to one way ANOVA test (p-value <0.001), LSD multiple comparison performed, the main differences came from the first location Tukrah as shown in the table (4-5).

4.1.5. Seeds size (cm^2):

Ten Carob seeds individually measured for their dimensions in the four study locations, the mean of the size of theses seeds calculated, higher mean noticed in the first location (mean = 0.547), the other three locations showed very comparable means (0.452, 0.489 and 0.406), as shown in the table (4-6) and figure (4-5), one way ANOVA test showed no significant differences in the mean of the size in the four locations (p-value < 0.05).

4.1.6. Carob trees width:

Carob trees trunks width measured in the four study locations and the mean of the trunks width compared, apparently the trees in the fourth location showed wider trunks compared with the other locations (mean =87.8 cm), followed by the carob trees trunks in the first and second locations (means = 81.6 and 84.8 cm) respectively, but relatively narrower trunks of carob trees noticed in the third location (mean = 67.6 cm), as shown in the table (4-7) and figure (4-6). No significant differences in the mean of the trunks in theses four locations found according to one way ANOVA test (p- value >0.05).

4.1.7. Carob trees height (m):

Carob tree height in meters measured in the four study locations, generally the Carob height ranges (12.2-16.8 m), the mean of these measurements calculated and compared higher carob trees found in the first location (mean = 15.07 m) followed by the third location (mean = 14.9 m) the means of height very comparable in the other two locations (means = 14.3 and 14.03 m), as shown in the table (4-8) and figure (4-7). The differences in the means of these measurements totally not significant according to one way ANOVA test (p- value > 0.05).

4.1.8. Correlating morphological characters of Carob in the four location:

In this part of calculations interrelations of each morphological parameter evaluated in all locations according to multi-correlation test. No significant correlations found between all growth parameters, as represented in table (4-9).

Table (4-1): Carob pods sizes in four locations in El-Jabal El-Akhdar.

Tukrah	Al Himadah	Wadi El-Kouf	Albyadah
13.53	17.16	16.07	15.12
18.10	17.93	14.98	11.80
12.48	22.93	13.76	14.48
14.04	15.44	15.44	12.15
20.64	20.28	16.54	12.73
14.39	14.63	16.68	11.27
12.82	16.22	23.12	13.94
16.68	14.98	12.21	15.29
20.25	14.46	13.62	19.39
18.25	15.29	15.41	16.33
Mean (cm^2) ± STD			
16.1±2.66	16.9±2.77	15.8±2.94	14.25±2.46
ANOVA		**Sig.**	0.209

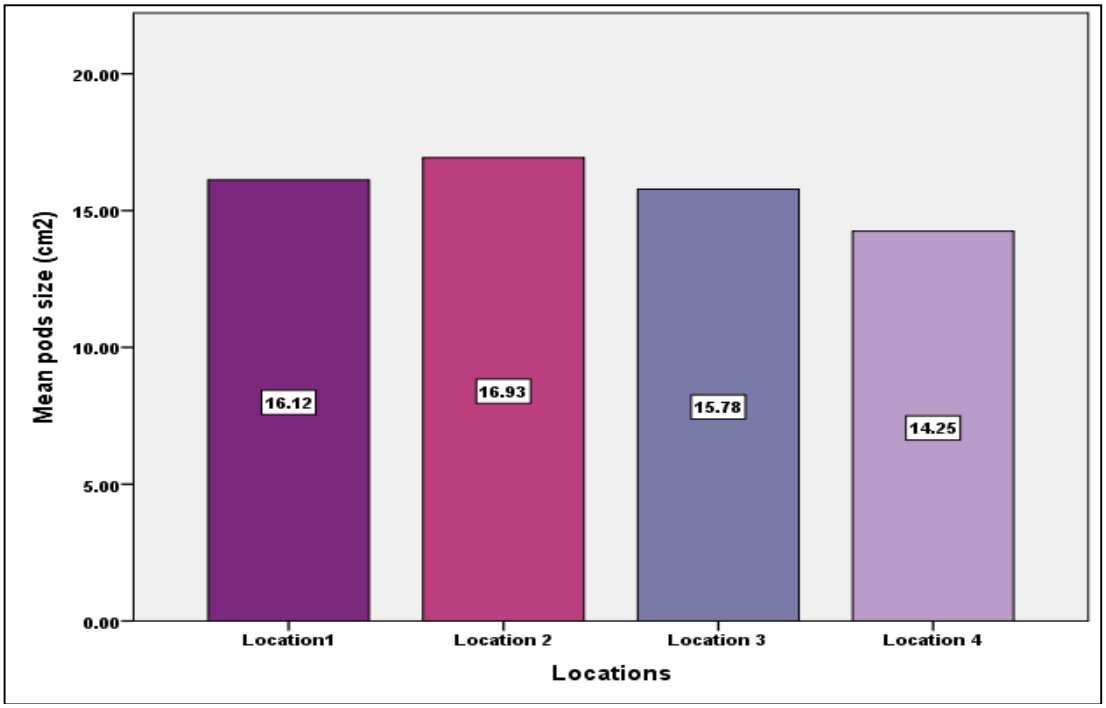

Fig. (4-1): Carob pods dimensions in four locations in El-Jabal El-Akhdar.

Table (4-2): Carob seeds weight (g) in four locations in El-Jabal El-Akhdar.

Tukrah	Al Himadah	Wadi El-Kouf	Albyadah
18.33	15.21	13.19	15.12
17.92	16.82	15.82	17.23
18.10	18.19	16.41	14.06
17.43	14.15	18.47	13.54
15.26	17.31	12.28	15.87
Mean (g) ± STD			
17.408±1.25	16.336±1.63	15.234±2.5	15.1640 ±1.5
ANOVA		***Sig.***	0.192

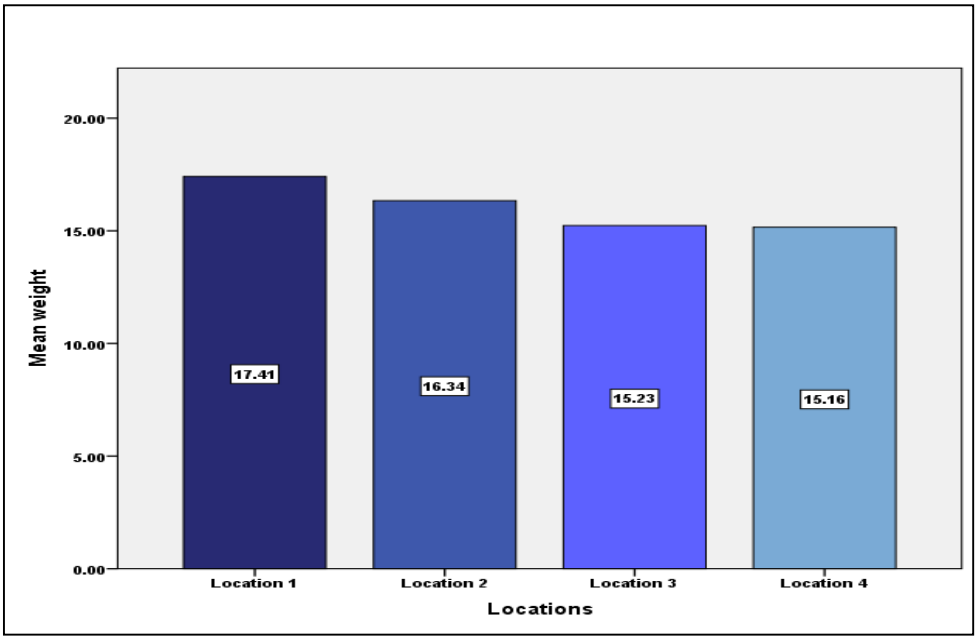

Fig. (4-2): Carob seeds weight in four locations in El-Jabal El-Akhdar.

Table (4-3): Carob seeds number in four locations in El-Jabal El-Akhdar.

Tukrah	Al Himadah	Wadi El-Kouf	Albyadah
7	15	5	8
5	7	8	12
7	11	5	5
10	13	10	5
9	9	8	7
10	11	7	8
12	6	7	9
13	9	6	8
11	9	6	11
12	8	13	5
Mean± STD			
9.6±2.59	9.8±2.74	7.5±2.46	7.8±2.44
ANOVA		Sig.	0.109

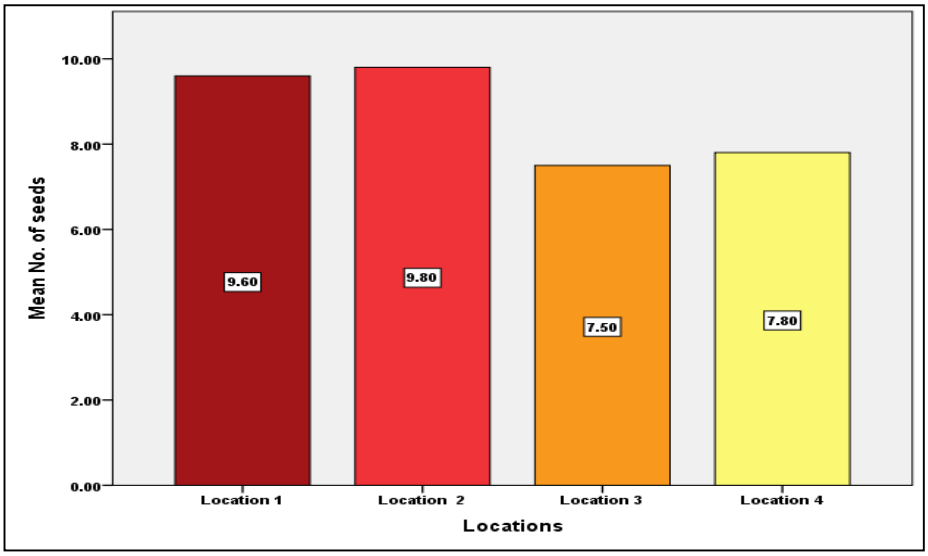

Fig. (4-3): Carob seeds number in four locations in El-Jabal El-Akhdar.

Table (4-4): Carob leaf areas in four locations in El-Jabal El-Akhdar.

Tukrah	Al Himadah	Wadi El-Kouf	Albyadah
7.61	8.1	8.89	9.13
6.08	8.89	8.16	7.56
4.86	8.19	8.78	6.49
3	8.26	10.26	4.59
3.78	8.21	10.15	6.98
4.16	8.87	8.78	8.51
4.61	7.74	9.36	8.19
3.52	7.2	7.2	8.49
3.42	8.72	8.21	7.01
Mean (cm^2) ± STD			
4.56 ±1.47	8.24 ± 0.55	8.87 ± 0.97	7.44 ± 1.37
ANOVA		***Sig.***	0.000

Table (4-5): LSD for the carob leaf area in the four locations.

Locations	Locations	Mean Difference	Sig.
Location1	Location 2	-3.68222*	0.000
	Location 3	-4.30556*	0.000
	Location 4	-2.87889*	0.000
Location 2	Location1	3.68222*	0.000
	Location 3	-0.62333	0.259
	Location 4	0.80333	0.148
Location 3	Location1	4.30556*	0.000
	Location 2	0.62333	.259
	Location 4	1.42667*	0.013
Location 4	Location1	2.87889*	0.000
	Location 2	-0.80333	0.148
	Location 3	-1.42667*	0.013

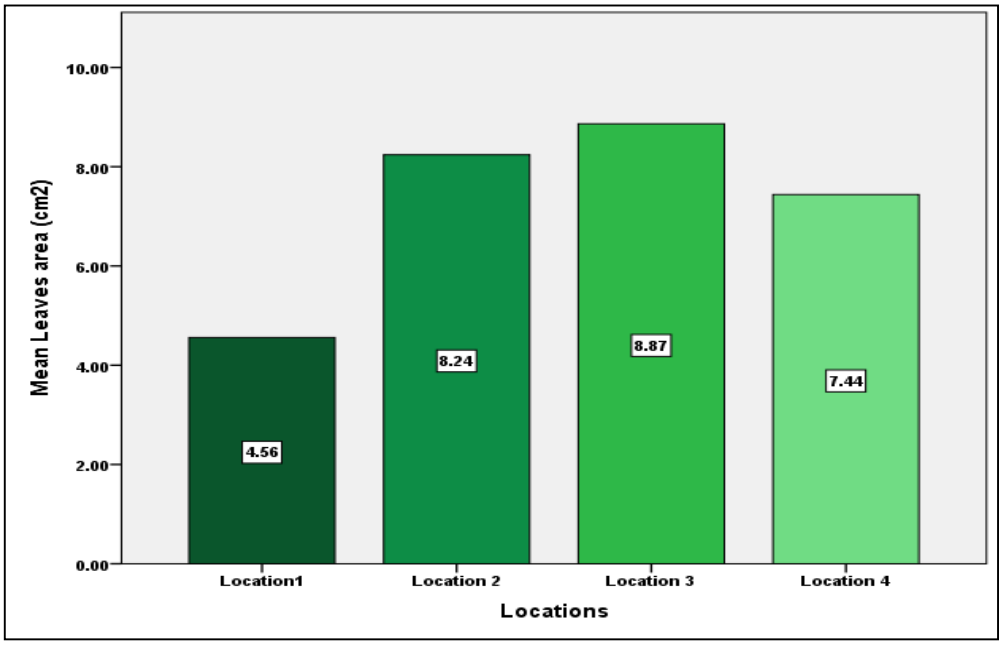

Fig. (4-4): Carob leaf volume in four locations in El-Jabal El-Akhdar.

Table (4-6): Carob seeds size in four locations in El-Jabal El-Akhdar.

Tukrah	Al Himadah	Wadi El-Kouf	Albyadah
0.5	0.4	0.5	0.5
0.5	0.6	0.84	0.4
0.5	0.4	0.4	0.45
0.84	0.48	0.56	0.45
0.56	0.5	0.5	0.4
0.55	0.52	0.4	0.6
0.52	0.27	0.6	0.52
0.6	0.5	0.39	0.4
0.4	0.4	0.22	0.04
0.5	0.45	0.48	0.3
Mean (cm^2) ± STD			
0.547 ±0.115	0.452±0.9	0.489±0.16	0.406±0.15
ANOVA		Sig.	0.132

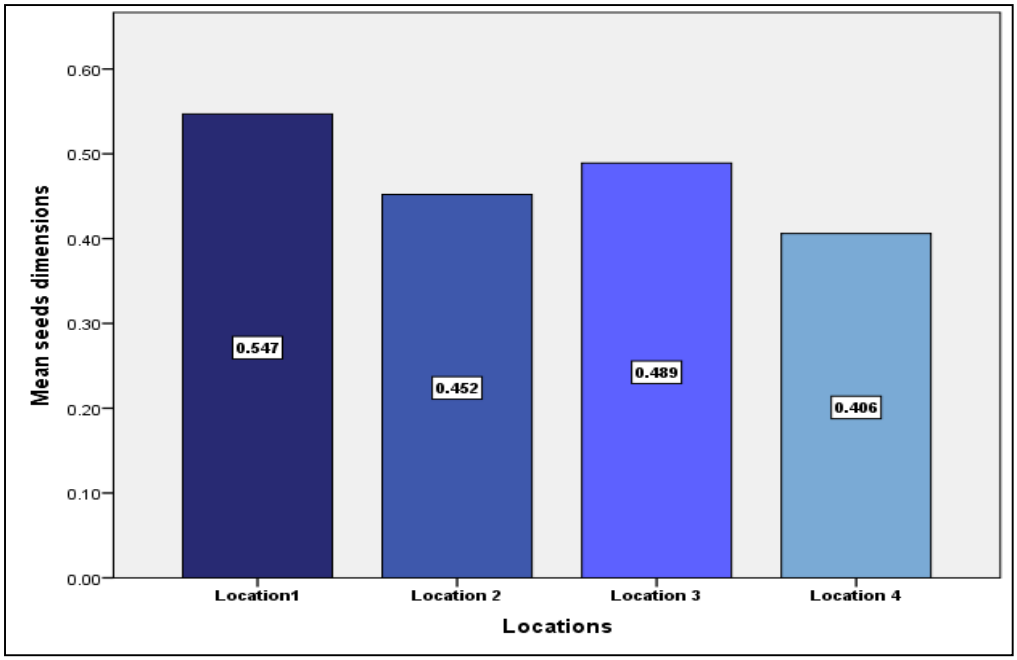

Fig (4-5): Carob seeds volume in four locations in El-Jabal El-Akhdar.

Table (4-7): Carob trunk width in four locations in El-Jabal El-Akhdar.

Tukrah	Al Himadah	Wadi El-Kouf	Albyadah
59	73	65	85
87	82	50	80
113	54	79	59
67	109	85	112
82	106	59	101
Mean (cm) ± STD			
81.6±20.85	79.5±23.08	69.75±14.35	84±20.35
ANOVA		Sig.	0.426

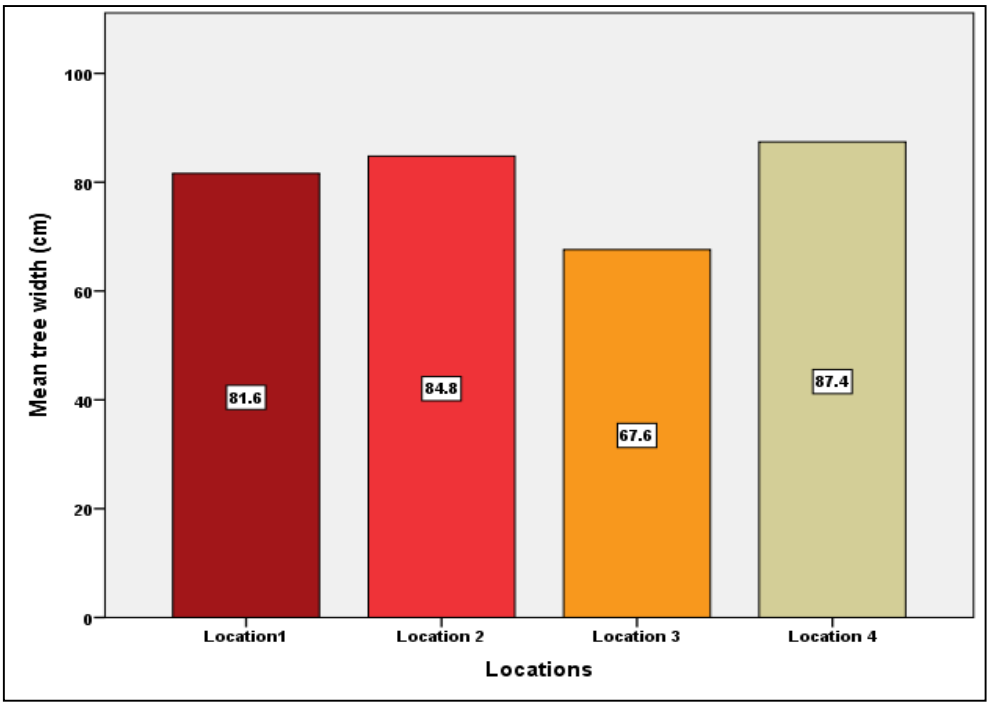

Fig. (4-6): Carob trunk width in four locations in El-Jabal El-Akhdar.

Table (4-8): Carob tree height in four locations in El-Jabal El-Akhdar.

Tukrah	Al Himadah	Wadi El-Kouf	Albyadah
12.6	14.7	16.5	12.2
16.8	14.8	12.8	14.2
15.8	13.5	15.4	15.7
Mean (m) ±STD			
15.07±2019	14.3± 0.723	14.9±1.9	14.03± 0.87
ANOVA		Sig.	0.871

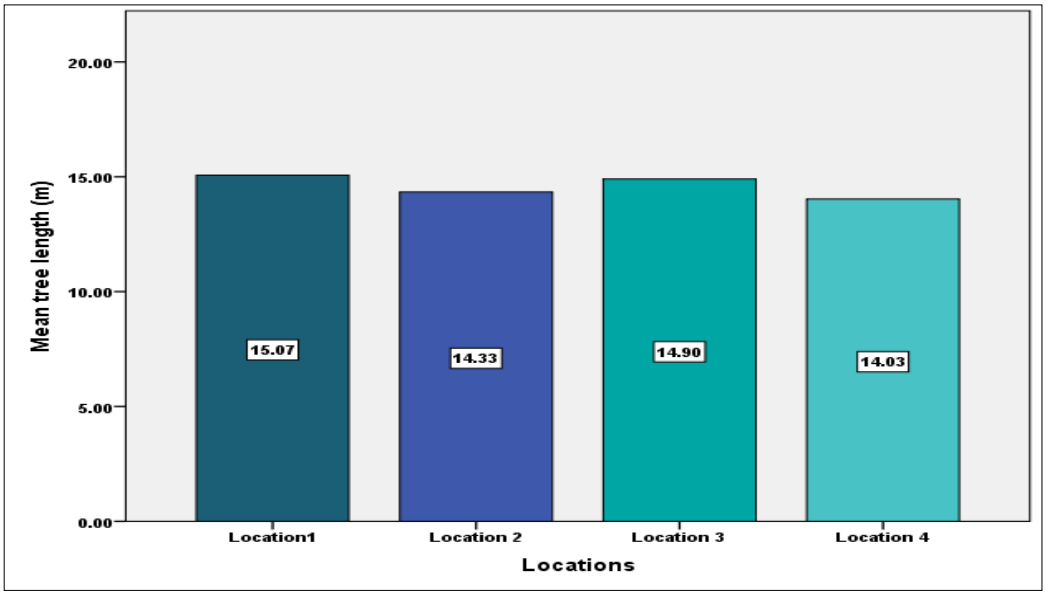

Fig. (4-7): Carob tree height in four locations in El-Jabal El-Akhdar.

Table (4-9): Evaluating morphological parameters relations of Libyan Carob in El-Jabal El-Akhdar.

Parameters		Pods size	No. of seeds	Leaf areas	Seeds sizes
Pods size	**Correlation**	-	0.088	0.192	-0.169
	Sig.	-	0.302	0.128	0.159
No. of seeds	**Correlation**	0.088	-	0.01	-0.005
	Sig.	0.302	-	0.477	0.489
Leaf area	**Correlation**	0.192	0.010	-	0.026
	Sig.	0.128	0.477	-	0.439
Seeds size	**Correlation**	-0.169	-0.005	0.026	-
	Sig.	0.159	0.489	0.439	-

4.2. Germination experiment:

4.2.1. Boiling water pre-sowing treatment:

The germination of the 25 carob seeds after treatment with boiling water evaluated, 24 seeds (96%) germinated starting from the fifth day, the results was higher the than the control which show only lower germination 32%, as represented in table (4-10) and figure (4-8).

4.2.2. Mechanical scarification pre-sowing treatment:

In the second treatment the germination of the 25 carob seeds treated with mechanical scarification evaluated, only 10 seeds (40%) germinated starting from the fifth day, the results was higher the than the control which show only lower germination 12%, as represented in table (4-11) and figure (4-9).

4.2.3. Sulfuric acid for 5 minutes pre-sowing treatment:

In this treatment only 8 seeds (32%) germinated, about half of the number 4 seeds germinated in the control treatment (16%), as shown in table (4-12) and figure (4-10).

4.2.4. Sulfuric acid 10 for minutes pre-sowing treatment:

In this treatment only 10 seeds (40%) germinated, only 4 (16%) seeds germinated in the control treatment, as shown in the table (4-13) and figure (4-11).

4.2.5. Sulfuric acid for 15 minutes pre-sowing treatment:

In this treatment about 15 seeds (60%) germinated, in the control treatment only 7 (28%) seeds germinated, as represented in the table (4-14) and figure (4-12).

4.2.6. Regular tap water pre-sowing treatment:

In this treatment no seeds showed any germination during the period of the experiment, 0% germination was recorded.

Comparing the results of carob seeds germination in the six treatments, showed that relatively higher germination percentage was noticed in the seeds treated with boiling water 96%, followed by carob seeds treated with sulfuric acid for 15 minutes which showed 60% germination, the mechanical scarification and sulfuric acid for 10 minutes showed similar germination percentages 40%, the seeds treated with sulfuric acid for 5 minutes showed relatively the smaller germination percentage 32%, while

the treatment with regular tap water showed zero germination as shown in the figure (4-13).

4.2.7. Mean germination time (MGT):

The mean germination time was calculated individually in each treatment, relatively longer mean germination time was noticed in mechanical scarification treatment, the seeds needs about (12.5 days) to germinate, followed by the seeds treated with sulfuric acid 5 minutes (7.8 days), followed by seeds treated with sulfuric acid 10 minutes which germinated in about (6.9 days), seeds treated with boiling water showed germination in (5.79 days), short germination time was noticed in seeds treated with sulfuric acid for 15 minutes (5.125) as shown in the table (4-15) and figure (4-14)

The accumulated germination is plotted versus (vs) time in the figure (4-15), faster germination was noticed in sulfuric acid 15 minutes pretreatment (4 days) which reached maximum germination by the seventh day of incubation, followed by seeds pretreated with boiling water (5 days) which reached the maximum germination by the seventh day of incubation, sulfuric acid 10 minutes pretreatment also started to germinate in (5 days) but delayed final germination to the tenth day of incubation was noticed, seeds pretreated with sulfuric acid 5 minutes started to germinated during (6 days) also delayed final germination to the ninth was noticed , mechanical scarification showed slower rate (10 days) the final germination was delayed to 14 days.

4.2.8. Seedling vigour index (SVI):

Carob seeds showed different vigour indices in different pre treatments, which was higher in seeds treated with boiling water, followed by seeds treated in sulfuric acid for 15 minutes, similar results found in seeds treated with mechanical scarification and sulfuric acid for 5 minutes , lower seedling vigour index was recorded by seeds treated with sulfuric acid for 5 minutes. compared with control treatment all pre-sowing treatments SVI significantly increased, as represented in table (4-16) and figure (4-16).

Table (4-10): Germination in Boiling water pre-sowing treatment.

Boiling water	Test	Control
Seeds No.	24	8
Percentage %	96%	32%

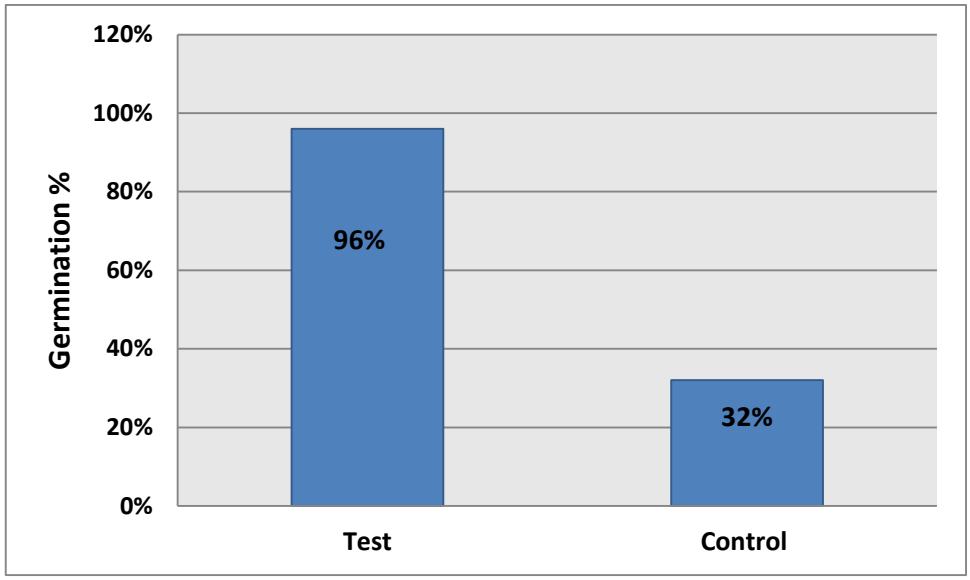

Fig. (4-8): Germination in Boiling water pre-sowing treatment.

Table (4-11): Germination in Mechanical scarification pre-sowing treatment.

Mechanical scarification	Test	Control
Seeds No.	10	3
Percentage %	40%	12%

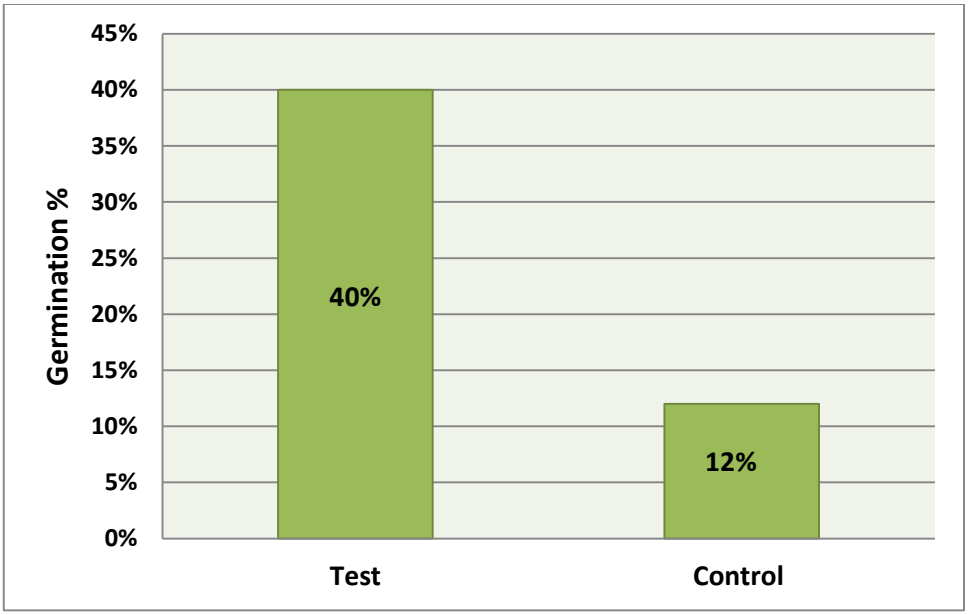

Fig. (4-9): Germination in Mechanical scarification pre-sowing treatment.

Table (4-12): Germination in H_2SO_4 for 5 min.

H_2SO_4 for 5 min	Test	Control
Seeds No.	8	4
Percentage %	32%	16%

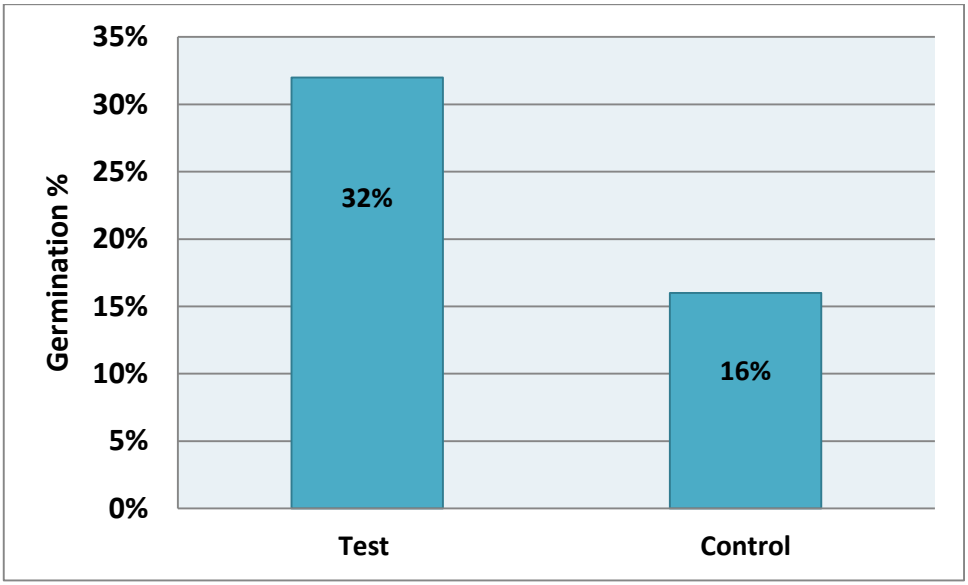

Fig. (4-10): Germination in H_2SO_4 for 5 min.

Table (4-13): Germination in H_2SO_4 for 10 min.

H_2SO_4 for 10 min	Test	Control
Seeds No.	10	4
Percentage %	40%	16%

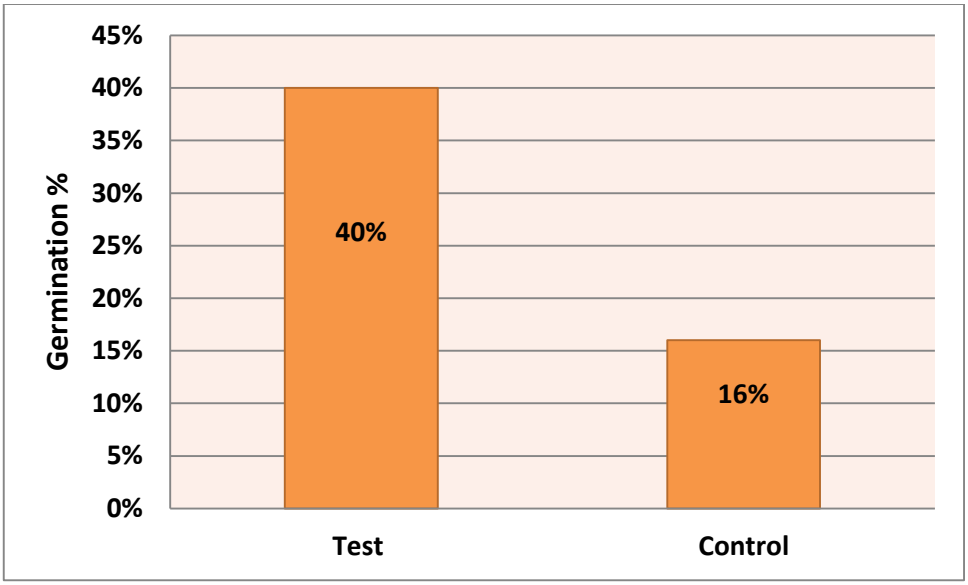

Fig. (4-11): Germination in H_2SO_4 for 10 min.

Table (4-14): Germination in H_2SO_4 for 15 min.

H_2SO_4 for 15 min.	Test	Control
Seeds No.	15	7
Percentage %	60%	28%

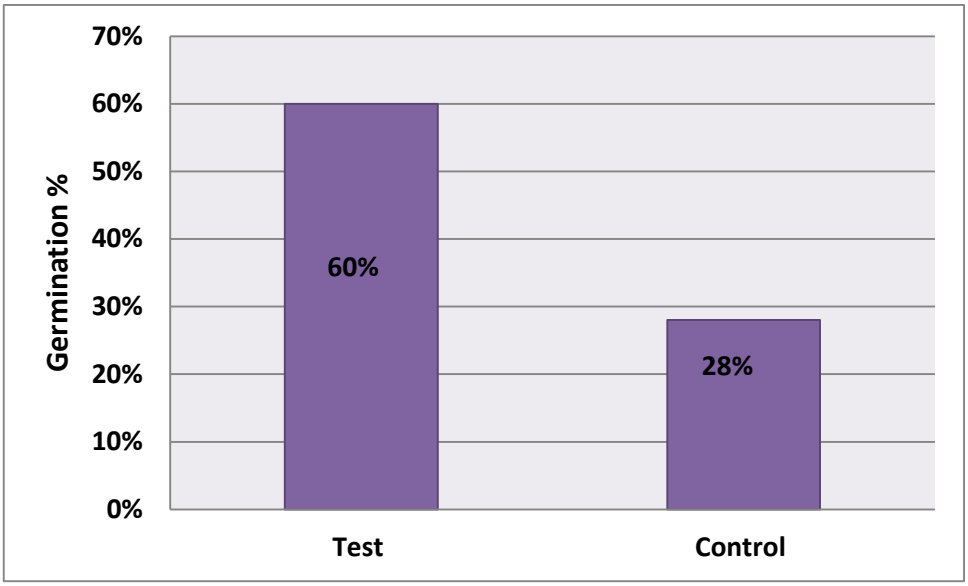

Fig. (4-12): Germination in H_2SO_4 for 15 min.

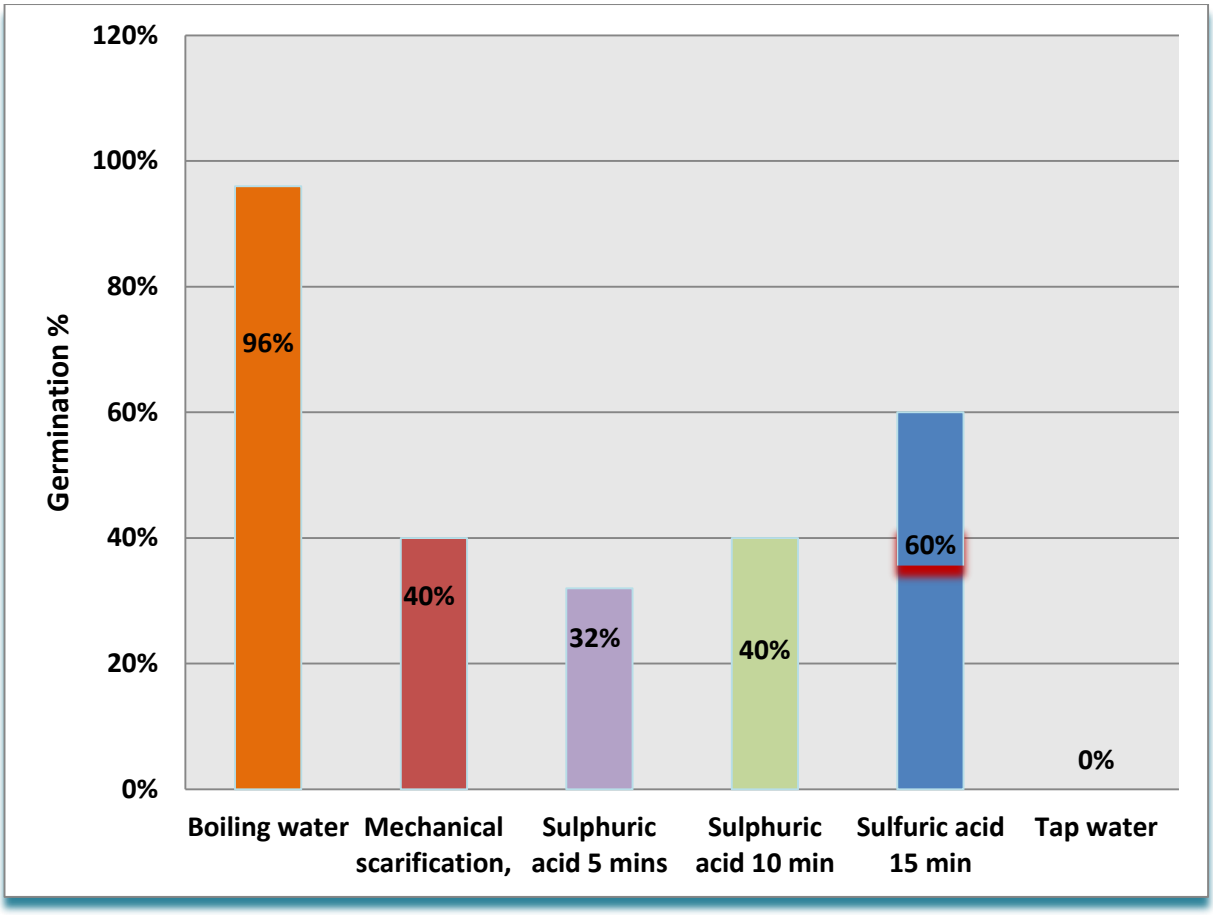

Fig. (4-13): Comparing germination percentage in the all pre-sowing treatments.

Table (4-15): Mean germination time for Carob seeds in different treatments.

Days	Boiling water	Mechanical scarification	H_2SO_4 5 min	H_2SO_4 10 min	H_2SO_4 15 min
1	-	-	-	-	-
2	-	-	-	-	-
3	-	-	-	-	-
4	-	-	-	-	8 (32%)
5	7 (28%)	-	-	4 (16%)	9 (36%)
6	18 (72%)	-	2 (8%)	7 (28%)	13(52%)
7	24 (96%)	-	3 (12%)	9 (36%)	16(60%)
8	-	-	7 (28%)	9 (36%)	-
9	-	-	8 (32%)	9 (36%)	-
10	-	2 (8%)	-	10(40%)	-
11	-	2 (8%)	-	-	-
12	-	4 (16%)	-	-	-
13	-	7(28%)	-	-	-
14	-	10(40%)	-	-	-
15	-	-	-	-	-
MGT	**5.79**	**12.5**	**7.8**	**6.9**	**5.125**

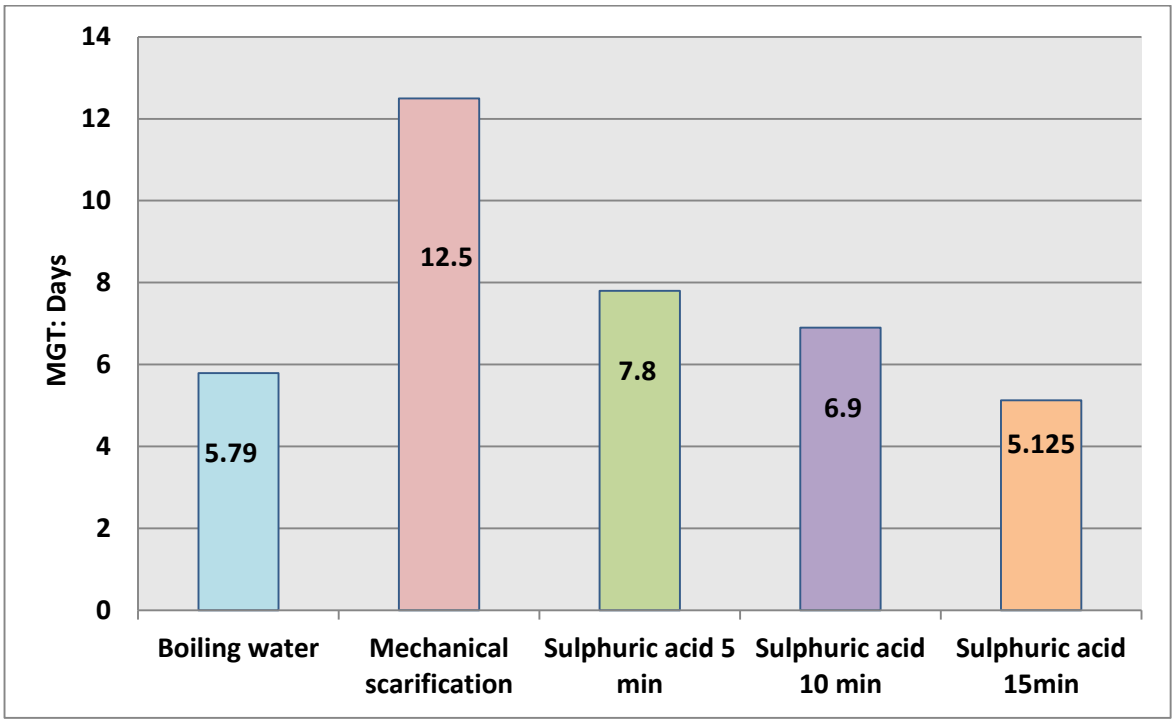

Fig. (4-14): Mean germination time for Carob seeds in different treatments.

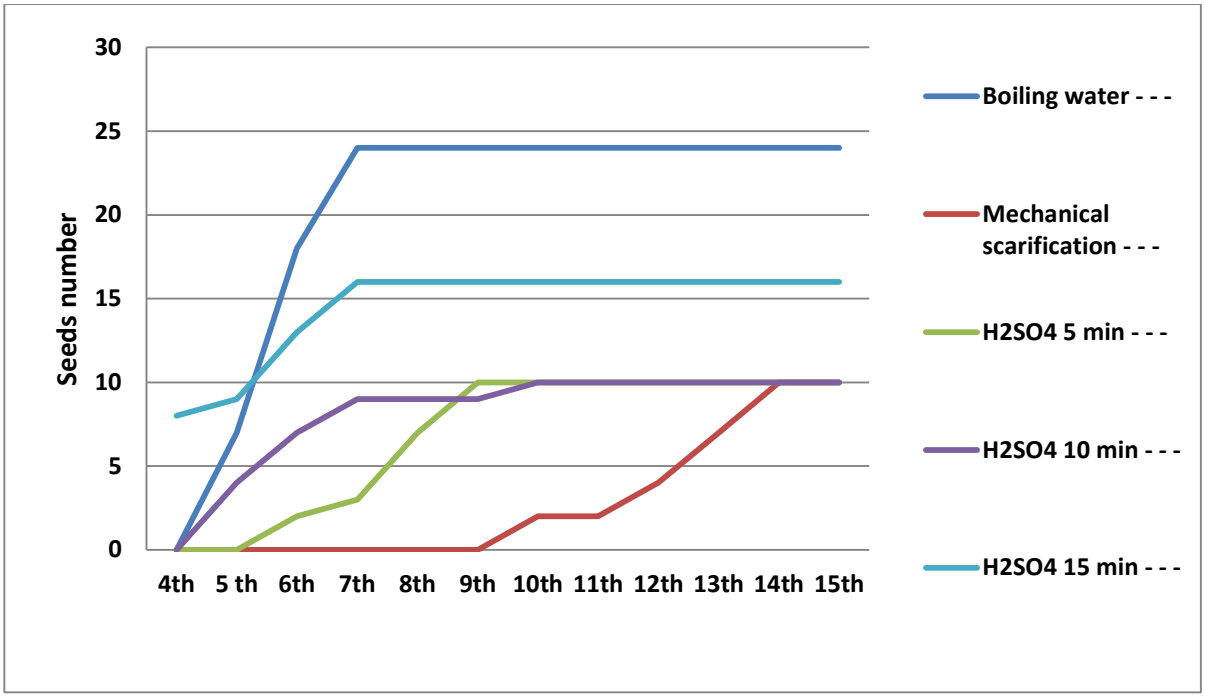

Fig (4-15): Accumulated germination process of all pre-sowing treatments

Table (4-16): Seedling vigours index in different Carob seeds treatments.

Treatment	Boiling water	Mechanical scarification	H_2SO_4 5 min	H_2SO_4 10 min	H_2SO_4 15 min	Control
Seedling length	7.63	7.2	7.5	7.2	8.02	4.3
Germination%	96	40	32	40	60	16
SVI	732.5	288	240	288	481.2	68.8

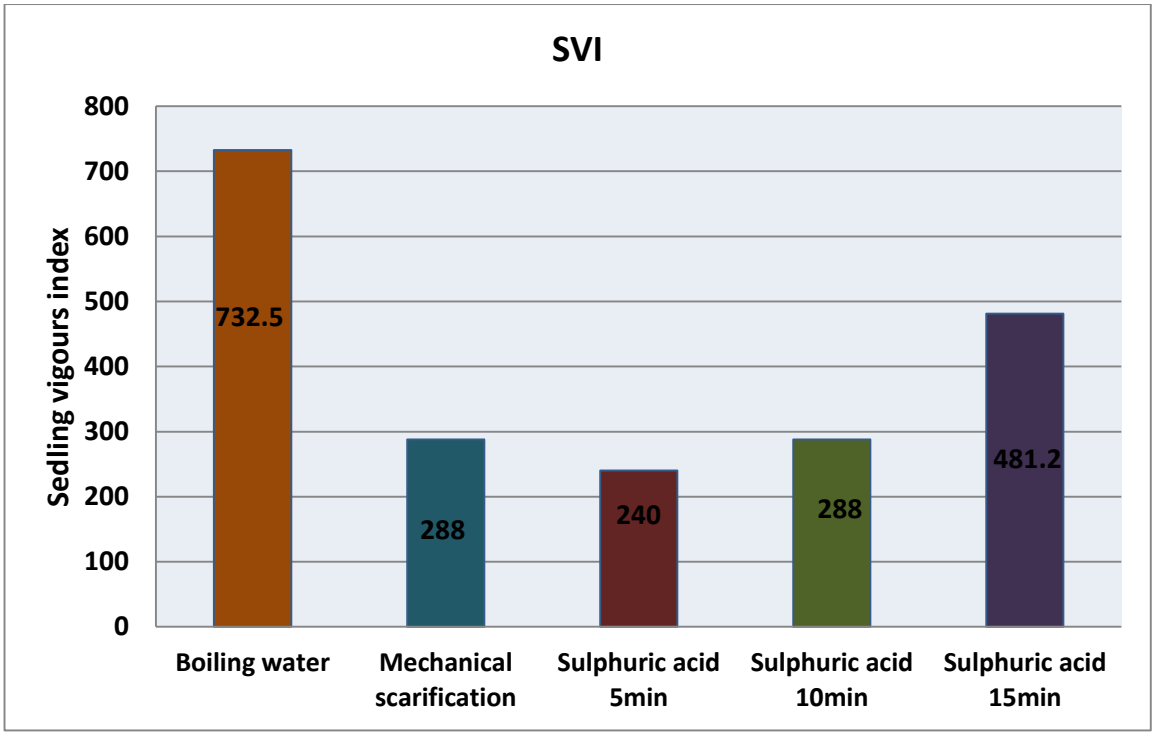

Table (4-16): Seedling vigour index in different Carob seeds treatments

4.3.Evaluation of seedling development of Carob plant:

The germinated seeds in each treatment underwent different measurements for their seeds fresh and dry weight, root and shoot lengths also measured these parameters was collected individually and the mean of the parameters calculated and compared with the control using independent T test.

4.3.1. Seedling development in boiling water pre-sowing treatment:

In this treatment the means the fresh weights of seeds treated with boiling water and control significantly different (p- value < 0.05). the mean of the dry weights was not significant (p- value > 0.05), in other hand the differences in the mean of the root and shoot lengths of the germinated seeds and control was highly significant (p-value < 0.05) for both root and shoots, as shown in the table (4-17), figures (4-17) and figure (4-18).

4.3.2. Seedling development in mechanical scarification pre-sowing treatment:

In this treatment the means the fresh weights of seeds treated with mechanical scarification and control significantly different (p- value < 0.05). the means of the seeds dry weights and control also significant (p- value < 0.05), the means of the root

length of the seedling and control also significant (p- value < 0.05), while the means of the shoot length of the seedling and control not significant (p- value > 0.05), as shown in the table (4-18), figures (4-19) and (4-20).

4.3.3. Seedling development in Sulfuric acid 5 minutes pre-sowing treatment:

In this treatment the means the fresh weights of seeds treated with sulfuric acid 5 minutes and control significantly different (p- value < 0.05). the means of the seeds dry weights and control not significant (p- value > 0.05), also the means of the root and shoots length of the seedling compared with control also not significant (p- value > 0.05). as shown in the table (4-19), figures (4-21) and (4-22).

4.3.4. Seedling development in Sulfuric acid 10 minutes pre-sowing treatment:

In this treatment the means the fresh weights of seeds treated with sulfuric acid 10 minutes and control significantly different (p- value < 0.05). the means of the seeds dry weights and control not significant (p- value > 0.05), also the means of the root and shoots length of the seedling compared with control also not significant (p- value > 0.05), as shown in the table (4-20), figures (4-23) and (4-24).

4.3.5. Seedling development in Sulphuric acid 15 minutes pre-sowing treatment:

The mean of all parameters in seeds treated with sulfuric acid 15 minutes compared with control treatment not significant (p- values > 0.05), which means comparable results found for each parameter compared with control, as shown in the table (4-21), figures (4-25) and (4-26).

4.3.6 Comparisons:

One way ANOVA test of variance was used to determine if there a differences in the means of seedling parameters in all pre-sowing treatments, the results of the test showed no significant differences in the mean of fresh and dry weight, root and shoot lengths among all pre-sowing treatments (p- value > 0.05).

Table (4-17): Evaluation of seedling characters in boiling water.

Group Statistics				Independent Samples	
Parameter		N	Mean ± STD	T	Sig.
Fresh weight	Treatment	24	0.43±0.08	7.793	0.000
	Control	8	0.18±0.05		
Dry weight	Treatment	24	0.14±0.16	1.405	0.17
	Control	8	0.066±0.043		
Root length	Treatment	24	4.18±0.86	6.597	0.00
	Control	8	2.07±0.45		
Shoot length	Treatment	24	3.45±0.59	4.396	0.00
	Control	8	2.33±0.72		

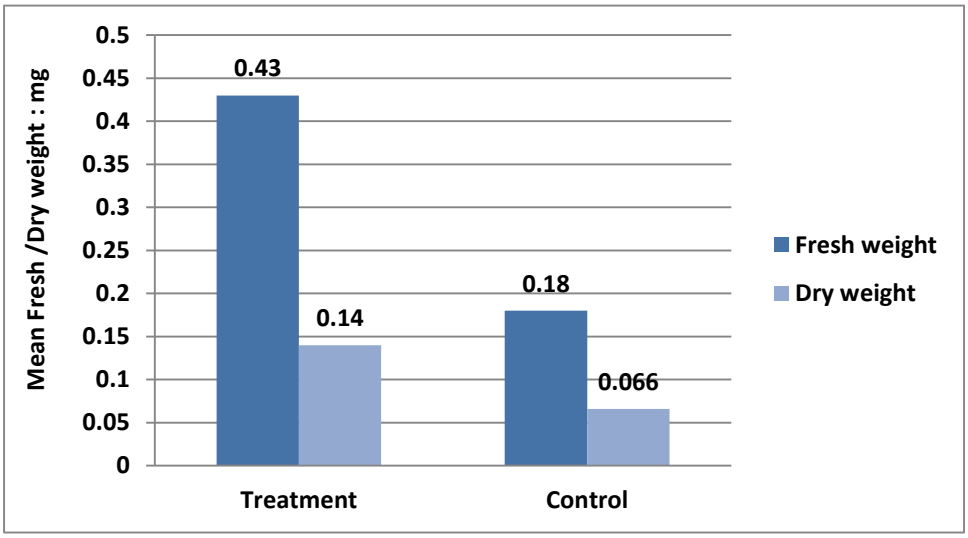

Fig. (4-17): Fresh and dry weight in the boiling water.

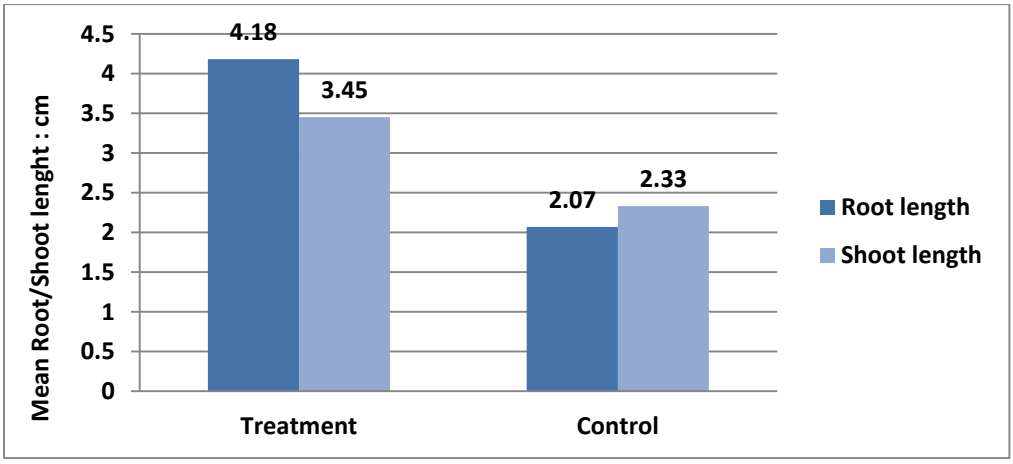

Fig. (4-18): Root and shoot length in boiling water.

Table (4-18): Evaluation of seedling character using mechanical scarification.

Group Statistics				Independent Samples Test	
Parameters		N	Mean ± STD	T	Sig.
Fresh weight	Treatment	10	0.41±0.08	3.698	0.004
	Control	3	0.21±0.07		
Dry weight	Treatment	10	0.15 ±0.04	3.513	0.005
	Control	3	0.04 ±0.04		
Root length	Treatment	10	3.76±0.7	2.609	0.024
	Control	3	2.57±0.5		
Shoot length	Treatment	10	3.55±0.88	0.968	0.354
	Control	3	3.03±0.4		

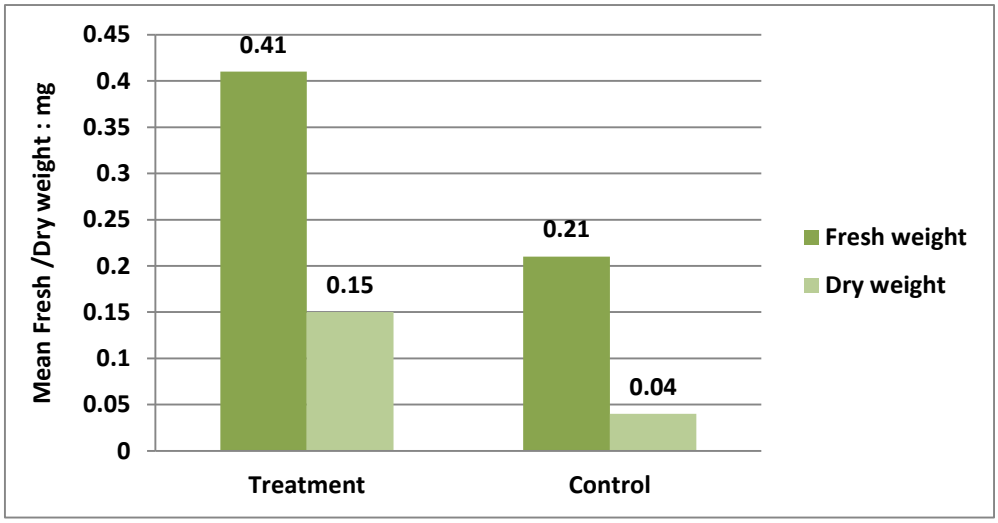

Fig. (4-19): Fresh and dry weight in mechanical scarification.

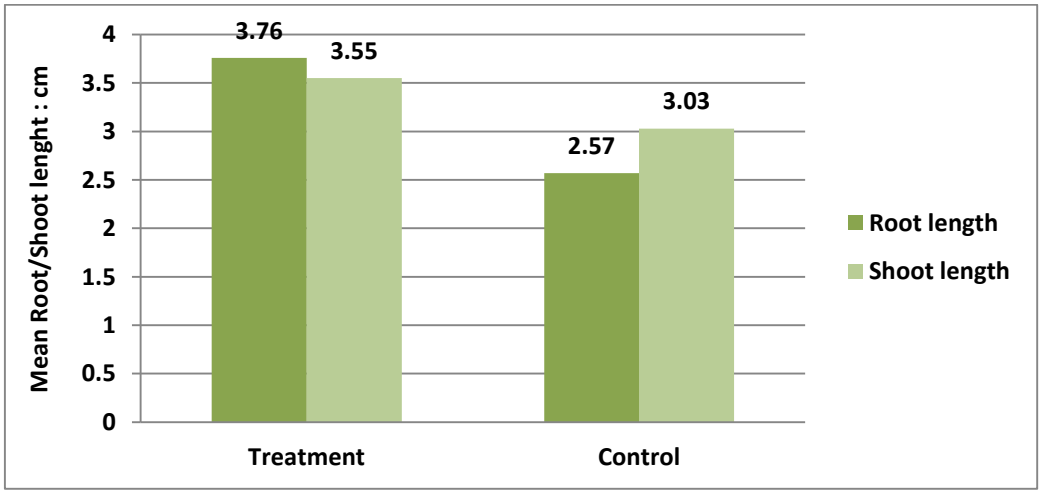

Fig. (4-20): Root and shoot length in mechanical scarification.

Table (4-19): Evaluation of seedling characters using H_2SO_4 for 5 min.

Group Statistics				Independent Samples Test	
Parameters		N	Mean ± STD	T	Sig.
Fresh weight	Treatment	8	0.42±0.065	-2.125	0.06
	Control	4	0.49±0.025		
Dry weight	Treatment	8	0.15±0.035	-0.728	0.483
	Control	4	0.17±0.016		
Root length	Treatment	8	3.63±0.87	0.428	0.678
	Control	4	3.4±0.8		
Shoot length	Treatment	8	3.86±0.72	1.144	0.279
	Control	4	3.35±0.76		

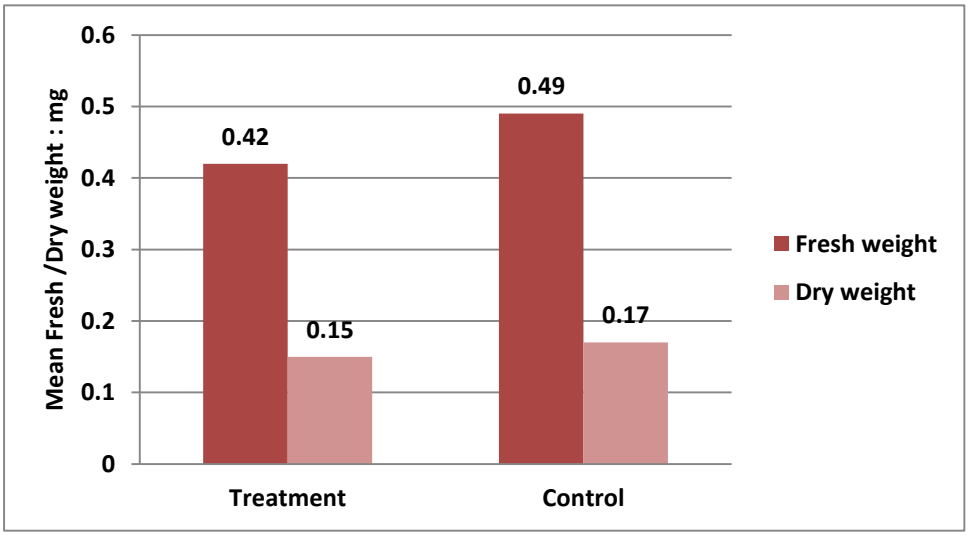

Fig. (4-21): Fresh and dry weight in H_2SO_4 for 5 min.

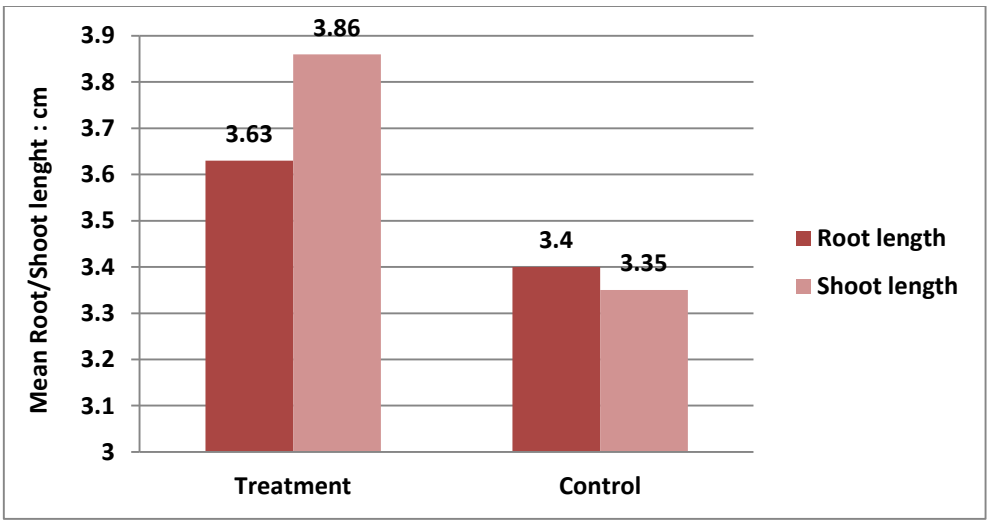

Fig. (4-22): Root and shoot length in H_2SO_4 for 5 min.

Table (4-20): Evaluation of seedling characters using H_2SO_4 for 10 min.

Group Statistics				Independent Samples Test	
Parameters		N	Mean ± STD	T	Sig.
Fresh weight	Treatment	10	0.34±0.07	-2.228	0.042
	Control	7	0.43±0.09		
Dry weight	Treatment	10	0.14±0.027	-0.958	0.353
	Control	7	0.15±0.033		
Root length	Treatment	10	3.53±0.87	0.409	0.688
	Control	7	3.33±1.17		
Shoot length	Treatment	10	3.73±1.03	-0.39	0.702
	Control	7	3.96±1.37		

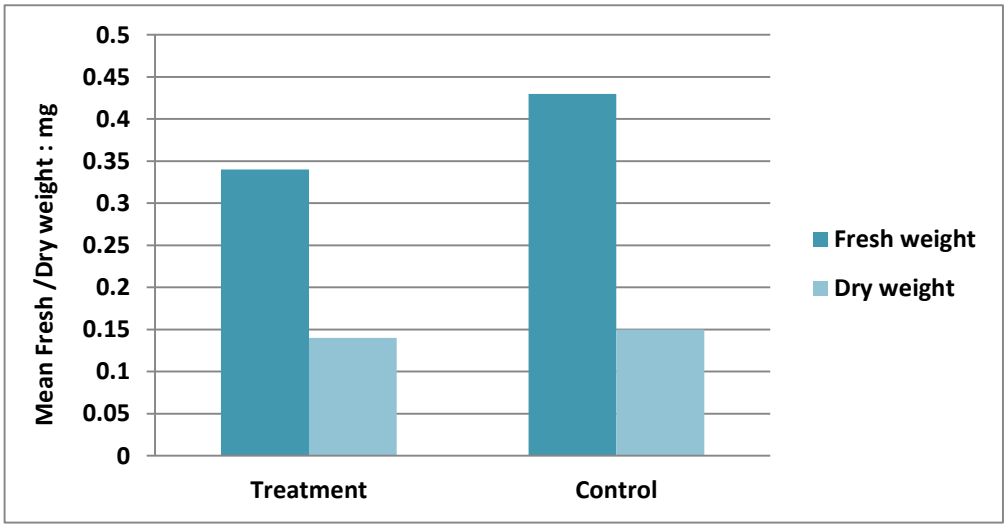

Fig. (4-23): Fresh and dry weight in H_2SO_4 for 10 min.

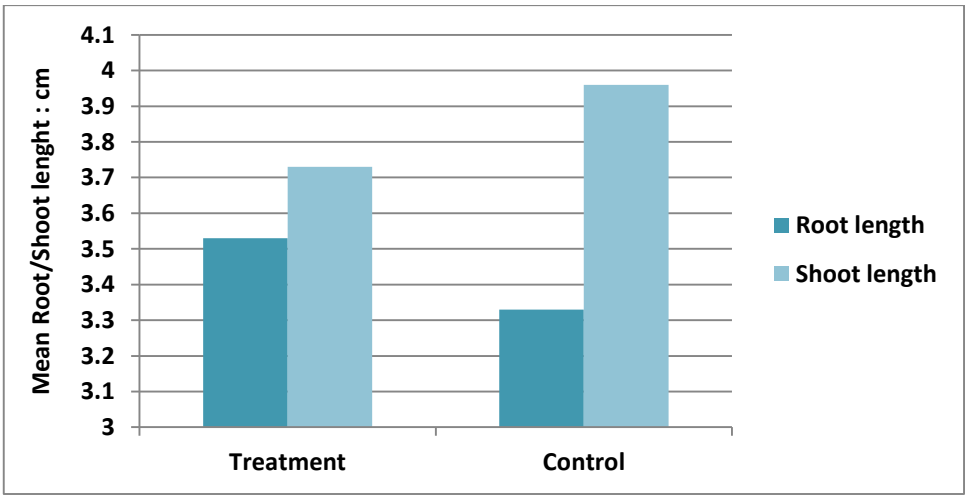

Fig. (4-24): Root and shoot length in H_2SO_4 for 10 min.

Table (4-21): Evaluation of seedling characters using H_2SO_4 for 15 min.

Group Statistics				Independent Samples Test	
Parameters		N.	Mean ± STD	T	Sig.
Fresh weight	Treatment	16	0.43±0.089	-2.054	0.052
	Control	8	0.50±0.051		
Dry weight	Treatment	16	0.16±0.036	-1.524	0.143
	Control	8	0.18±0.013		
Root length	Treatment	16	4.36±0.73	0.693	0.495
	Control	8	4.14±0.79		
Shoot length	Treatment	16	3.66±0.77	-0.613	0.546
	Control	8	3.89±1.06		

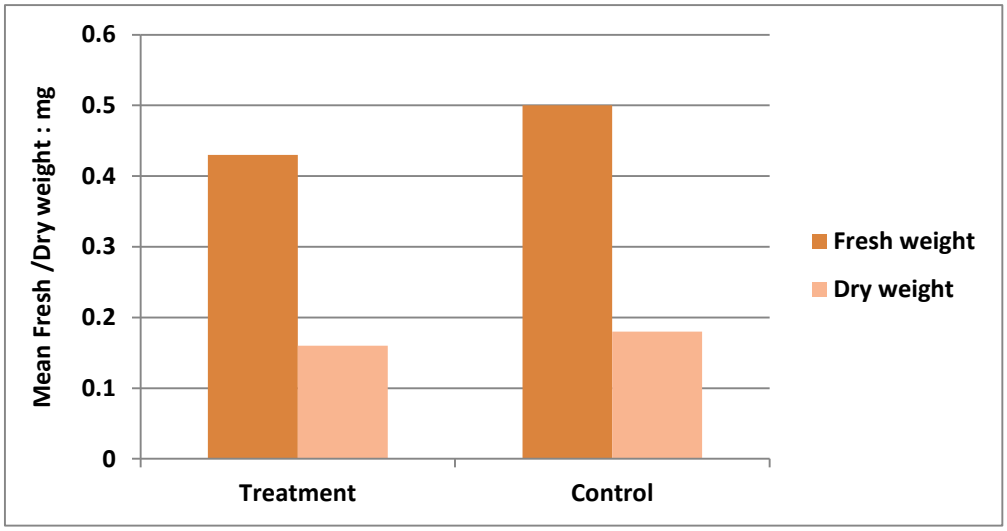

Fig. (4-25): Fresh and dry weight in H_2SO_4 for 15 min.

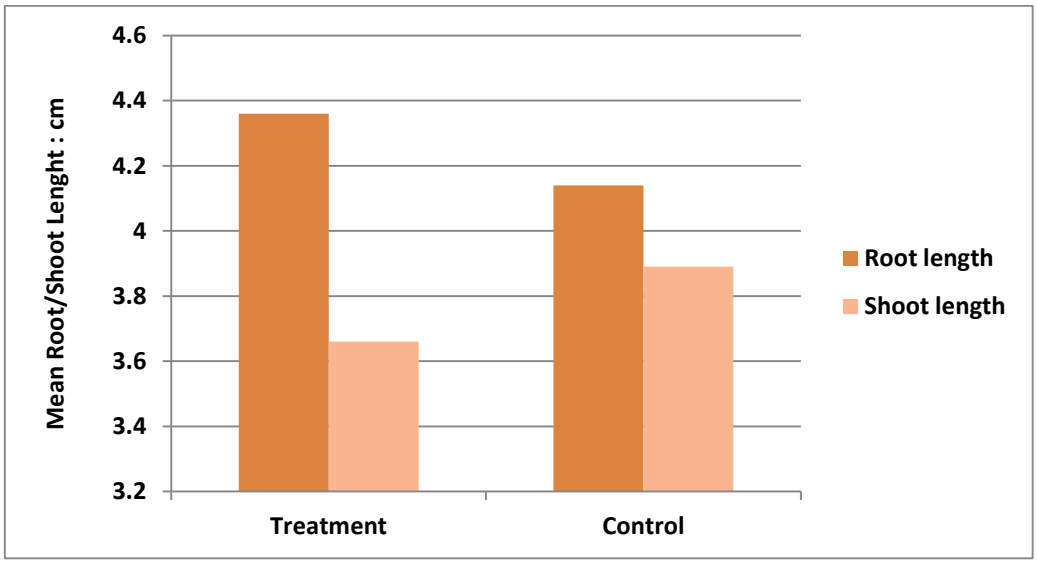

Fig. (4-26): Root and shoot length in H_2SO_4 for 15 min.

Chapter Five

5. Discussion

Carob (*Ceratonia siliqua* L.) is one of the most environmentally and economically an important tree and is among the most difficult to propagate fruit and slow-growing species , the carob tree may live more than a 100 years and begins fruiting after 6 or 7 years of growth. The pods of *Ceratonia siliqua* L. (*Fabaceae*) have taken foot in the ever-growing diet industry as chocolate replacements. The demand for the production of pods has subsequently increased and the up scaling of production is required. The horticultural practices to achieve such results are not as easily achieved with the carob tree as is commonly known. This is because *C. siliqua* is a slow growing tree, with different genders. Only the female trees produce the pods of interest. Another obstacle to overcome is seed dormancy that is attributed to its seemingly impervious seed coat. While, the seeds contain 60% Proteins. Both pods and seeds are used in food and pharmaceutical industries (Loeb *et al*., 1989; Gruendel *et al*., 2006). Carob tree serves as ornamental tree and roadside tree as the tree helps protection of soil from erosion and as fence against sand and some storms. Carob tree seeds with hard coat and it is impermeable to water. In addition to that, carob tree seeds pass through some physiological dormancy based on the environmental conditions where the tree lives in (Gruendel *et al*., 2006). APAT (2003) reported that average germination of carob seeds was 60-95% after treatment because of tough and impermeable seed coat that impede water absorption, thus hindering germination. Without any treatment, germination percentage rarely exceeds 10%. The treatments: Scarification, mechanical with corrosive material, soaking in boiled water for 12-24 hours, acid as 90% H_2SO_4 for 20 min. and even some alkalis. The natural germination of the seeds is lower than 10% (Piotto and Di Noi, 2003). Treatments enhancing seed germination presented in bibliography investigated in this study.

5.1. Evaluation of morphological characters of Libyan carob in four locations:

The first part of the experiment was done to assess the morphological differences of carob population diversity in 4 different location in El-Jabal El-Akhdar region in Libya, five characters used to evaluate the morphological differences of carob plant pods, seeds number, weight, leaf sizes and seeds sizes. Several authors had

resort to the use of pod characteristics to identify, label or to characterize different carob populations, collections, or germaplasms (Marakis *et al*., 1988; Tous *et al*., 1996; Battle and Tous, 1997; Gharnit *et al*., 2001). Moreover, morphological characters of pods and seeds are widely used as quantitative markers to identify populations of carob according to certain criteria such as productivity, resistance to disease, and environmental stress (Konaté *et al*., 2007). The results of this study revealed that, the pod size of the Libyan carob is considered to be medium (14 – 26.5cm). Other studies reported that the average pod size may range from 10 to 30cm, and classified pods size into three categories: long, medium, and short pods (Battle and Tous, 1997). The pods sizes compared in the four locations and no significant differences in the mean of the volume of carob pods in all the study locations. Libyan carob showed no significant differences among the studied populations for all the examined characters except leaf sizes as highly significant differences found in Carob leaf in the four locations. Carob leaf, in Wadi El-Kouf location showed larger area compared with other locations, followed by Alhmeda, then Albyadah, but small leaf found in Tukrah. Other morphological parameters like seeds weight, pods yield (number of seeds per pod), seeds size, trees height and diameter showed no significant differences among the four study locations. These results in disagreement with the results of many authors who had confirmed that carob showed a high diversity in morphological parameters, in Libyan study (Ali *et al*., 2019), Spanish study (Albanell *et al*., 1991), Italian study (Garbgallo *et al*., 1997), Portuguese study (Barracosa *et al*., 2007), Moroccan study (Konaté *et al*., 2007), and Tunisi study (Naghmouchi *et al*., 2009).

5.2. Correlation of morphological characters of Libyan carob:

In this part of the study interrelations of each morphological parameter evaluated in each location according to Pearson correlation test, Correlation is significant at the 0.05 level. The Pearson coefficient correlation among morphological parameters of the Libyan carob revealed that the characteristics describing the pods showed a positive or negative correlation among each other. On the other hand, there are no correlations among characteristics describing the pods and those describing the seeds except for seeds size in Albyadah location. In agreement with our results, many authors have underlined that significant correlations, which found among

characteristics describing pod (El Kahkahi *et al*., 2014; Elfazazi *et al*., 2017; Srečec *et al*., 2016, Ali *et al*., 2019).

5.3. Evaluation of germination experiment results:

The natural germination of the seeds is lower than 10% (Piotto and Di Noi, 2003). Since the tree is growing in many areas in the Mediterranean area, sometimes farm animals eat the pods, the seeds undergo some exposure to acids and digestion enzymes in the stomach of those animals, and they grow naturally better, El-Shatnawi and Ereifej, 2001). This fact withdrew the attention of researchers to apply some artificial pre-sowing treatments to enhance the seed germination. Those treatments include mechanical scarification, soaking in hot/cold water, soaking in mineral acids such as hydrochloric, sulfuric, nitric, and phosphoric acid. Some plant hormones also used to do so such as gibberellin. Concentrated 98%, Sulfuric acid was used to treat the seeds for 30 minutes and the germination percent was raised to 93%, (Gubbuk *et al*., 2009).

The results have shown that germination of control seeds was drastically lower that of mechanically scarified ones (25% and 99%, respectively). These results are in accordance with those obtained by Piotto and Di Noi (2003). The impermeable seed coat, as occur in many *Fabaceae* taxa (Baskin and Baskin, 1998, 2004; Eisvand *et al.*, 2006; Finch-Savage and Leubner-Metzger, 2006; Silveira and Fernandes, 2006; Gresta *et al.*, 2007; Pérez-García, 2008; Pérez-García *et al.*, 2008), is the cause of the physical dormancy of carob seeds. Most carob seeds do not germinate due seed coat impermeability hindering the uptake of water and they will not germinate unless the seed coat is scarified (Piotto and Piccini, 1996).

In this study all pretreatments enhanced the seed coat permeability to varying degrees resulting in higher imbibitions in carob compared with control treatments, results of carob seeds germination in the pre-sowing treatments, showed that relatively higher germination percentage was noticed in the seeds soaked in boiling water 96%, followed by carob seeds treated with sulfuric acid for 15 minutes which showed 60% germination, the mechanical scarification and sulfuric acid for 10 minutes showed similar germination percentages 40%, the seeds treated with sulfuric acid for 5 minutes showed relatively the smaller germination percentage 32%, while the treatment with regular tap water showed zero germination. Other literature in disagreements with our study who found carob seeds germination improved to 95.69% by mechanical

scarification and making some cracks on the seed coat after concentrated sulfuric acid treatment, (Karaguzel *et al*., 2002). Also Perez-Garcia (2009) used various treatments to enhance seed germination in carob and found the lowest germination rate in the control seeds 25% and highest 99% in scarified seeds. Similarly, Tsakaldimi & Ganatsas (2001) found that the highest germination rate 87% was found on seeds dipped in concentrated sulfuric acid for 15 minutes. also Gunes *et al*., (2013) recorded highest germination percentage was obtained with mechanical scarification and also with sulfuric acid treatment,

As far as it is known that all seeds when they passed natural or artificial dormancy due to different conditions or requirements, these seeds start germination and form new plants. Whenever you put the seed in wet environment and oxygen available, they will grow as they start absorb water and the breaking down of reserved food start conversion into usable metabolites and germination happen. Before the germination when the seed is dry, the seed is a dry object and the metabolisms are at the lowest range. The seed coat is tough and does not allow water or gases to go in and let germination takes over. All dry seeds pass through this stage. In germination, water gets into the seed through seed coat and start swelling, the metabolites in seed changed into soluble materials and hydrolytic reactions occur in addition to gases exchange, in order for the germination to start. If the seed coat is not permeable for water due to physiological or natural dormancy, water cannot go into the seed and no germination will occur (Xiaodong *et al.*, 2013). This phenomenon is normal in plant life cycle to preserve the species in life cycle or overcome some unfavorable environmental conditions. However, when the plant seeds exposed to physiological dormancy due to some growth regulators such as abscisic acid, which extends the physiological dormancy (Xiaodong *et al*., 2013), which confirmed occurrence of that dormancy in newly harvested seeds.

The seed dormancy in plant family Fabaceae species (as carob is a member of this plant family), is very much due to the hard seed coat which does not allow water or gases to go in or being exchanged. However, in this case it was needed to treat these seeds to enhance germination when plant them for these purposes. Many chemical, physical, and mechanical methods applied to do so on dormant seeds. In this experiment, hot water treatment increased germination from 23.33% to 97.67% by treating the seeds in hot water for 60 min. The increased germination to 97.67% may be due to increase the permeability of seed coat to water in which the hot water

softened the seed coat and allowed water absorption and solubilize the reserved food substances in cotyledons of the seed and germination occurred. This finding agreed with Longer and Degago, (1996), as the germination enhanced by water absorption and gases exchange in addition to activation of metabolites conversion into usable formulas for metabolism. The gases exchange and water absorption could release the inhibitors in some of the seed embryo parts as phenolic compounds, (Mohamed-Yaseen *et al*., 1994) overcome the problem of seed coat hardness.

5.4. Mean germination time and seedling index:

Faster germination was recorded by seeds pre-treated with sulphuric acid for 15 minutes, seeds started to germinate by the fourth day of incubation with mean germination time (MGT 5.125) started with 32% germination in the fourth day and the final germination was recorded in the seventh day 60%, germination recorded by carob seeds pre-treated with boiling water started to germinate in the fifth day of incubation 28% with mean germination time (MGT 5.79), the final germination was achieved in seventh day of incubation 96%, seeds pre-treated with sulfuric acid 10 minutes started to germinated in the fifth day of incubation by 16% with mean germination time 6.9 days the final germination was recorded in the tenth day 40%, seeds pre-treated with sulfuric acid 5 minutes and mechanical scarification showed somewhat delay in germination 6 and 10 days respectively, mean germination times 7.8 and 12.5 days respectively with higher final germination for the mechanical scarification treatment. few authors evaluated the mean germination time in carob seeds, Gunes *et al*., (2013) found the highest germination percentage was obtained with mechanical scarification and also with sulfuric acid treatment, but the shortest mean germination time was found using scarification (Gunes *et al*., 2013), another author found that after five days incubation the germination rate of seeds that are treated with boiling water has reached a level of 42.5%, 90% for the seeds treated with concentrated sulfuric acid and 12.5% for the control (sterile distilled water). In twelve days of incubation of the germination rate gradually increases with time, for seeds soaked in sulfuric acid germination rate reaches a maximum value of 100%, the seeds are soaked in boiling water for 80% and soaked in sterile distilled water seeds reach 22.5% (El Kahkahi *et al*., 2014).

Carob seeds showed different vigour indices in different pre treatments, which was higher in seeds treated with boiling water, followed by seeds treated in sulfuric

acid for 15 minutes, similar results found in seeds treated with mechanical scarification and sulfuric acid for 5 minutes , lower seedling vigour index was recorded by seeds treated with sulfuric acid for 5 minutes. In contrast, the study conducted by Salih and Abdulrraziq (2018) revealed that, the treatment of scarifying with soaking in gibberellin was more effective than sulfuric acid and all other pre-treatments (Salih and Abdulrraziq, 2018).

5.5 Evaluation of seedling developments measurements:

The germinated seeds in each treatment underwent different measurements for their seeds fresh and dry weight, root and shoot lengths also measured these parameters was collected individually and the mean of the parameters calculated and compared with the control using independent T test. seeds pretreated with soaking in boiling water showed significant differences in their fresh weight, root and shoot lengths compared with untreated control seeds while results of dry weight was not significant compared to control treatment. in previous study by (Saif, 2020) demonstrated that significantly highest root length was obtained with the hotbed treatment compared to untreated seeds (control) treatment resulted in the highest percentages not only in germination parameters but also in seedlings growth parameters. The results of boiling water treatment and its effect on germination can be discussed according to Baskin and Baskin, (2014) who reported that high temperature through the germination process can lead to improvement of seed germination by break physical dormancy for seeds which have hard husk. Also, Kozlowski and Pallardy (1997) reported that some seeds leguminous plants sometimes required heat of more than 50^{o}C to break its seeds dormancy. In mechanical scarification pretreatment the means of fresh and dry weights in addition to root length was highly significant compared to untreated control treatment, while shoot length showed no significant differences compared to control. The scarification treatment improved morphological measurements of carob seed. These findings in agreement with that reported by (Saif *et al*., 2020).

All sulfuric acid pretreatments showed no significant differences in seedling parameters (dry weight, fresh weight, shoot and root lengths) compared to control seeds. As a comparison between theses 5 pretreatment performed, the greatest fresh weight was obtained by seeds pretreated with boiling water and sulfuric acid 15 minutes, the greatest dry weight was obtained by pretreatment with sulfuric acid 15

minutes, the greatest root lengths obtained in germinated seeds pretreated with sulfuric acid 15 minutes but the maximum shoot length was obtained by soaking in sulfuric acid 5 minutes. generally no significant differences in the mean of each individual parameter in each pretreatment, so dry weights, fresh weigh, shoot and root lengths showed no significant differences in each pretreatments. El Deen *et al*,. (2014) studied carob seed propagation. Seeds and cuttings scarified and disinfected under aseptic circumstances with some treatments to increase the germination percentage. Six treatments applied to the seeds: control (untreated seeds), soaking in water at 30^{o}C for 72 h, soaking in hot water for 10 min, dipping in 60% H_2SO_4 for 30 min, dipping in GA_3 and dipping in 80% acetone. Their findings illustrated that the highest values of seed germination percentage, the fastest germination, the greatest plant length, number of leaf/plant, root length and dry weight acquired by soaking seeds in 60% H_2SO_4. Tsakaldimi and Ganatsas. (2001) reported that the shortest root and shoot lengths obtained from hot water treatment due to damage to the embryo. Gunes *et al*., (2013) reported the maximum root length found in sulfuric acid treatment and followed by mechanical scarification of the seeds . However the greatest shoot length was determined with mechanical scarification and the lowest hot water treatment (Gunes *et al*., 2013).

Conclusion

1. In this study four different locations in El-Jabal El-Akhdar region in Libya evaluated for carob plant populations differences. The pod size of the Libyan carob is considered to be medium (11.26 – 23.12 cm^2), seeds number per pod ranged between (3-10.26 cm^2), the range of leaf size measurements was (6-22 cm^2), seeds sizes ranged between (0.2-0.84 g).
2. Generally no significant morphological differences found among theses population except in leaf size which was highly significant, Tukhra area showed smaller leaf sizes compared with other locations.
3. No significant correlations between pod size and other morphological characters of carob in all locations except in Albyadah in which a significant negative correlation was found between pods sizes and seeds sizes.
4. Compared to control, generally all pretreatments significantly enhanced the germination of carob seeds, relatively higher germination percentage was noticed in the seeds soaked in boiling water 96%, followed by carob seeds treated with sulfuric acid for 15 minutes which showed 60% germination, the mechanical scarification and sulfuric acid for 10 minutes showed similar germination percentages 40%, the seeds treated with sulfuric acid for 5 minutes showed relatively the smaller germination percentage 32%, while the treatment with regular tap water showed zero germination.
5. Faster germination was recorded by seeds pre-treated with sulfuric acid for 15 minutes, followed by germination recorded by carob seeds pre-treated with boiling water, then seeds pre-treated with sulfuric acid 10 minute, seeds pre-treated with sulfuric acid 5 minutes and mechanical scarification showed somewhat delay in germination.
6. Carob seeds pretreated with soaking in boiling water showed significant differences in their fresh weight, root and shoot lengths compared with untreated control seeds, while results of dry weight was not significant compared to control treatment.

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Appendix

1. Pods sizes:

Descriptive Statistics				
Locations		N	Mean	Std. Deviation
Location1	pods dimensions	10	16.1180	3.06277
	Valid N (listwise)	10		
Location 2	pods dimensions	10	16.9320	2.76652
	Valid N (listwise)	10		
Location 3	pods dimensions	10	15.7830	2.93883
	Valid N (listwise)	10		
Location 4	pods dimensions	10	14.2500	2.45712
	Valid N (listwise)	10		

ANOVA					
Pods dimensions					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	37.819	3	12.606	1.590	0.209
Within Groups	285.375	36	7.927		
Total	323.194	39			

2. Weigh of 100 seeds:

Descriptive Statistics						
Locations		N	Minimum	Maximum	Mean	Std. Deviation
Location1	Weight	5	15.26	18.33	17.4080	1.24554
	Valid N (listwise)	5				
Location 2	Weight	5	14.15	18.19	16.3360	1.63300
	Valid N (listwise)	5				
Location 3	Weight	5	12.28	18.47	15.2340	2.50510
	Valid N (listwise)	5				
Location 4	weight	5	13.54	17.23	15.1640	1.46841
	Valid N (listwise)	5				

ANOVA					
Weight					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	16.880	3	5.627	1.779	.192
Within Groups	50.599	16	3.162		
Total	67.479	19			

3.Number of seeds:

Descriptive Statistics						
Locations		N	Minimum	Maximum	Mean	Std. Deviation
Location1	No. of seeds	10	5.00	13.00	9.6000	2.59058
	Valid N (listwise)	10				
Location 2	No. of seeds	10	6.00	15.00	9.8000	2.74064
	Valid N (listwise)	10				
Location 3	No. of seeds	10	5.00	13.00	7.5000	2.46080
	Valid N (listwise)	10				
Location 4	No. of seeds	10	5.00	12.00	7.8000	2.44040
	Valid N (listwise)	10				

ANOVA					
No. of seeds					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	42.675	3	14.225	2.169	.109
Within Groups	236.100	36	6.558		
Total	278.775	39			

4.Leaf area:

Descriptive Statistics				
Locations		N	Mean	Std. Deviation
Location1	Leaf area	9	4.5600	1.47006
	Valid N (listwise)	9		
Location 2	Leaf area	9	8.2422	0.54826
	Valid N (listwise)	9		
Location 3	Leaf area	9	8.8656	0.97183
	Valid N (listwise)	9		
Location 4	Leaf area	9	7.4389	1.37395
	Valid N (listwise)	9		

ANOVA					
Leaf area					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	97.771	3	32.590	24.625	0.000
Within Groups	42.351	32	1.323		
Total	140.122	35			

Multiple Comparisons						
Dependent Variable: Leaf area						
LSD						
		Mean			95% Confidence Interval	
(I) Locations	(J) Locations	Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Location1	Location 2	-3.68222*	.54231	.000	-4.7869	-2.5776
	Location 3	-4.30556*	.54231	.000	-5.4102	-3.2009
	Location 4	-2.87889*	.54231	.000	-3.9835	-1.7742
Location 2	Location1	3.68222*	.54231	.000	2.5776	4.7869
	Location 3	-.62333	.54231	.259	-1.7280	.4813
	Location 4	.80333	.54231	.148	-.3013	1.9080
Location 3	Location1	4.30556*	.54231	.000	3.2009	5.4102
	Location 2	.62333	.54231	.259	-.4813	1.7280
	Location 4	1.42667*	.54231	.013	.3220	2.5313
Location 4	Location1	2.87889*	.54231	.000	1.7742	3.9835
	Location 2	-.80333	.54231	.148	-1.9080	.3013
	Location 3	-1.42667*	.54231	.013	-2.5313	-.3220
*. The mean difference is significant at the 0.05 level.						

5. Seeds area:

Descriptive Statistics				
Locations		N	Mean	Std. Deviation
Location1	Leaf area	9	4.5600	1.47006
	Valid N (listwise)	9		
Location 2	Leaf area	9	8.2422	.54826
	Valid N (listwise)	9		
Location 3	Leaf area	9	8.8656	.97183
	Valid N (listwise)	9		
Location 4	Leaf area	9	7.4389	1.37395
	Valid N (listwise)	9		

ANOVA					
seeds dimensions					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.107	3	.036	1.997	.132
Within Groups	.641	36	.018		
Total	.747	39			

6. Trees width:

Descriptive Statistics				
Locations		N	Mean	Std. Deviation
Location1	tree width	5	81.60	20.852
	Valid N (listwise)	5		
Location 2	tree width	5	84.80	23.080
	Valid N (listwise)	5		
Location 3	tree width	5	67.60	14.346
	Valid N (listwise)	5		
Location 4	tree width	5	87.40	20.354
	Valid N (listwise)	5		

ANOVA					
tree width					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	1168.150	3	389.383	.981	0.426
Within Groups	6350.400	16	396.900		
Total	7518.550	19			

Multiple Comparisons

(I) Locations	(J) Locations	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Location1	Location 2	-8.18333*	1.25069	.000	-10.7224	-5.6443
	Location 3	-9.18456*	1.21902	.000	-11.6593	-6.7098
	Location 4	-6.14010*	1.19249	.000	-8.5610	-3.7192
Location 2	Location1	8.18333*	1.25069	.000	5.6443	10.7224
	Location 3	-1.00122	1.21902	.417	-3.4760	1.4735
	Location 4	2.04323	1.19249	.095	-.3776	4.4641
Location 3	Location1	9.18456*	1.21902	.000	6.7098	11.6593
	Location 2	1.00122	1.21902	.417	-1.4735	3.4760
	Location 4	3.04445*	1.15923	.013	.6911	5.3978
Location 4	Location1	6.14010*	1.19249	.000	3.7192	8.5610
	Location 2	-2.04323	1.19249	.095	-4.4641	.3776
	Location 3	-3.04445*	1.15923	.013	-5.3978	-.6911

*. The mean difference is significant at the 0.05 level.

Comparing fresh weight and dry weight/ root and shoot lengths in carob seeds treated with boiling water:

Independent Samples Test										
1st treatment		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Fresh weight	Equal variances assumed	2.387	0.132	7.793	31	0.000	0.247565	0.031766	0.182777	0.312353
	Equal variances not assumed			9.917	19.646	0.000	0.247565	0.024962	0.195434	0.299696
Dry weight	Equal variances assumed	0.395	0.534	1.405	31	0.170	0.078795	0.056067	-.035554	0.193144
	Equal variances not assumed			2.282	30.805	0.030	0.078795	0.034532	0.008349	0.149241
Root length	Equal variances assumed	1.732	0.198	6.597	30	0.000	2.10417	0.31898	1.45272	2.75561
	Equal variances not assumed			8.876	23.493	0.000	2.10417	0.23707	1.61431	2.59402
Shoot length	Equal variances assumed	0.132	0.719	4.396	30	0.000	1.12917	0.25685	0.60462	1.65372
	Equal variances not assumed			4.004	10.450	0.002	1.12917	0.28199	0.50451	1.75382

Comparing fresh weight and dry weight/ root and shoot lengths in carob seeds treated with mechanical scarification:

Independent Samples Test										
2nd treatment		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Fresh weight	Equal variances assumed	0.074	0.791	3.698	11	0.004	0.194533	0.052604	0.078752	.310314
	Equal variances not assumed			4.044	3.825	0.017	0.194533	0.048108	0.058514	.330552
Dry weight	Equal variances assumed	0.034	0.857	3.513	11	0.005	0.102800	0.029262	0.038395	.167205

	Equal variances not assumed			3.323	3.075	0.043	0.102800	0.030931	0.005710	.199890
Root length	Equal variances assumed	0.376	0.552	2.609	11	0.024	1.19333	0.45739	0.18662	2.20005
	Equal variances not assumed			3.214	4.893	0.024	1.19333	0.37124	0.23271	2.15395
Shoot length	Equal variances assumed	3.979	0.071	.968	11	0.354	0.51667	0.53375	-0.65810	1.69143
	Equal variances not assumed			1.427	8.054	0.191	0.51667	0.36217	-0.31752	1.35085

Comparing fresh weight and dry weight/ root and shoot lengths in carob seeds treated with Sulfuric acid 5 min:

Independent Samples Test										
3rd treatment		**Levene's Test for Equality of Variances**		**t-test for Equality of Means**						
		F	**Sig.**	**T**	**Df**	**Sig. (2-tailed)**	**Mean Difference**	**Std. Error Difference**	**95% Confidence Interval of the Difference**	
									Lower	**Upper**
Fresh weight	Equal variances assumed	1.022	.336	-2.125	10	.060	-.073250	.034477	-.150069	.003569
	Equal variances not assumed			-2.778	9.812	.020	-.073250	.026368	-.132155	-.014345
Dry weight	Equal variances assumed	4.917	.051	-.728	10	.483	-.013750	.018881	-.055820	.028320
	Equal variances not assumed			-.916	9.991	.381	-.013750	.015011	-.047201	.019701
Root length	Equal variances assumed	.066	.803	.428	10	.678	.22500	.52589	-.94676	1.39676
	Equal variances not assumed			.436	6.396	.677	.22500	.51631	-1.01963	1.46963
Shoot length	Equal variances assumed	.207	.659	1.144	10	.279	.51250	.44786	-.48539	1.51039
	Equal variances not assumed			1.122	5.793	.306	.51250	.45693	-.61531	1.64031

Comparing fresh weight and dry weight/ root and shoot lengths in carob seeds treated with Sulfuric acid 10 min

Independent Samples Test										
		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Fresh weight	Equal variances assumed	3.992	0.058	-2.054	22	0.052	-0.070687	0.034415	-0.142059	0.000684
	Equal variances not assumed			-2.451	21.396	0.023	-0.070687	0.028842	-0.130600	-0.010775
Dry weight	Equal variances assumed	19.501	0.000	-1.162	22	0.258	-0.015625	0.013442	-0.043501	0.012251

	Equal variances not assumed			-1.524	20.838	0.143	-0.015625	0.010252	-0.036956	0.005706
Root length	Equal variances assumed	0.044	0.835	0.693	22	0.495	0.22500	0.32451	-.44800	0.89800
	Equal variances not assumed			0.673	13.070	0.513	0.22500	0.33422	-0.49665	0.94665
Shoot length	Equal variances assumed	3.409	0.078	-0.613	22	0.546	-0.23125	0.37736	-1.01384	0.55134
	Equal variances not assumed			-0.548	10.750	0.595	-0.23125	0.42210	-1.16293	0.70043

Comparing fresh weight and dry weight/ root and shoot lengths in carob seeds treated with Sulfuric acid 15 min

Independent Samples Test										
		Levene's Test for Equality of Variances		**t-test for Equality of Means**						
									95% Confidence Interval of the Difference	
4 th treatment		**F**	**Sig.**	**T**	**df**	**Sig. (2-tailed)**	**Mean Difference**	**Std. Error Difference**	**Lower**	**Upper**
Fresh weight	Equal variances assumed	0.280	0.604	-2.228	15	0.042	-0.088857	0.039888	-0.173877	-0.003837
	Equal variances not assumed			-2.141	11.185	0.055	-0.088857	0.041498	-0.180011	0.002296
Dry weight	Equal variances assumed	0.781	0.391	-0.958	15	0.353	-0.014043	0.014662	-0.045294	0.017208
	Equal variances not assumed			-0.925	11.396	0.374	-0.014043	0.015186	-0.047326	0.019240
Root length	Equal variances assumed	0.776	0.392	0.409	15	0.688	0.20143	0.49207	-0.84739	1.25025
	Equal variances not assumed			0.387	10.475	0.706	0.20143	0.51983	-0.94975	1.35261
Shoot length	Equal variances assumed	1.283	0.275	-0.390	15	0.702	-0.22714	0.58224	-1.46815	1.01386
	Equal variances not assumed			-0.370	10.578	0.719	-0.22714	0.61369	-1.58446	1.13017

ملخص الدراسة

تعتبر شجرة الخروب (*Ceratonia siliqua* L) من الأشجار المهمة بيئيًا واقتصاديًا وهي من بين أصعب أنواع الاشجار تكاثراً و ابطأها نمواً ، وهي من الأنواع البرية المتوطنة دائمة الخضرة والتي توجد بشكل طبيعي في منطقة الجبل الأخضر التي تقع على الفور جنوب الحزام الساحلي في المنطقة الشمالية الشرقية من ليبيا

الهدف والمنهجية: الجزء الأول من الدراسة كان لفحص الخصائص الخارجية لنبات الخروب في 4 مواقع مختلفة في منطقة الجبل الأخضر (البياضة ، توكرة ، وادي الكوف ،الحمدة) ، وتم قياس خمس سمات على القرون التمييزية لمعرفة (حجم القرن ، عدد البذور لكل قرن ، وزن 100 بذرة ، حجم البذور ، حجم الأوراق) ، تم قياس معاملين آخرين مباشرة في موقع الدراسة وهما قطر الساق وارتفاع الشجرة بأكملها ، الجزء الثاني من الدراسة كان لفحص تأثير معاملات ما قبل البذر المختلفة الموجودة في الأدبيات السابقة على نمو بذور الخروب ، كانت (الماء المغلي ، ماء الصنبور ، الخدش الميكانيكي وحمض الكبريتيك). تم تقييم كل من نسبة الإنبات ، متوسط وقت الإنبات ، مؤشر قوة انبات وتطور الشتلات بشكل فردي ومقارنتها مع التحكم لكل معالجة ، تم تنفيذ الإجراءات وفقًا لبروتوكول مطور بالكامل لإنبات البذور في المختبر للخروب الموجود في الأدبيات السابقة.

النتائج فيما يتعلق بدراسة الصفات الخارجية بشكل عام ، لم يتم العثور على فروق شكلية معنوية بين هذه المجموعات باستثناء حجم الأوراق الذي كان عالي الدلالة ، وأظهرت منطقة توكرة أحجام أوراق أصغر مقارنة بالمواقع الأخرى. لا توجد علاقة ارتباط معنوية بين حجم القرون والصفات الخارجية الأخرى للخروب في جميع المواقع ما عدا منطقة البياضة حيث وجد ارتباط سلبي معنوي بين أحجام القرون وأحجام البذور. مقارنةً بالسيطرة ، عززت جميع المعالجات بشكل عام إنبات بذور الخروب ، ولوحظت نسبة إنبات أعلى نسبيًا في البذور المنقوعة في الماء المغلي 96٪ ، تليها بذور الخروب المعالجة بحمض الكبريتيك لمدة 15 دقيقة والتي أظهرت إنبات 60٪ ، والآثار الميكانيكية. أظهرت الخدوش وحمض الكبريتيك لمدة 10 دقائق نسب إنبات متشابهة 40٪ ، البذور المعالجة بحمض الكبريتيك لمدة 5 دقائق أظهرت لمعاملة بماء الصنبور العادي لم تظهر اي إنبات. تم تسجيل إنبات أسرع بالبذور المعالجة مسبقًا بحمض الكبريتيك لمدة 15 دقيقة ، يليها الإنبات المسجل ببذور الخروب المعالجة مسبقًا بالماء المغلي ، ثم البذور المعالجة مسبقًا بحمض الكبريتيك لمدة 10 دقائق ، والبذور المعالجة مسبقًا بحمض الكبريتيك 5 دقائق وأظهر الخدش الميكانيكي تأخيرًا إلى حد ما في الإنبات. أظهرت بذور الخروب المعاملة بالنقع في الماء المغلي اختلافات معنوية في وزنها الطازج وأطوال جذورها وأطوالها مقارنة ببذور المقارنة غير المعالجة ، بينما لم تكن نتائج الوزن الجاف معنوية مقارنة بالمعاملة الضابطة.

دراسة بيئية فسيولوجية لنبات الخروب في منطقة الجبل الأخضر

قدمت من قبل:

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

جامعة بنغازي

كلية العلوم

أغسطس 2020