Allelopathic effect of Plant extract from *Ambrosia artemisiifolia* on inhibition of *Orobacnhe aegyptiaca* seed germination and plant host interaction

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This study was submitted in partial fulfillment of the requirements for the Master's Degree of Science in Agriculture biotechnology

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Palestine Technical University-Kadoorie (PTUK)

November, 2021

التأثير البيوكيميائي (Allelopathy) لمستخلص اوراق نبات (*Ambrosia artemisiifolia*) لمنع نمو بذور الهالوك المصري وتفاعله مع العائل

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المشرف

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قدمت هذه الاطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في

التكنولوجيا الحيوية الزراعية

كلية الدراسات العليا

جامعة فلسطين التقنية–خضوري

تشرين اول ٢٠٢١

Committee Decision

This thesis /dissertation (Allelopathic effect of Plant extract from *Ambrosia artemisifolia* on inhibition of *Orobacnhe aegyptiaca* seed germination and plant host interaction).

Was successfully defended and approved on ……..

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أقر بأن ما اشتملت عليه هذه الرسالة إنما هو نتاج جهدي الخاص، باستثناء ما تم الإشارة إليه حيثما ورد، وأنّ هذه الرسالة ككل، أو أي جزء منها لم يُقدم من قبل لنيل درجة أو لقب علمي أو بحثي لدى مؤسسة تعليميّة أو بحثيّة أخرى.

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Dedication

I dedicate this achievement to my father soul and I hope you are here, also to my mother thank you for your love and moral, spiritual, emotional and financial support.

Acknowledgment

No appreciation phrases can be enough to emphasize my deeply thanks for the one and only person who loved, understand, and walked this road daily with me; my mother. Who still encourages and helps me through the difficulties up to this present moment, I'm nothing without her, thanks a lot and I love you very much.

I would like to thank my supervisor, Professor Mazen Salman for guidance, encouragement and advice he has provided throughout my time as his student. I have been extremely lucky to have a supervisor who cared so much about my work, and who responded to my questions and queries so promptly.

Many words of appreciation are also forward to those who taught me the meaning of happiness, humility, self-loving and the meaning facing challenges no matter what they are; such as Eyad Abed-alrazeq my soldier.

To my souly second sister Sajeda Iwissat and my best friend Mai Sowan.

Special thanks for my second soul my sister Sajeda Iwissat, and my best friend Mai Sowan.

I also would like to thank the lab technician Basima AbuRumaila Khadeja Najjar and Bassma Bushnaq whose nothing could be done without their sincere help.

Special thanks and respect to Dr. Ghadeer Omar for her help and instructions. Last but not least,

I would like to thank all who contributed, or provided, and implemented any requirements for completing this work either by action or with supporting words, it’s meant a lot for me.

I also acknowledge Palestine Technical University Kadoorie for providing this master program, scholarship and labs to fulfill this thesis.

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Abstract

Orobanche species are a root holoparasitic plant lack of chlorophyll and completely depending on the host to complete their life cycle. They are classified as the most important parasitic plants in the world because of economic and financial losses in crops that may reach 100%. Due to the lack of sufficient information available to farmers, and the non-viability of traditional, chemicals and anti-herbicides to eliminate this herb. And also the possibility of killing the host plants instead of the parasitic one. For these reasons new biocontrol ways were developed. *Ambrosia artemisiifolia* plant leaf extracts can be a promising biocontrol herbicide. Because *Orobanche* seeds can stay dormant for long period, seeds were incubated for 7 days in dark to break their dormancy. During this period plant, leaves were collected and 10% (w\v) extract was prepared. Extract (10%) was experimented on seeds germination and using growth regulator (GR24) instead of root exudates. This step confirmed that *Ambrosia artemisiifolia* plant leaf extracts inhibited seed germination, while seeds with GR24 grew normally. Seeds were treated and incubated for 5 days with different extract dilutions (0.0, 0.5, 1.0, 1.5, 2, 2.5, 5 and 10%) to estimate their germ tubes length and to calculate

germination percentages, where germination percentages at 2.5%, 5 % and 10% were 0% without germ tube elongation, and the highest germination percentages were 94% at 0.5%, and other germination percentages were: 84% at 1%, 32% at 1.5% and 24%at 2%. Then different solvent were used to dissolve plant extract (water, ethanol, and methanol), water was detected the appropriated one where it completely inhibit seed germination.

After comparing the effect of the pesticide (Chlorsulfuron) with the effect of the extract, it was found that the effects of the extract on preventing seed germination were higher than the effects of the pesticide, which proves that plant extract has a huge ability to compete with the chemical herbicides.

To prove this, a potting experiment was conducted to study the effect of the extract (2.5% and 5%) on the germination of *Orobanche* seeds, in addition to studying its effect on the germination of model plant. It was noted that the extract had the ability to prevent seed germination by a percentage, but at the same time, it did not affect the growth of the model plant.

Introduction

General Introduction

The Parasitic weeds of the family *Orobanchaceae* are considered to be the most serious agricultural pests causing economic loses in many parts of the world including warm-temperate, subtropical and tropical regions. There are more than 150 species of *Orobanche, (*Mondal, B, *et al.,* 2020)

Only five species (*Phelopanche ramosa*, *Orobanche cernue*, *Orobanche cumana*, *Orobanche crenata* and *phelipanche aegyptiaca*) are considered significant parasites of the agronomic crop in the world (Punia *et al.,* 2012). The genus *Orobanche* vary in their infection range (i.e. from narrow to broad range of infection) (Cochavi A.,*et al*, 2017).

As an obligate holoparasitic weeds (they have no green parts that contain chlorophyll and cannot synthesize their own foods), they are considered as a major biotic limiting factors to the production of many plants (Cartry D,*et al*., 2021).

These parasites can infest different types of crops such as: hemp, carrot, cabbage, chickpea, faba bean, sunflower, lentil, eggplant, potato, pea, celery, tobacco and vetch (Eizenberg *et al.,* 2001). The amount of damage caused by *Orobanche* depends on the degree of infestation and type of the host crop cultivated. Yield

losses as a result of infestation by *Orobanche* may range from 5-100 % depending on the region and the crop (Ennami M,*et al*., 2017).

Moreover, *Orobanche* species can reduce crop quality and reduce the area under cultivation. In addition to that, the presence of parasitic plant material in harvested crop products may reduce the value of the crop or make it unmarketable (Habimana *et al*., 2014).

Orobanche species are difficult to be controlled because of their underground location in close association with host plant roots, complex mechanisms of seed dispersal and nature of seeds germination and longevity.

The most common methods to control *Orobanche* include traditional methods such as crop rotation, trap and catch crops, sowing dates and cropping density, chemical and physical methods.

Unfortunately, these methods are not enough to reduce *Orobanche* seeds germination and also it may damage host plant and lead to develop resistant *Orobanche* species (Eizenberg *et al*., 2001).

In recent years, biological control has been applied to reduce *Orobanche* infestation (Müller-Stöver *et al*., 2005). Biological control is one of the most effective and safe alternatives which depend on organisms or another plants to replace herbicides and limit their use. Some biocontrol agents such as *Fusarium*

oxysporum sp. *orthoceras* in fields contains Orobanche resulted in 90% control of *Orobanche* in sunflower or tomato (Nazer Kakhaki *et al*., 2017).

The use of plant extracts might also be one of the alternatives to overcome problems caused by *Orobanche*. Extraction is the separation of medicinally mixture of many plant secondary metabolites, such as alkaloids, glycosides, phenolics, terpenoids, and flavonoids using selective solvents through standard procedures. The aim of all solvent extraction methods is to separate the soluble plant metabolites, leaving behind the insoluble cellular marc. Plants extracts are becoming very important due to their uses mainly as a source of therapeutic compounds that may lead to the development of novel drugs (Dekebo, A. 2019). also it was used to effect on growth and photosynthetic fluorescence (Shi *et al*., 2020), and it was found that have effects on ecological safety by reductions some poisons elements.

In this study, *Ambrosia artemisiifolia* will be tested for the possibility to control *Orobanche aegyptiaca.*

Objectives

- Determination of effect of *A. artemisiifolia* leaf extract on seed germination of *Orobanche* under in vitro conditions.
- Testing the effect of extract on haustorial and germ tube elongation.

- Identifying the ED_{50} of the extract.
- Examining the inhibition of tubercle attachment to the roots of host plants.
- Examining the possibility of integrating chemical herbicides with leaf extract to control *Orobanche* parasitism.

Literature Review

Morphology and biology of *Orobanche* species

Orobanche are dicotyledonous annual plants that differ in length depending on the species which can reach more than 30 cm (Punia *et al.*, 2012), and are known for their yellow to straw-colored stems and yellow, white, or blue, snap dragon-like flowers, with triangular scaled leaves that lack of chlorophyll (Yoder, 2001). On the leaf axial white and tubular flowers appear, with fruits located in capsules that contain numerous tiny black seeds.

Orobanche reproduces by seeds which are oval-shaped and dark brown in color, the seeds are characterized by their tiny size, which is 0.35 x 0.25 mm, and weigh 3 to 6 μg which make them difficult to be recognized by the naked eye (Figure 1).

A single *Orobanche* plant flower can produce thousands of seeds per year. Seeds are released when capsules dry out and break open. The Seeds can stay dormant in the soil for about 20 years or more until environmental conditions become appropriate for germination (Rodenburg and Bastiaans, 2016). Before germination, seed must undergo conditioning period under suitable temperature and moisture conditions, to ensure that only seeds within the rhizosphere of an appropriate host root will germinate and contact to the host (Mauromicale *et al.*, 2000). After

conditioning the Seeds start to germinate and the seedling must contact immediately with the host root to survive (Scher and Walters, 2010).

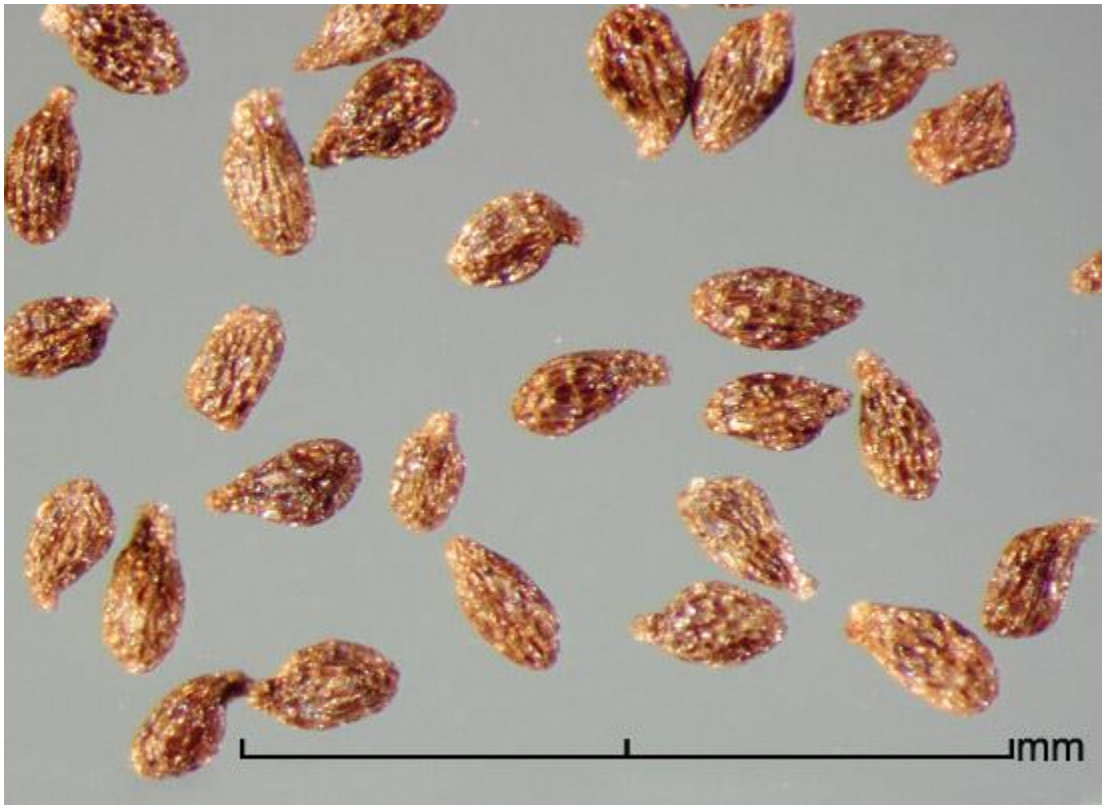


Figure 1. Orobanche seeds (Source: Federal Noxious Weed Disseminules of the United States. https://idtools.org/id/fnw/gallery.php?show[]=fruit&remove[]=seed&page=3).

Spreading of seeds is very easy due to their lightweight. The Seeds generally stay dormant and require a post-harvesting ripening period for their germination before response to chemical stimulation from the root of a host plant. Chemical stimulant stability is very short-lived in the soil, so seeds must undergo a conditioning period under suitable temperature and moisture conditions before their germination. These conditions confirm that only seeds with the rhizosphere of an appropriate host root will be germinated and make contact with a host root before exhausting its limited

energy resources (Morozov *et al.*, 2000). The optimum temperatures for conditioning and germination are different between different *Orobanche* species, which is generally reflected its geographical distribution (Mauromicale *et al.*, 2000).

After the conditioning phase, germinated seeds produce a germ tube or radical in close proximity to the host's plant roots. This radical elongated by cell division attaches itself to the host root, mainly in the region of root elongation and absorption to develop an attachment organ called haustorium (Song *et al.*, 2005). The haustorium acts as a bridge between the parasitic plant and host plant to drive water, mineral nutrients and carbohydrates from the host plant to the parasitic plant (Matusova *et al.*, 2004).

Enlargement in the radical tip leads the haustorial tissue to penetrate the epidermis and cortex tissues, and ultimately fuse to the root vascular system and establishes connections with the host root vascular system by enzymatic degradation. After that, the seedling swells outside the root of the host plant to form a tubercle. Due to the *Orobanche* being parasitic, they cannot synthesize their food, but instead draw their nutrient from the host plant by direct connection to the host phloem and drain the host plant of carbohydrates. Therefore, they force host plant to increase their photosynthesis rate (Domina, 2018)

The shoot buds are developed from the tubercle and produce flowering spike, which elongates and breaks through the soil surface within 1-2 weeks. After 43-58 days of transplantation, the spike begins to emerge above the ground, where the flowering is completed in 7-13 days after emergence. The stem dries completely in 26-38 days after the emergence of the spike and it completes its life cycle within 37-70 days after emergence (Figure 2), and spills thousands of seeds per plant (Benvenuti *et al.*, 2005).

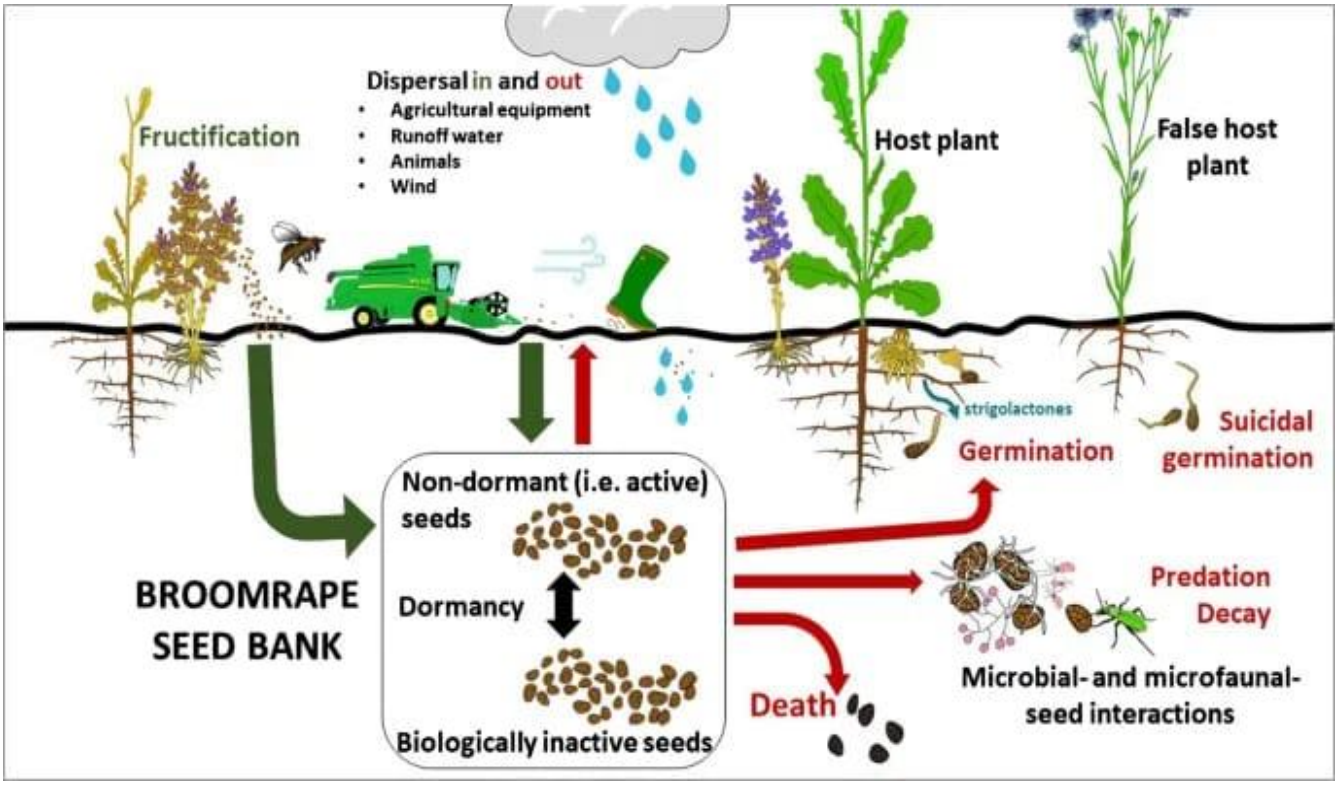


Figure 2. Life cycle of *Orobanche* species (Source: Cartry, *et al.*, 2001).

Orobanche aegyptiaca

Orobanche aegyptiaca is the most destructive parasite due to its ability to parasitize a large variety of crops, such as watermelon, tobacco, carrot, tomato, potato, lentil, cabbage, maize and others. It is widely spread in the Mediterranean countries and the Near East (Hasannejad *et al.*, 2006).

The pubescent glandular parasite grows up to 20-50 cm, is usually regular shaped and branched from the bottom with elongated branches that contain spikes with low flowers and subsessile. The flowers have 8-14 mm Calyx and a 20-35 mm Corolla which is pubescent outside with white lower side and blue or lilac upper side (Figure 3) (Darvishzadeh *et al*., 2010).

Figure 3. *Orobanche egyptiaca* infesting eggplant (Source: Author).

The induction of germination occurs by interaction between host and parasite through host root exudates (germination stimulants).The exudates are secondary

metabolites which are produced in low quantities by the hosts. In response to the exudates, the parasite produces a radicle that connects with the host root. the radical tip differentiates into a haustorium that penetrates the host root which forms the physical and physiological bridge between the parasite and host that carries water and nutrients from the host to the parasite leading to damages of the. A single plant can produce hundreds of thousands of seeds, which can remain viable in the soil for more than10 years (Rubiales 2003).

Management of *Orobanche*

Orobanche control is very difficult due to its underground growth. Close association with host plant roots, complex mechanisms of seed dispersal, seed germination, and long seed shelf life (more than 20 years) (Punia, 2014).

Furthermore, when the plant becomes visible above ground most of the damage has already been done, which means control would be not effective. Easy dispersal of tiny seeds and in different ways such as wind, water and livestock, are also major factors that reduce the efficiency of control measures (Punia, 2014).

Several management practices of *Orobanche* have been made over the years but with limited effectiveness.

Preventive method

The aim of an eradication program must be to reduce the seed bank by minimizing the production of new seeds and seeds dispersal to new places as well as to avoid spreading the infestation into neighboring fields. However due to continuous production and easy dispersal of seeds, it is almost impossible to prevent seed transfer from a heavily infested field to its close surroundings. To avoid *Orobanche* seeds dispersal from infested fields to a new area some phytosanitary measures can be taken into consideration, such as certified planting material, machinery and types of equipment cleaning , deep tillage must be done to place *Orobanche* seeds below a 20 cm soil depth and collection and burning of the parasite before flowering (Qasem, J. R. 2021).

Cultural methods

Crop rotation

The main aim of crop rotation in controlling *Orobanche* is to reduce parasite infestation as a result of repeated planting of non-host crops for many seasons which will in turn reduce the seeds bank in the soil. Monoculture with the same *Orobanche* host crop on the other hand will increase *Orobanche* infestation (Duca, M, *et al*., 2019).

Trap and catch crops

‘Trap crops are very important method that cause a suicidal seed germination such as wheat. The method might be considered a good management option for reducing *Orobanche* seed bank in infested fields. The catch crops exude stimulants to induce *Orobanche* seeds germination and establishment of *Orobanche* roots without viable attachment to host. The parasite seedling wilts and the seed bank can be reduced. The crops that induce *Orobanche* seeds germination but do not support the seedling after attachment may serve an excellent trap crops as well (Aksoy, E, *et al.*, 2016)

The use of trap and catch crops is limited due to the huge amount of *Orobanche* seeds dispersal in the soil. Small amounts of seeds can be exposed to germination stimulants in the rhizosphere. The feasibility and economics of growing these crops sometimes are very low (Abebe *et al*., 2005).

Sowing dates and cropping density

Early planting dates are beneficial in certain instances, to help in reducing the parasitism of *Orobanche*, germination of *Orobanche* tends to be very much reduced below 8 °C and further development is greatly reduced at low temperatures. Delaying the planting date affects *Orobanche* more than its hosts(Yadav *et al.*, 2005) , for example results in faba bean showed that shifting

sowing from October to November, December or January reduced numbers and dry weight of attached and emerged broomrapes both *O. crenata* and *O. foetida* (Grenz , 2005).

Water management

Less infestation of the parasitic weed has been observed growing under flooded irrigation compared to sprinkler irrigation. *Orobanche* seeds can grow heavily under conserved moisture than seeds under extended period of inundation. While not all plants can grow underwater flooded making this method not effective (Punia, 2014).

Nutrient management

Orobanche tends to be associated with less fertile soil conditions. High levels of nitrogen fertilizer or chicken manure showed a suppressive effect. The main effects could be due to, reduction of stimulant exudation, direct damage to *Orobanche* seeds and seedlings in the soil, reduced osmotic pressure in the parasite relative to the host, a toxic effect of nitrogen on the parasite development and alternation of host roots and shoots balance (Labrousse et al., 2010). Other studies (Haidar and Sidahmed, 2006) showed that application urea or ammonical forms of nitrogen during germinating phases can reduce radical germination and *Orobanche*

proliferation. Adequate amount of phosphorus and potash fertilization are required to raise and maintain the crop productivity.

Physical methods

Hand weeding/hand pulling

This method can be effective and practical if it applied before flowering. However, the emergence of new inflorescence from below-ground plant part within 7-10 days, thus requires frequent repetitive weeding practices. This method can limit seed production but doesn't compensate for the damage in terms of yield losses. The method can only be accepted in an area with recent infestation, in combination with other methods to reduce seed bank (Banik, D, *et al.*, 2020).

Tillage

Deep tillage during summer months leads to seeds drying and places seeds below root zone that prevent seeds germination. However, it's not an effective method due to aging 20 years at viability .In addition to that tillage may retrain seeds in root zone (Haidar and Sidahmad 2006).

Soil solarization

This method is effective to kill the seeds and reduce seed bank by covering the soil with polyethylene sheet the treatment increased maximum soil temperature by around 10 C, and at 5 cm below the soil surface temperature reach more than 45 C

(Haidar and Sidahmad 2006). This treatment can kill about 95% of viable seed, and induced secondary dormancy in the remaining 5%.

Chemical methods

This method is complicated because it depends on use of prophylactic treatment and unknown level of infestation level. The herbicide has low persistence and the parasite can germinate and grow continuously and develop new infections (Perez-de-Luque *et al*. 2010).

Control with Glyphosate

Glyphosate is systemic herbicides that is used to control *Orobanche,* on different crops that are tolerated to glyphosate such as faba bean, carrot cabbage and celery (Rubiales and Fernandez-Aparicio, 2012).

Glyphosate is a broad spectrum nonselective foliar herbicide. Its efficacy in *Orobanche* management is quite useful limited and needs critical precaution to

achieve effective results. Moreover the higher dose of glyphosate at early crop stages because localized phytotoxicity with many symptoms like marginal leaf chlorosis, slow leaf growth, interveinal leaf bleaching, and slight elongation of apical leaves (Pathak and Kannan, 2014).

Soil fumigants

Methyl bromide was one of the most common soil fumigants that were used to control *Orobanche* in the field. However, due to its high negative impacts on human health and environment, the product has been banned by World Health Organization (WHO) and Agricultural authorities. Other compounds such as methyl isothiocyanate was suggested for *Orobanche* eradication. The compound is only effective in deep soil layers due to its rapid evaporation (Mauro *et al.*, 2015)

Seed treatment

Seeds coating with some chemical such as imidazolinones and/or pronamide was effective for controlling *Orobanche* germination, in bean sunflower seed (Matthews 2002).

Biological methods

Several biological control agents were test for their efficacy to control Oroabanche. The fly *Phytomyza orobanchia*, fungus *Fusarium oxysporum* sp. *orthoceras* and some bacterial isolates showed promising results as alternative to chemical treatments or other control mechanisms ((Nosratti, *et al*., 2020).

Ambrosia artemisiifolia

The plant *Ambrosia artemisiifolia* is an annual herb belongs to phylum angiosperms (Angiospermatophyta), class of Dicotyledonouses (Dicotyledonopsida), the order of composites (Asterales), family Daisies (Asteraceae), subfamily disc florets (Tubuliflorae) and genus of ragweeds (Wayne *et al.,* 2002). This genus Ambrosia contains 42 species. It's originally introduced from North American and can also be found in Europe, Asia and South America (Gaudeul *et al*., 2011).

The plant grows in modest and in poor soil and reaches more than 150 cm in rich soil (Ploetz *et al.*, 2013).

Ambrosia artemisiifolia is usually grow in cultivated fields, along with roadsides, and in open disturbed habitats due to little competition during early growth (Pinke *et al.,* 2011). During the growing period, the *A. artemisiifolia* has high water use

efficiency and high nitrogen efficiency and its photosynthesis ratio is very high during blooming. While it grows rarely in soil containing high Na, K and Mn concentrations (Kiss and Beres, 2006).

It can tolerate leaves loss due to the good root system (Gard *et al*., 2013). It is the monoecious allergenic herb in which male inflorescences contain numerous flowers located on the upper terminal branches. The female flowers are located in lower leaf axils near the stem (Figure 4).

Figure 4. *A. artemisiifolia* (Source: Author).

Despite its harmful effect on agriculture, human health, biodiversity and environment, *A. artemisiifolia* can be used in animal feed (Ploetz *et al*., 2013). Moreover it can use in Phytoremediation for extracting heavy metal content from

the soil. In this case (Taylor, 2005). In addition to that, *A. artemisiifolia* is used in traditional medicines in native North Americans (Mamedov, *et al.*, 2015). A recent study conducted by Kleef and Salman (2021) reported that leaf extract of *A. artemisiifolia* could inhibit germination of fungal spores under in vitro condition.

Materials and Methods

Orobanche seed collection

Seeds were collected from mature plants during spring in fields grown with Faba bean plant and infected with *Orobanche eagyptiaca*. The seeds were kept in dark at room temperature until use.

Seed sterilization

Surface sterilization was carried out by placing seeds in tea-bag after remove tea from them, which was then washed for 10 min under running tap water. The seeds then were placed in 10% ethanol for 5 min. Followed by washes 5 times (each with100ml autoclaved tap water).The seeds were transferred to beaker containing 100ml of 1% hypochlorite solution containing 250µl of tween-80 for another 5 min. After 5 times washes, seeds were allowed to dry on Whatmann No. 1 filter paper inside Petri dishes. The dried seeds were collected and stored in sterile screw-cap tubes. (Barghouthi and Salman 2010)

Seeds Preconditioning

Autoclaved Glass fiber filter papers disks (GFP, 1 cm diameter) were placed in 9 cm petri dishes containing sterilized Whatman No 1 filter paper and soaked with 3 ml tap water. *Orobanche* seeds were aseptically sprinkled on GFP (~ 30 seeds per

disk). The plates were sealed with Parafilm and incubated in the dark for 7 days at 25°C (Figure 5).

Figure 5. Preconditioning of *Orobanche* seeds on GFP, 1 cm diameter placed in 9 cm petri dishes

Preparation of Growth regulator GR24 stock solution

Stock solution (1000 ppm/ml) of the Strigol analogue GR24 was prepared by dissolving 1mg in 1ml acetone and stored at -20°C in the dark, a working solution

Contained 2 ppm/ ml of GR24 was used in further experiment.

Preparation of A. artemisiifolia water leaf extract

A. artemisifolia plants were collected from the PTUK farm. The leaves were picked up wrapped in the aluminum foil and dried in the oven at 65°C for 48 h. The dried leaves were cleaned from the vines and mixed to have a fine powder. A 10% (w/v) extract was prepared by soaking 10 g of the powder in 100 mL distilled water for 24 h on a horizontal shaker at 110 rpm. The extract was filtered through a double layer of cheesecloth followed by filtration through Whatman No 1 filter paper the extract was stored at 4°C until use.

Leaf extraction with different solvents

A. artemisiifolia fresh leaves were picked. Then they were chopped in to small pieces with mixer. After that, 30 grams of the sample were taken and soaked in 300ml of ethanol and methanol. The sample was put on the magnetic stirror for (1, 2 and 3 day). Then again leaves were picked and put on aluminum foil. Then they were put in the oven at 65°C for 3 days. After that, the dried leaves were cleaned from the large veins. Then were ground with the mixer to have fine powder, 30 grams of the sample was taken and soaked with 300 ml of ethanol and methanol, samples were put on a magnetic stiller for (1, 2 and 3 day). After (1, 2 and 3 days), the samples were left to stand for 15 min, filtrated by filter paper. The filtrated solutions were extracted using (1:1, V: V) Ethel Estate® by the separatory funnel,

to extract the organic matter from the solution, separatory funnel was shaken for 30 to 60 second put them for 2 to 3 min until the solution separates into two layers. The upper layer was taken and put in a small beaker. Magnesium sulfate was added to the solution in the beaker, to remove excess water.

Then it was filtrated with filter paper, solution was taken and put into rotary evaporator at 55 °C and 80 rounds per minute. Finally, the residues were taken by spatula, put in eppendorf tube, and kept at 4°C until use.

Methanol and ethanol leaf extract (1, 2 and 3 days), samples were left until rest for 15 min. After that, the samples were filtrated by filter paper. Then the solutions were taken and put directly on a rotary evaporator at 45°C and 80 rounds per minute to evaporate ethanol and methanol. Finally, the residues were taken by spatula and put in eppendorf tube, kept on 4 °C until use.

Stock solution

A100 mg of each stock was taken separately, and then it was dissolved in 1000 µL acetone with mixing at high speed and stored at 4 °C. Serial dilutions were done by completing the volume with sterile distil water up to 3 ml to have a final concentration of 0.5, 1, 1.5, 2, 2.5 and 5 %.

Six plates for each treatment were done, the autoclaved filter papers were put inside plates. Then the solution drained in each plate, after transferring

preconditioned seeds to the plate. After that, plates were sealed with parafilm and incubated in dark for 5 days at room temperature. Seed germination was examined microscopically using dissecting microscope.

The effect of the Extract on Orobanche seeds germination

To test the effect of *A.artemisiifolia* on germination of *Orobanche* seeds. Preconditioned seeds (on GFP) were placed in petri dishes containing Whatman NO 1 filter paper as mentioned before. The seeds were then treated with 3 ml of the following solutions (autoclaved distilled water as a negative control), 10% plant extract, GR24 (2 ppm/ml in water as a positive control) and (GR 24 ppm/ml in plant extract (0.5, 1, 1.5, 2.0, 2.5, 5 and 10%) the petri dishes sealed with parafilm and incubated in the dark for five days at 25°C. Seed germination was examined under microscope at 40 X magnification power. The radical length was estimated on a scale relative to seed length (usually from 0 to 5).

Determining the ED50 of the extract

The effective dose for 50% of the population which the dose that produces a specific biological effect in 50% of the population that took the dose. Different dilutions of the extract were made contains GR24. Drained on filter papers inside plates. The preconditioning seeds on glass fibre filter paper were transferred to the drained plate. Finally, the plates were sealed with parafilm and incubated in the

dark for five days at 25°C. Seed germination was examined microscopically with a dissecting microscope.

Effect of chemical herbicides (Chlorosulfuron) and leaf extract combination on controlling *Orobanche* seeds germination

A solution from Chlorosulfuron herbicides was prepared by dissolving 16 mg in 10L water as mentioned in product's instruction. In order to test the effect of herbicide and leaf extract solution combinations on seeds germination. A combination of both solution was prepared as by combination (0.1, 0.5, and 1%) of herbicide each one with (0.5, 1, 1.5, 2, 2.5, and 5%) of extract. After that germination of preconditioned *Orobanche* seeds was tested as explained before.

Pot Experiment

Field experiment was conducted in in the greenhouse of the Palestine Technical University – Kadoorie, during February 2020.

Tomato TR20 seedlings were used as a model plant to test the effect of *A. artemisiifolia* and herbicide in inhibiting *Orobanche aegyptiaca* seeds germination.

All accessions were transplanted in (2L) plastic pots filled with soil (field soil) and peat moss (3:1 v/v) and mixed with *Orobanche* seeds. Each pots were arranged as

triplicates in a single row, first row was irrigated with 2.5% of plant extract and pots were containing *Orobanche* seeds inside, next one was irrigated with 5 % of plant extract and also containing *Orobanche* seeds, next one was irrigated with 2.5 and 5 % of extract and combination with herbicide Chlorosulfuron which was prepared by socking 16mg/10L water as mentioned in the instruction, pots also containing *Orobanche* seeds inside them, another row was irrigated with herbicide Chlorosulfuron only and it was containing *Orobanche* seeds . The last two rows were irrigated with water and used as control, one was containing *Orobanche* seeds inside the pots as (control positive) and the other don't include seeds as (control negative).

Each triplicate were irrigated with equal amount by Graduated cylinder at the same time and when they need.

In April the effect of the extracts were recorded by tracing the growth of the *Orobanche* seeds and the appearance of the *Orobanche* above the soil surface, also fresh and dry weights of roots and stems as well as the length of tomato seedlings were recorded.

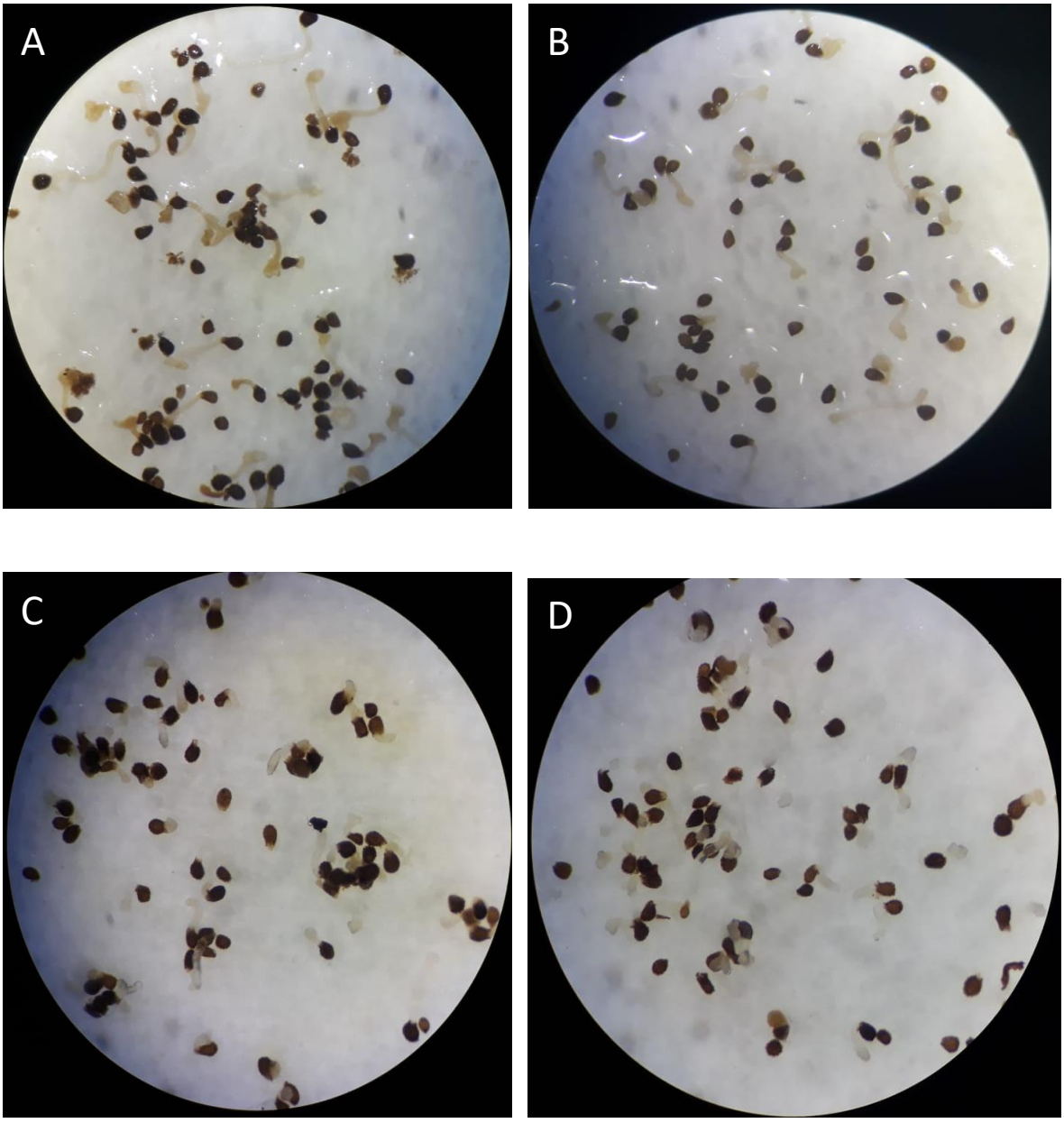
Statistical analysis

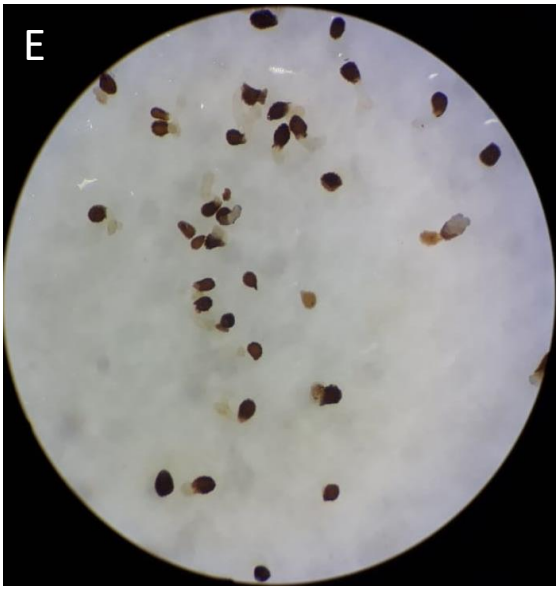
All experiments were done in triplicates and repeated three times. Statistical Analysis was done using ANOVA after Tukeys HSD test at $p \leq 0.05$.

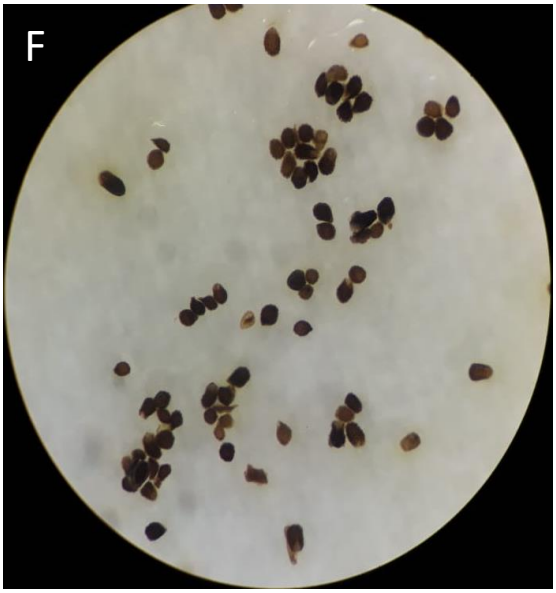
Results

Effect of extract on *Orobanche* seeds germination

Germination of seeds was reduced with increasing plant extract concentrations (Figure 6 A-I). Normal germination was obvious in seeds treated with GR24 (Figure 6A). Few seeds were able to germinate in 0.5-2.0% leaf extract (Figure 6 B-6 E). In 2.5-10% leaf extract, complete inhibition of seed germinations was obvious (Figure 6 F-6 H). No germination was reported in seeds treated with water. The percent of seed germination was significantly different ($p < 0.05$) in seeds treated with 1.5 and 2% extract. Lower concentrations of the extract (0.5-1.0%) did not affect seed germination significantly compared to the seeds treated with GR24 (6 ppm). As shown in figure (7) no germination of the seeds was recorded at higher concentrations (2.5-10%) of the leaf extract.


A
B
C
D


E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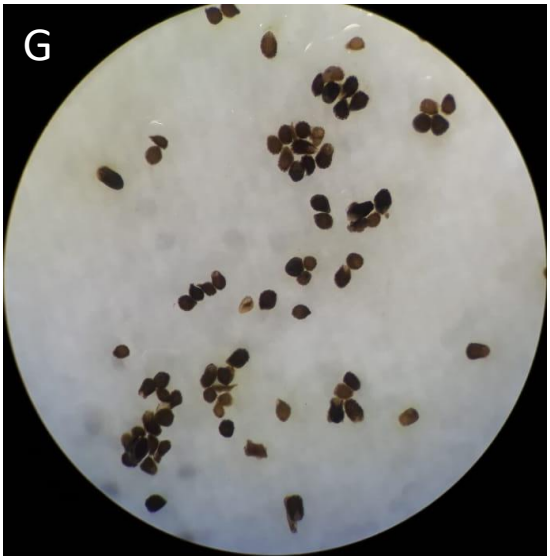
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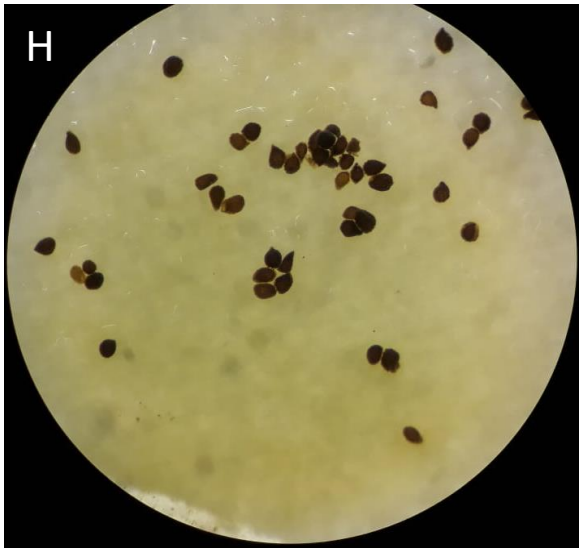
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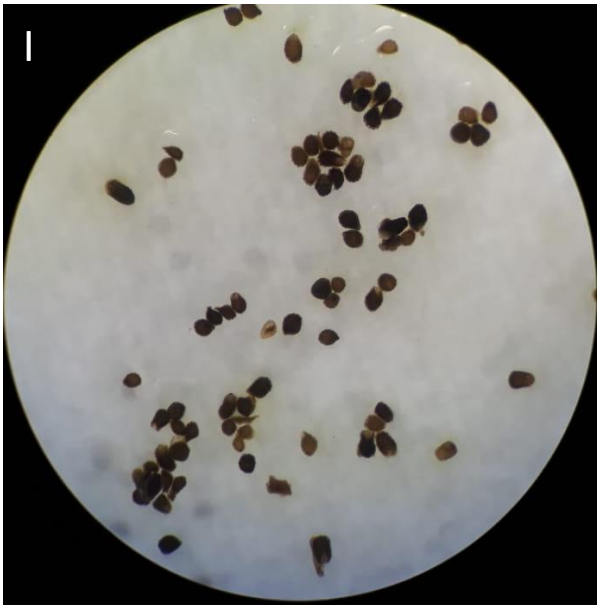

Figure 6.A: Seeds germination of *Orobanche* in water with GR24 (control+), B: Seed germination under 0.5% leaf extract, C: Seed germination under 1.0% leaf extract, D: Seed germination under 1.5% leaf extract, E: Seed germination under 2.0% leaf extract, F: Seed germination under 2.5% leaf extract, G: Seed germination under 5.0% leaf extract, H: Seed germination under 10% leaf extract, I: Seed germination under 0.0% leaf extract in water with no GR24 (as a negative control)

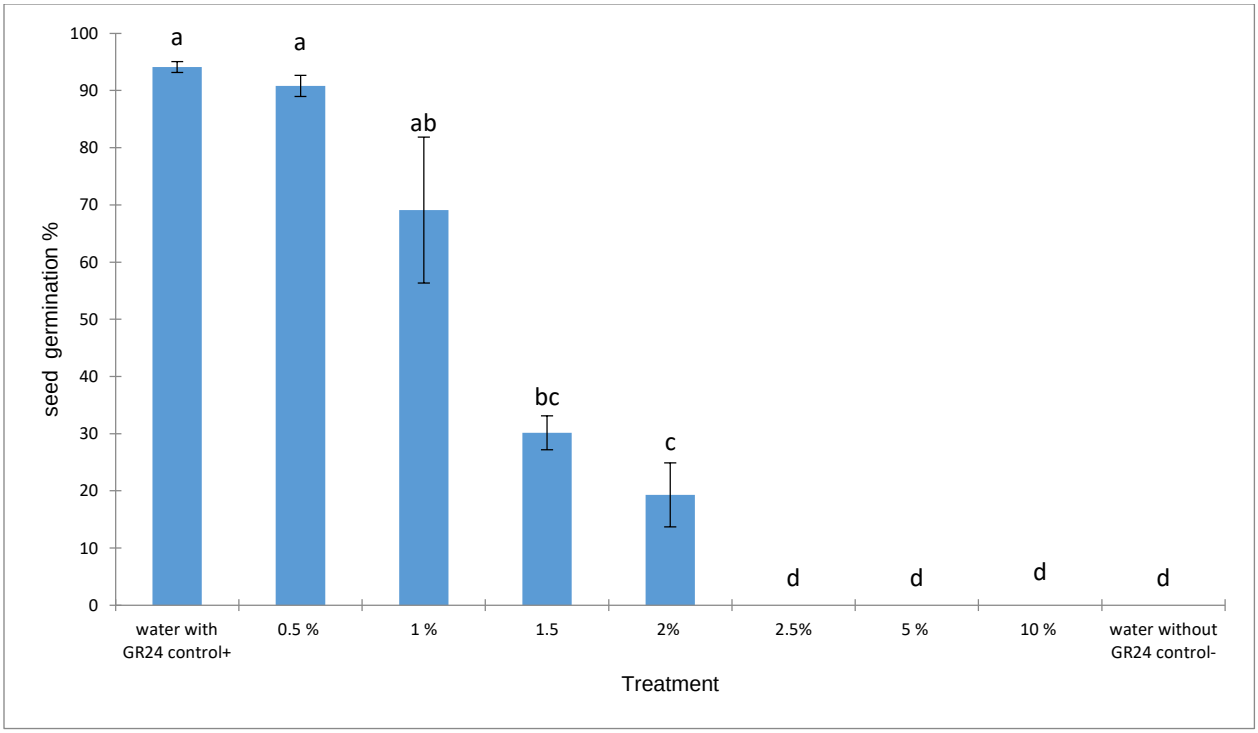

Figure 7. Percentage of germination at different concentration of *A.artemisiifolia* extract and water with GR24 (control+), water without GR24 (control-).

Effect of Extract on radical length

Not only the extract reduced the germination percentage of *Orobanche* seeds (as see above), but also the length of the germ tube was reduced (Figure 8). At lower concentrations of the extract, the length of the germ tubes was significantly ($p<0.05$) lower than that in the seeds germinated with GR24 (Figure 8). The results showed that the length of the germ tube in seeds treated with 2.0% leaf extract was shorter than that in seed treated with 1.5% extract, the first day of measure the germ tube is the five day of incubation the preconditioning seeds.

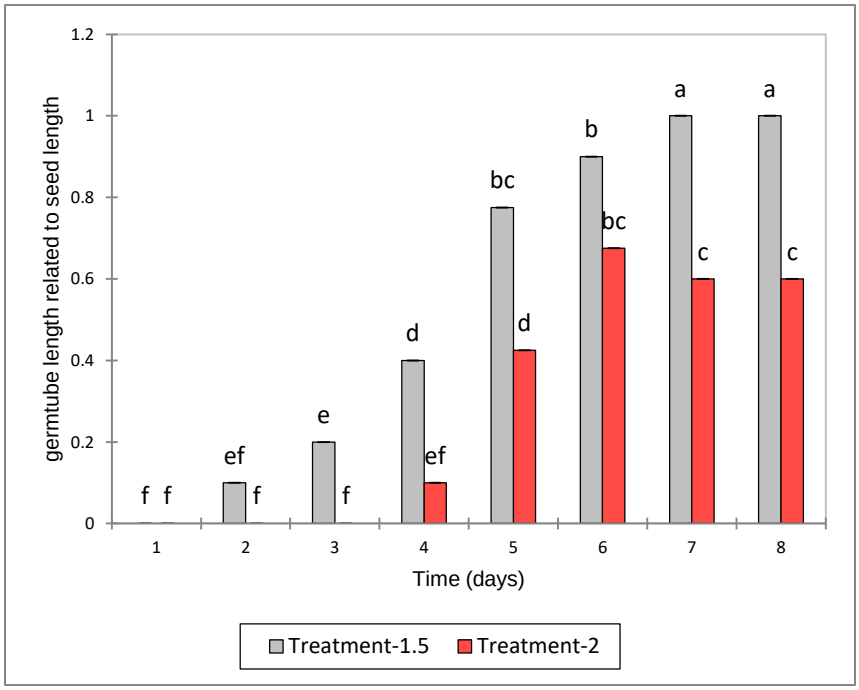

Figure 8. Germ tube length related to seed length treated in 1.5 and 2 % of plant extract.

Identifying the ED_{50} of the extract

The ED50 (median effective dose) is the dose that produces a specific effect in 50% of the population , Knowing this point is necessary for the possibility of combination the extract with other herbicides or others materials and reducing the negative impact of the high concentration of the extract.

Our results (figure 9) showed that extract concentration 1.3% could inhibit the germination of 50% of *Orobanche* seeds in in vitro conditions.

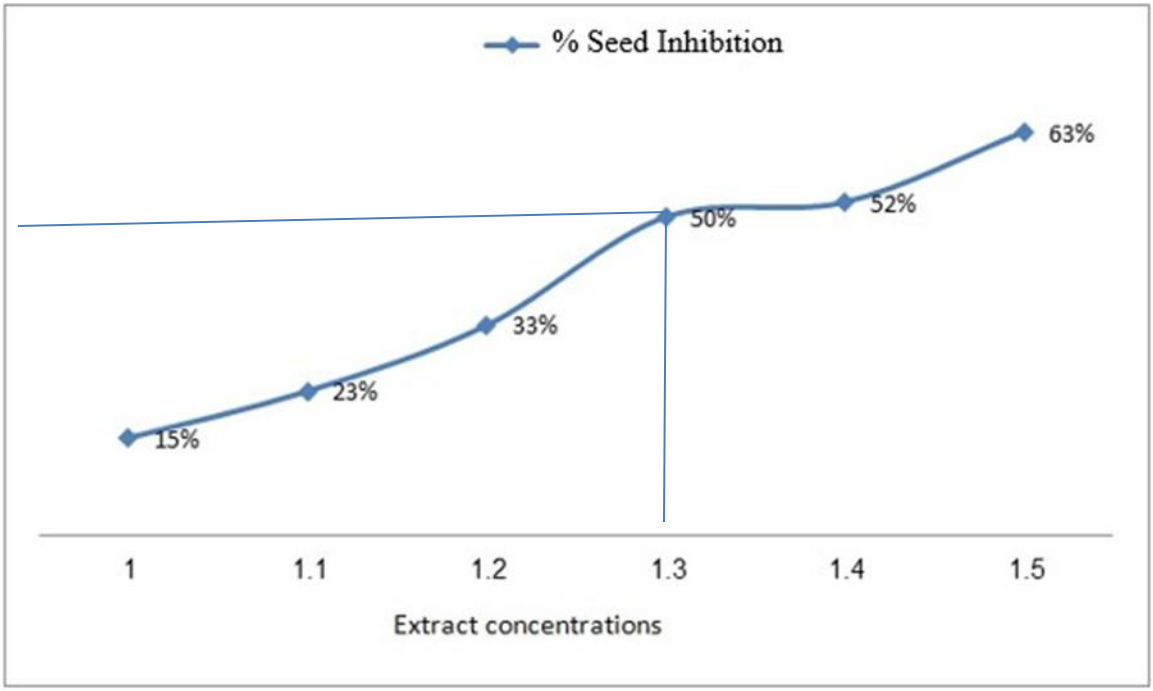

Figure 9. ED_{50} of the plant extract.

Effect of extraction method on seed germination

Figures (10, 12 and 14) show the results of using different solvents (ethanol, methanol and water) for active ingredients extraction for different periods of soaking (1, 2 and 3 day) of fresh and dry plant leaves.

As shown in the figures below, complete inhibition of seed germination was when soaking dry leaves in water at (1, 2 and 3 day).

in the other solutions ethanol and methanol there were decreasing in seeds germination percentage when dry plant leaves were used, on the other hand there was no decrease in the fresh leaves when they dissolving in water, ethanol and methanol this may be due to chlorophyll pigments which obstruct dissolving.

In figures (11, 13 and 15) show that the best treatment is 5 % of extract in all three days, also figures show that 5 % extract decreasing in seeds number germination in all different solvent but in the dry leaf solvent was more effective and decreasing in germination numbers until complete inhibition in 5% dry leaf in water.

Also figures (10, 12 and 14) show increased inhibition of germination rates with time.

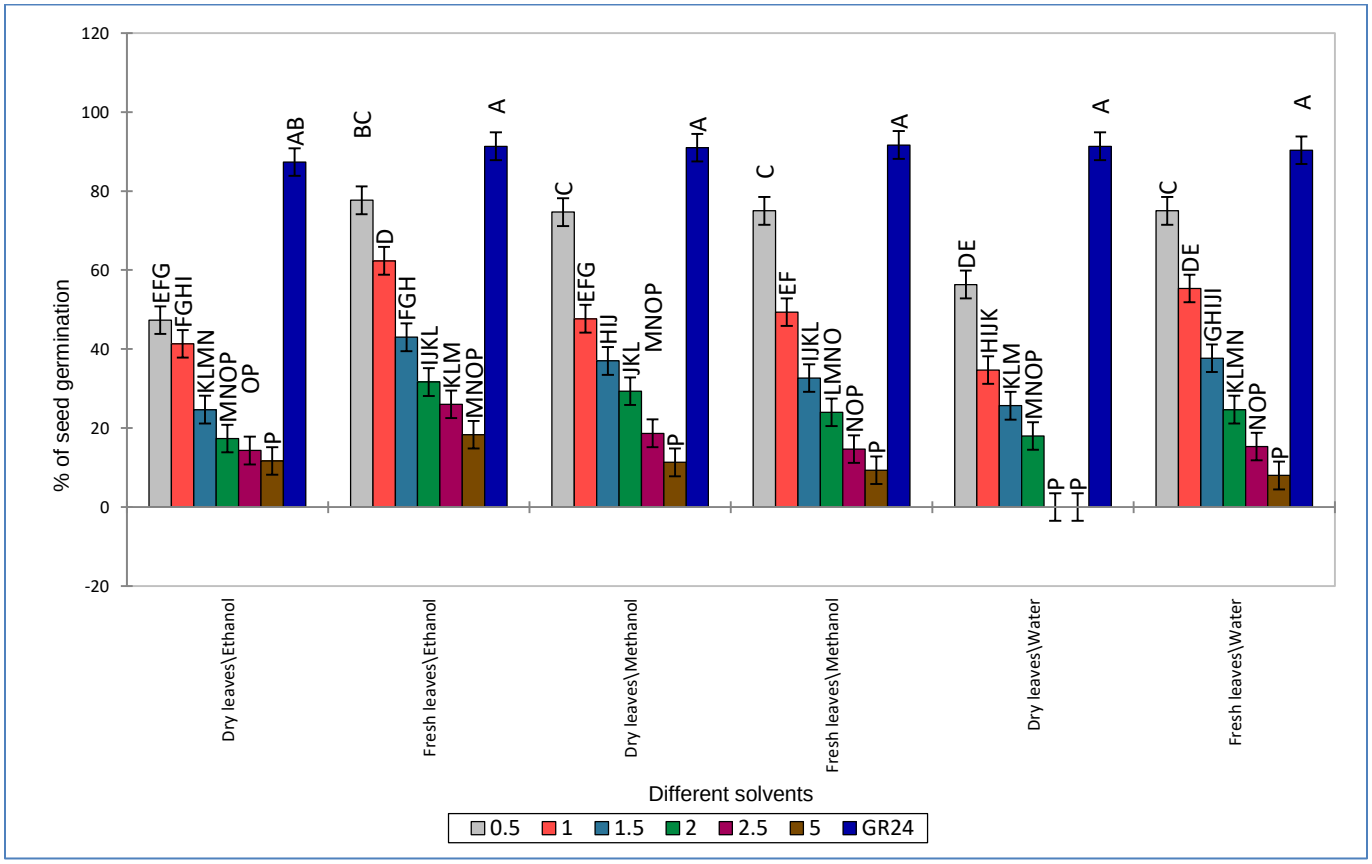

Figure 10. Percent of seeds germination in different solvents (ethanol fresh and dry, methanol fresh and dry, water fresh and dry) in one day .

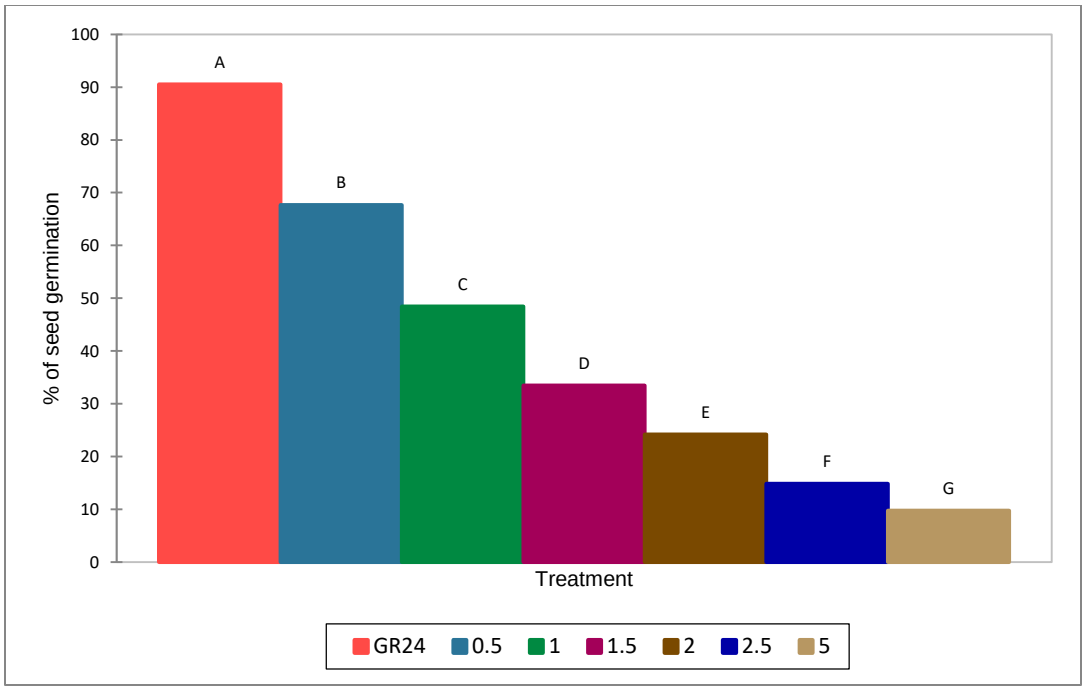

Figure 11. Percent of germination of *Orobanche* seeds after one day of treatment with different concentrations of *A. artemisiifolia* leaf extract. Data were pooled across all solvent type.

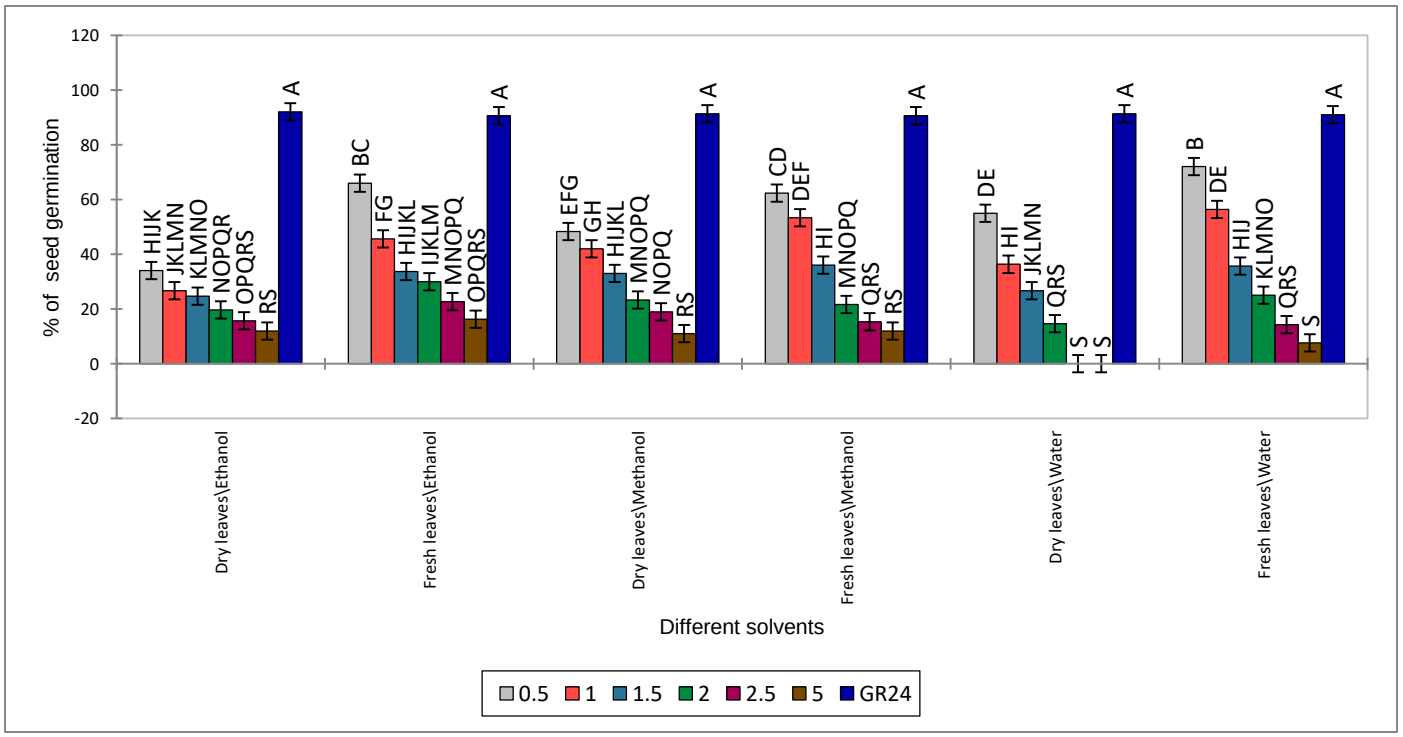

Figure 12. Percent of seeds germination in different solvents (ethanol fresh and dry , methanol fresh and dry, water fresh and dry) in second day.

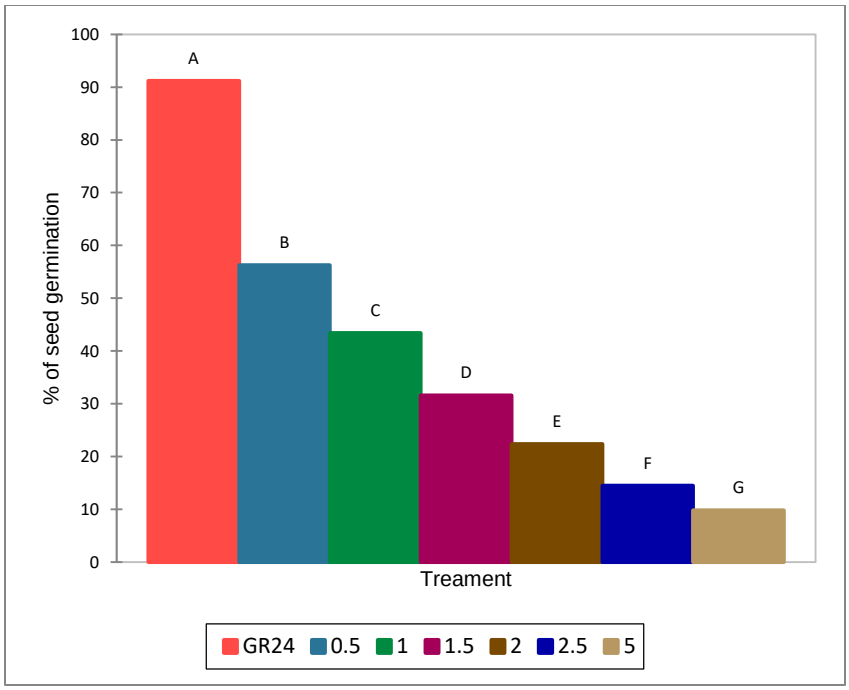

Figure 13 Percent of germination of *Orobanche* seeds after two day of treatment with different concentrations of *A. artemisiifolia* leaf extract. Data were pooled across all solvent type.

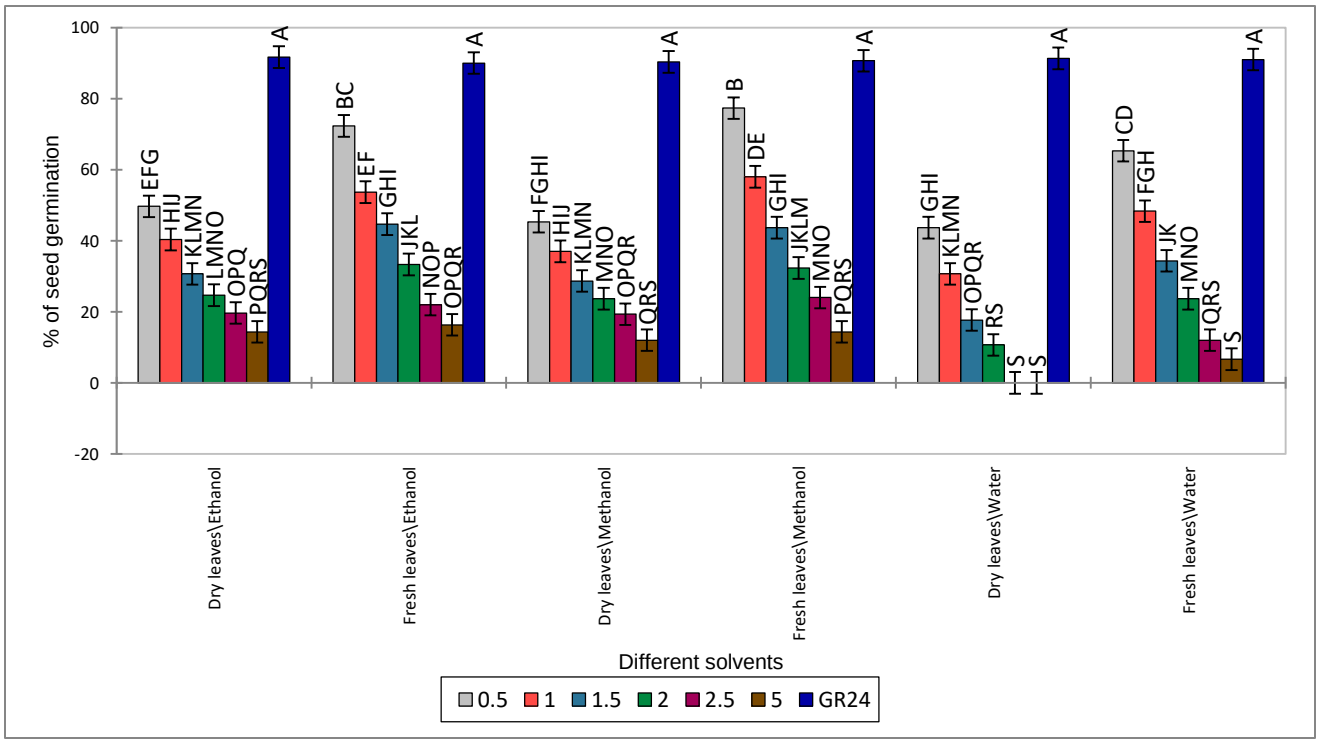

Figure 14. Percent of seeds germination in different solvents (ethanol fresh and dry , methanol fresh and dry, water fresh and dry) in the third day.

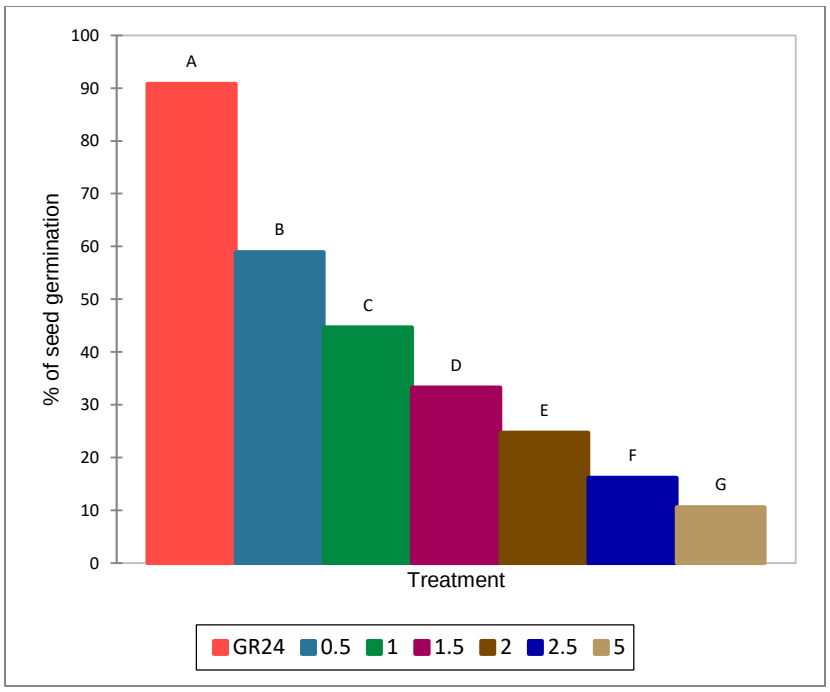

Figure 15. Percent of germination of *Orobanche* seeds after three day of treatment with different concentrations of *A. artemisiifolia* leaf extract. Data were pooled across all solvent type.

Integrated control of seed germination:

The figure (16) shows that there is no growth for the seeds that were treated with the combination (0: 5 and 0:2.5) herbicide: extract, and those that were treated with (1:0) herbicide: extract.

When a mixture was made between the herbicide and the extract with different combinations of each, it was found that there was seed growth with significant differences ($p < 0.05$) between each mixture and the other, where the seed growth percent was higher at combination (01:05) herbicide: extract.

The figure also shows that herbicide and extract combinations (0.5:2.5, 0.5:5, 1:2.5 and 1:5) herbicide: extract, were the most effective combined treatment against seeds germination with no growth of seeds.

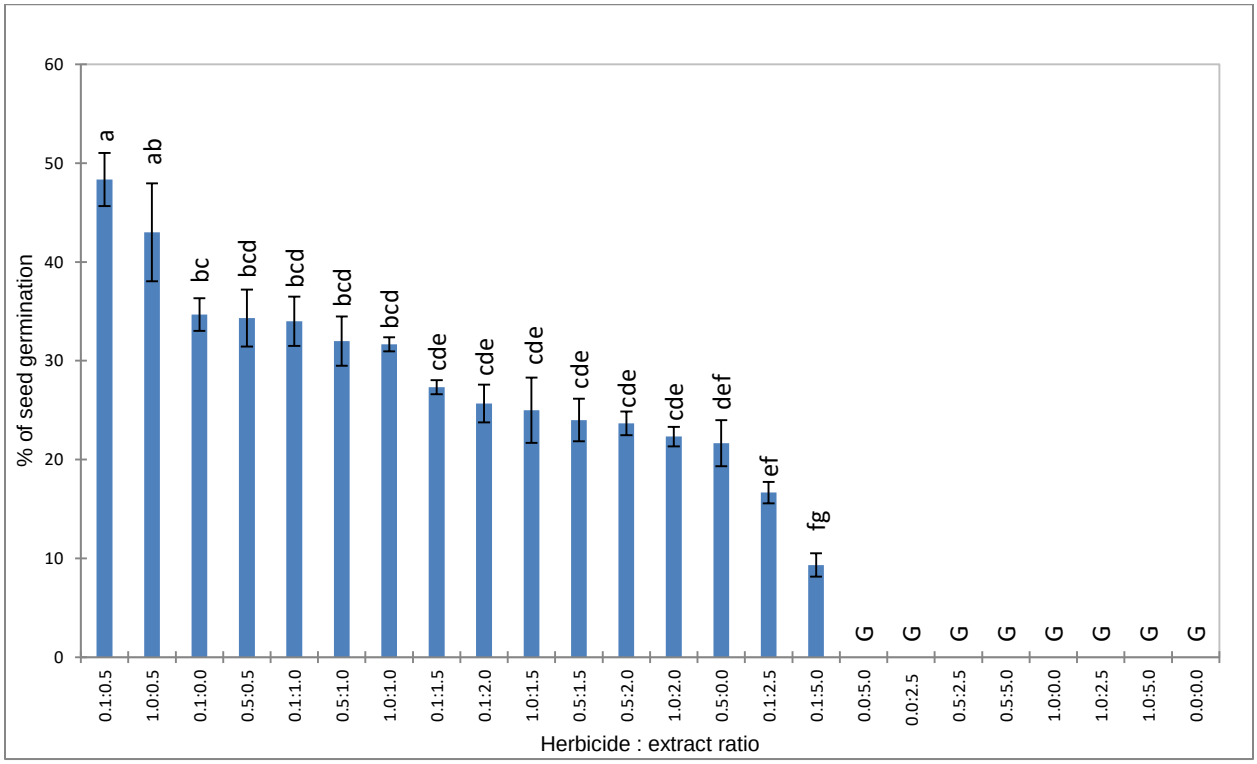

Figure 16. Percent of seeds germination in different treatments in herbicide and extract and herbicide extract combination.

Pot experiments

As for pot experiments on Tomato seedlings after 80 days of planting, extract application shows inhibition in *Orobanche* seed germination completely when irrigated with 5% extract, figure (17).

Figure 17. Tomato seedling irrigated with 5% extract.

On the other hands figure (18) shows small spikes on the pot surface, this spikes was started to appear at the surface after 75 days of planting seedlings and irrigations with extract, also figure appears the spikes number comparison with control positive pot, figure (19) which was irrigation with water and containing large numbers of *Orobanche*.

Figure 18. *Orobanche* seeds germination in tomato seedling irrigated with 2.5% extract.

Figure 19. *Orobanche* seeds germination in tomato seedling (Control positive) irrigated with water.

Moreover there were significant differences between the number of appeared *Orobanche* above the soil surface, the number of seed which grown under the soil in these pots compared to that in the positive control pots as shown in the table below.

Table1. The number of *Orobanche* above and under soil surface.

Pots name	Numbers of O.above	Numbers of O.under
Control -	0	0
Control -	0	0
Control-	0	0
Control+	55	0
Control+	44	10
Control+	51	0
Herbicide	0	0
Herbicide	0	0
Herbicide	0	0
2.5%	5	6
2.5%	0	3
2.5%	3	10
5%	0	0
5%	0	0
5%	0	0

(Figure 20) shows that there is no significant difference between the height of the plant that was irrigated with water and the plant that was irrigated with the extract at a concentration of 2.5%, and with the increase in the concentration of the extract to 5%, or using the herbicide, we notice the negative effect on the height of the

plant, which has no significant difference between it and the negative effect of control +.

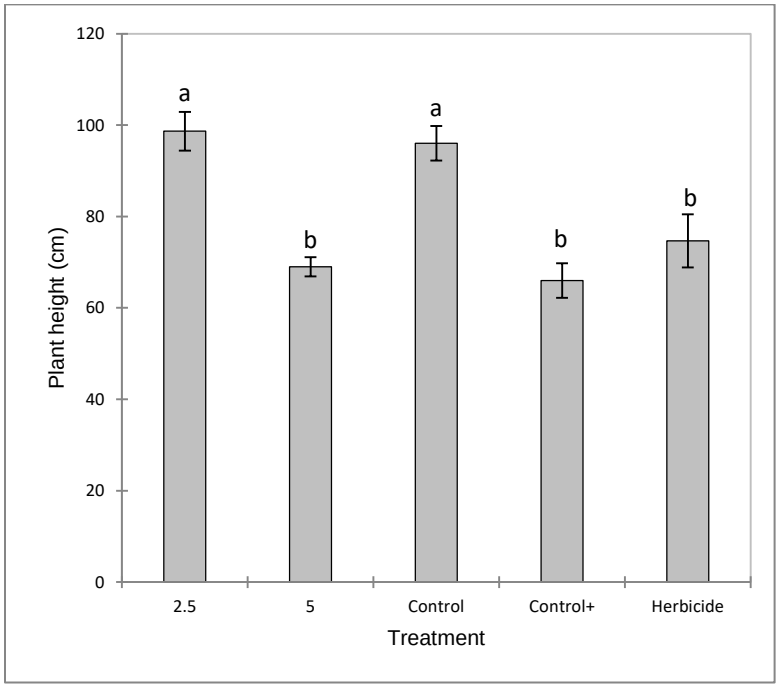

Figure 20. The effect of different treatment on plant height (cm).

The figures below show the positive effect of the extract at a concentration of 2.5% on both the dry and fresh weight of the shoot and the root, with significant differences between the weights of the plants which were irrigated with such extract concentration and those which were irrigated with the herbicide.

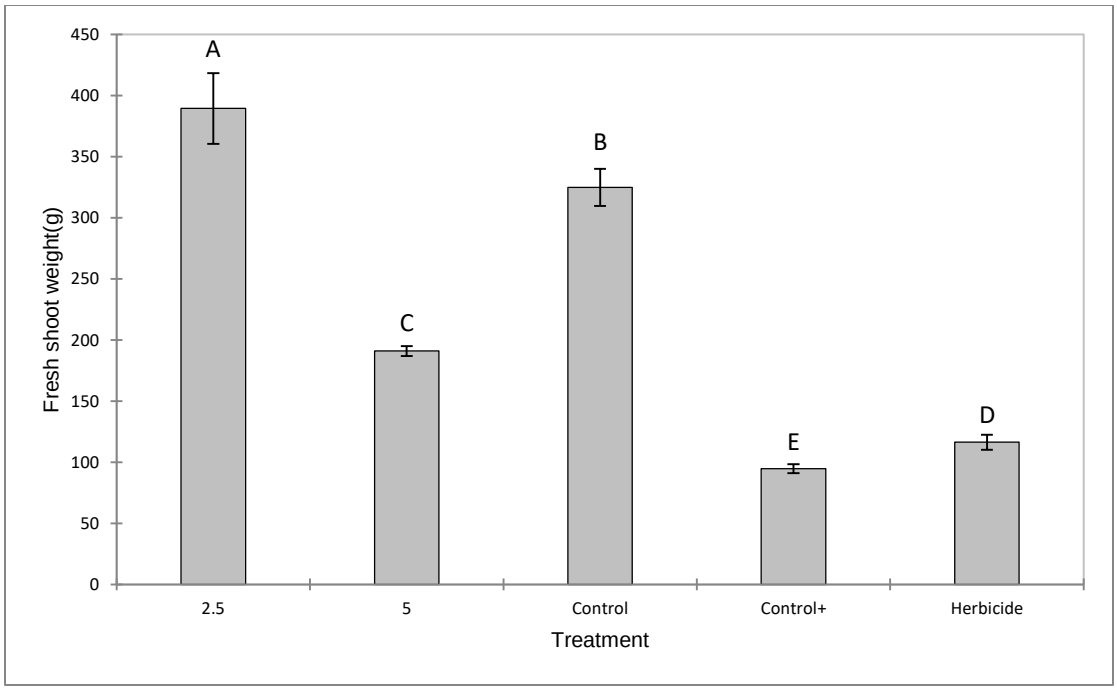

Figure 21. The effect of different treatments on shoot fresh weight of tomato seedling (g). Data of different letters are significantly different after Tukeys HSD test using ANOVA at $p<0.05$.

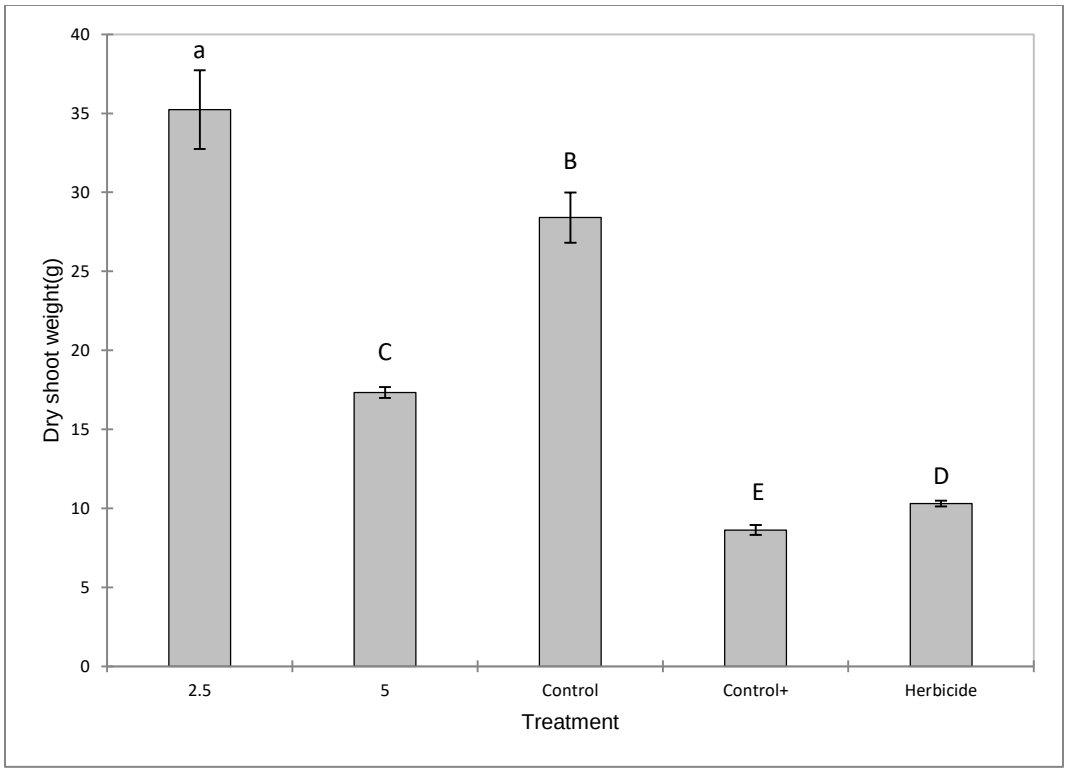

Figure 22. The effect of different treatments on shoot dry weight of tomato seedling (g). Data of different letters are significantly different after Tukeys HSD test using ANOVA at $p<0.05$.

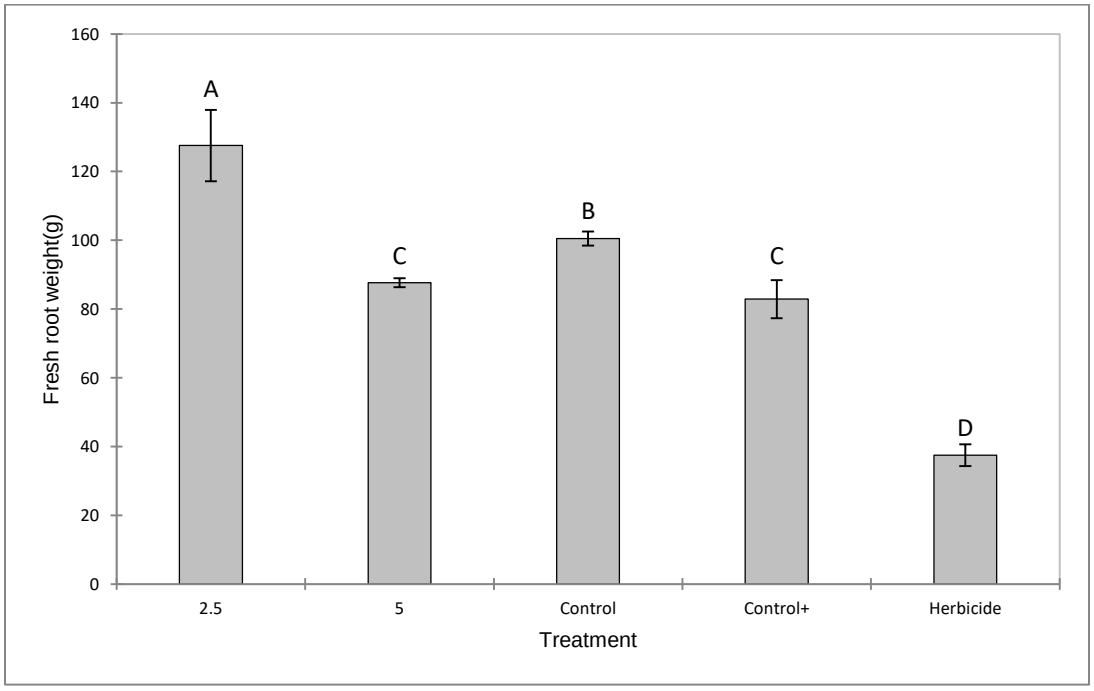

Figure 23. The effect of different treatments on root fresh weight of tomato seedling (g). Data of different letters are significantly different after Tukeys HSD test using ANOVA at $p<0.05$.

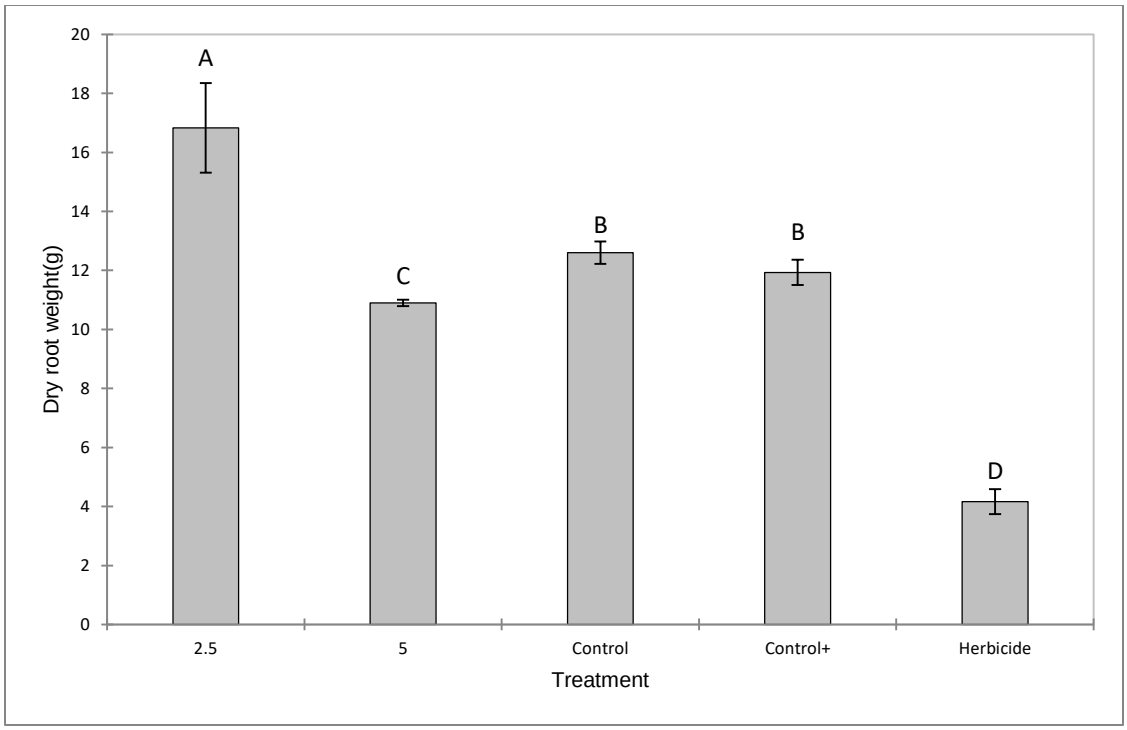

Figure 24. The effect of different treatments on root dry weight of tomato seedling (g). Data of different letters are significantly different after Tukeys HSD test using ANOVA at $p<0.05$.

Discussion

Palestine is one of the Mediterranean countries, which suffers from *Orobanche* problems. *Orobanche* affects a wide range of regions as well as a wide range of crops. Over years, traditional methods for preventing *Orobanche* problems were not effective, due to *Orobanche* ability to reduce yield (sometimes crops losing might reach 100%), large numbers of long lived seeds, and its easy dissemination (Silaev, 2021).

The results of the present study indicated that leaf extract from *A. artemisiifolia* plant inhibited *Orobanche* seeds germination and reduced germ tube length under in vitro conditions. It has been shown that *A. artemisiifolia* contains some secondary metabolites (e.g. sesquiterpene lactones) that have some molluscicidal activity against the small tropical freshwater snail (Sturgeon *et al.*, 2005).

Herbicide formulations contain active ingredients, components of the herbicide formulation that responsible for being phytotoxic to weeds. Active ingredient can be divided into two categories including solvents and adjuvants. Common solvents include substances such as water (Gao et al., 2019). A single active ingredient often dissolve in several different kinds of solvents, usually water. Moreover, not all herbicide active ingredients are soluble in water, usually fit into one of three solubility classes including water soluble, oil soluble, and non-soluble.

Solubility of herbicides in water generally decreases from salt to acid to ester formulations, but there are some exceptions. For example glyphosate is highly water-soluble and has a strong adsorption capacity (Helling *et al*., 1971). Due to the sesquiterpene lactones that located in the aerial part of the *A.attrimisiifolia* this may lead to make leaves extract dissolve in water more than others solvents (ethanol and methanol). In dry leaves extract form not in the fresh ones, may due to some pigments or food that storage in leaves which decrease the ingredients that inhibition *Orobanche* seeds germination.

Chlorsulfuron is an active ingredient used in herbicides which can control select broadleaf weeds and other undesirable grasses. Originally used for agricultural weed control and selective control of weeds in wheat and barley, it is 100 times more active than traditional herbicides so very low rates are used in the field (Ray, T. B. 1982).

In a previous experiment (Ibrahim, *et al*, 2012) three herbicides; chlorsulfuron, triasulfuron and ima-zaquin were tested to evaluate their efficiency in controlling the tomato broomrape. The herbicides significantly reduced the broomrape parasitizing tomato plants growing in pots without visible injury effect on the plants.in the experiments, triasulfuron increased the dead spikes from 77% to 84%; chlorsulfuron from 51% to 84% and imazaquin from 52% to 84% at the

concentrations (0.5 - 5 µg·ml−1) compared with the control, this emphasis our results that shown above about chlorsulfuron which inhibition seeds germination in both perti dishes and pot experiment which was no *Orobanche aegyptiaca* germination.

In our pot experiment the extract inhibit *Orobanche aegyptiaca* seeds germination in pots that were irrigated with 5% of extract but few *Orobanche* spikes appear in the pots that were irrigated with 2.5%, this concentration was inhibited seeds germination under vitro conditions this may be due to different conditions between vitro and field conditions, conditions in vitro was controlled but not in field, also nature of plants and root absorption system of active ingredients, may also due to soil nature and interactions with the extract and plant root system, the experiment carried in several months which are different in weather.

In pervious pot experiment biological control was carried using fungal pathogens *Fusarium oxysporum* sp. *orthoceras* was the predominant species, it is soil-borne fungi possess several advantages which render them suitable for the bioherbicide approach (Shabana et al, 2003), excellent control was repeatedly observed under laboratory and greenhouse conditions (Cohen et al., 2002a) observed reduction in *Orobanche aegyptiaca* attached to tomato in greenhouse experiments using host-specific strains of *F. oxysporum* ,this methods in the field are rare, the results

already indicate that *Fusarium* spp. in most cases do not provide the level of control desired by farmers. Thus there has been no successfully demonstrated control of this weed using potential inundative bioherbicides. (Sauerborn et al., 2007). This is in agreement with the results we determine

Conclusion

Germination of *Orobanche* seeds was inhibited by leaf extract from *A. attrimisiifolia* under in vitro and greenhouse conditions. Up to our knowledge, this study is the first of its kind that considers plant leaf extract to control *Oroabcnhe* infestation. It might open the way for development of a new bioherbicide depending on natural formulation, with an added benefits of environmentally safe and economically viable products.

The extract could be used in combination with chemical herbicide offering the possibility to reduce the costs of Orobanche management strategies by lowering the amount of herbicides.

Recommendations

Based on the results of our study we could recommend the following:

1. Determining the active ingredients of the leaf extract that affect Orobanche seed germination.
2. Testing the efficacy of the leaf extract under field conditions.
3. Production of suitable formulations from the leaf extract

الملخص بالعربي

التأثير البيوكيميائي(Allelopathy) لمستخلص اوراق نبات (*Ambrosia artemisiifolia*) لمنع نمو بذور الهالوك المصري وتفاعله مع العائل

الهالوك هو احدى انواع النباتات الطفيلية التي تفتقر الى مادة الكلوروفيل، وتعتمد على افرازات جذور النبات المضيف لإكمال دورة حياتها. وقد صنفت على انها اخطر انواع النباتات الطفيلية في العالم، وذلك بسبب ما تسببه من الخسائر الاقتصادية في المحاصيل والتي قد تصل احيانا في بعض انواع المحاصيل الى100%.

وبسبب قدرتها على انتاج كمية كبيرة من ملايين البذور الدقيقة، التي تتميز بالوزن الخفيف الذي يتيح لها الانتقال بسرعه كبيرة مع الرياح والماء والنشاطات الزراعية، كما ان لهذه البذور القدرة على البقاء في التربة لسنوات طويلة في طور الكمون الى ان تصبح الظروف ملائمة للنمو.

ولان هذه البذور تنمو ملتصقة في جذور النبات المضيف، يصعب فصلها عن جذوره ، ولا تتوفر المعلومات الكافية لدى المزارعين في كيفية التعامل مع هذا النبات الطفيلي، و نظرا لعدم نجاح المكافحات الكيمائية والفيزيائية والطرق التقليدية في التخلص منها، ويعود ذلك لقدرتها على تطوير اليات مقاومة ودفاع ضد المبيدات الكيميائية. كما ان للمبيدات الكيميائية اثار جانبية ضارة على النبات المضيف، وقد تتسبب احيانا في تدمير النبات المضيف مع بقاء النبات الطفيلي على قيد الحياة.

لهذه الاسباب مجتمعه كان لا بد من ايجاد بديل من مواد حيوية من اوراق نبات *Ambrosia* (*artemisiifolia*ودراسة تأثيرها على نمو بذور نبات النبات الطفيلي بالإضافة لدراسة تأثيرها على النبات المضيف كذلك.

بداية تم كسر فترة السكون للبذور، ودراسة التركيز الاصلي من المستخلص 10% (10 غم من مسحوق الاوراق المجففة في 100مل من الماءالمقطر) على نمو هذه البذور. وبعد التأكد من قدرة المستخلص على منع نمو البذور في داخل المختبر، تم اجراء تخفيفات من المستخلص بتراكيز مختلفة (0.5،1 ،1.5 ،2 ، 2.5،5 %) وتطبيقها على البذور لتحديد التركيز الاقل ذو القدرة على منع نمو البذور بشكل كامل. وقد تم ذلك باستخدام مادة بديلة للمواد التي يفرزها الجذر في النبات(GR24) وقد تم نقع البذور في المستخلص ذو التراكيز المختلفة، لمدة 5 ايام ثم حساب نمو الانابيب الجذرية ومدى استطالتها. وكانت التراكيز 2.5 و5 % هي التراكيز المناسبة، والتي ادت الى توقف انبات الانابيب الجذريه في البذور بشكل كامل بينما 0.5 % كانت اكثرها نموا للأنابيب الجذرية، حيث كانت نسب الإنبات عند (2.5 و 5 و10%) 0% بدون استطالة الأنابيب الجذرية بشكل كامل، وكانت أعلى نسب الإنبات 94% عند 0.5% ، وكانت نسب الإنبات الأخرى: 84% عند 1% ، 32% عند 1.5% و 24%عند 2%. وتم التأكد من طبيعة المادة وامكانية اذابتها في مذيبات مختلفة (ماء، إيثانول، ميثانول) وتحديد المذيب الافضل الذي يحقق اعلى قيمه منع لنمو البذور، وقد كان الماء هو أفضل المذيبات التي ادت الى ايقاف نمو البذور وتم فحص مستخلص النبات بحالتيه المجففه والخضراء وكانت المجففه المذابه في الماء افضل حيث تم منع النمو للانابيب الجذريه بشكل كامل . وهذه كانت اشارة جيدة نظرا لتوفر الماء. ولان المبيدات الكيمائية هي احدى طرق التي تستخدم لمقاومة نمو نبات الهالوك كان لا بد من دراسة احدى هذه المبيدات الكيمائية ومدى فاعليتها في المختبر

على البذور ومن خلال مقارنة المستخلص الحيوي مع المبيدات الكيميائية المستخدمة مسبقا في مكافحة الهالوك. تم التأكيد على قدرة المستخلص على المنافسة للمبيدات الكيميائية، وقدرته على ان يكون بديل امن وصديق للبيئة وللنبات العائل.

كما ان تطبيق المستخلص على نبات البندورة من نوع (TR20) في داخل البيت البلاستيكي لم يكن له أثر سلبي على نمو النبات المضيف وقد كان النبات المضيف ينمو بشكل طبيعي بالمقارنة مع النباتات التي تم سقايتها في الماء. بينما منع المستخلص بكل تراكيزه التي استخدمت في التجربة (2.5، 5%) نمو بذور الهالوك.

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