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Chemical and Biological Control of Broomrape (*Orobanche aegyptiaca*) on Tomato Plants

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List of abbreviations

Item	Abb.	Item	Abb.
Acetolactate synthase	ALS	Potato dextrose broth	PDB
Acytohydroxy acid synthase	AHAS	Potato dextrose agar	PDA
Pentachloronitrobenzene	PCNB	Wettable granules	WG
Part per million	ppm	Soluble solution	SL
Fusarium oxysporum	Foxy	Broomrape	Br.
Fusarium arthrosporioides	Farth	Control	CK
Species	Spp	Active ingredient	a.i
Scepter®	S	Variety	var
Glean®	G	Faculta 144	FA 144
Amber®	A	*Fusarium* isolate	Fu

Abstract

The broomrape (*Orobanche spp.*) belonging to the Orobanchaceae family, are obligate holoparasitic weeds that cause severe damage to many important vegetable crops, grain legumes and sunflower.

Many control strategies have been tried over the years, to control broomrape (*Orobanche* spp.) by using mechanical and physical methods, chemical herbicides and mycoherbicides with limited effectiveness.

In this investigation, the susceptibility of the most common vegetable and grain legume local cultivars to *Orobanche aegyptiaca* were tested. The results revealed that soybean, cowpea, bean and peas were immune while wooly vetch, bitter vetch, lupine and clover were resistant but tomato, broad bean, chickpea, sunflower, common vetch, and lentil, were susceptible.

In the chemical and biological control of tomato broomrape, three herbicides and 125 native *Fusarium* spp. isolates were tested. The herbicides, chlorsulfuron, triasulfuron and imazaquin significantly controlled broomrape parasitizing tomato plants growing in pots, irrigated open field and under greenhouse conditions at the concentrations (0.5-10 *ppm*) without visible effect on the plants. In the pot experiments, triasulfuron increased the dead spikes (%) by 79, 77, 84 and 84; chlorsulfuron, by 59, 51, 84 and 84 and imazaquin by 52, 59, 66 and 84 at the concentrations 0.5, 1, 3 and 5 *ppm*, respectively, compared to the control. In the irrigated open field experiment, triasulfuron increased the dead spikes (%) by 10.5, 13.5, and 26.6; chlorsulfuron by 13.6, 20.1 and 29.1 and imazaquin by 13.1, 22.2, and 28.9 at the concentrations 1, 3, and 5 *ppm*, respectively, compared to the control. In the greenhouse experiment triasulfuron, chlorsulfuron, and imazaquin increased the dead spikes (%) by 30 and 51; 60 and 68; 30 and 61 at the concentrations 5 and 10 *ppm*, respectively.

During a field survey, about 125 *Fusarium* spp isolates were recovered from 135 samples of the diseased broomrape spikes collected from fields in the Hebron agricultural areas. The pathogenicity of the isolates on broomrape were evaluated using both mycelia and conidia in the inoculum suspension. The most promising isolates; (Fu 20, Fu 30, Fu 52, Fu 59, Fu 87, Fu 119), significantly increased the broomrape dead spikes (%) by 33.6 - 72.7 compared to the control, and the isolates had no pathogenic effect on tomato plants. In addition, the two identified strains, *Fusarium oxysporum* strain EId (CNCM-I-1622) (Foxy) and *Fusarium arthrosporioides* strain E4a (CNCM-I-1621) (Farth) were tested and showed significant control to broomrape parasitizing tomato plants grown in pots; broomrape dead spikes (%) increased by 50.0 and 51.6, respectively . The native *Fusarium* spp. isolates (Fu 20, Fu 25 and Fu 119) were identified as *Fusarium solani*, while the isolates Fu 30, Fu 52, Fu 59, Fu 87 and Fu 12-04 were identified as *Fusarium oxysporum*.

Chapter 1

Introduction

1. 1. Background

The broomrape (*Orobanche spp*) belongs to the *Orobanchaceae* family. They are obligate holoparasitic weeds that cause severe damage to hosts. Broomrape hosts are many, but the most important include broad bean, pepper, tomato, and other important crops in the Mediterranean region and the Middle East (Parker & Riches, 1993). The estimated global annual food crop losses is about $ 1.3 billion to 2.6 billion (Parker & Riches, 1993). Egyptian broomrape (*Orobanche aegyptiaca*) together with branched broomrape (*Orobanche ramosa*) infests about 2.6 million hectars of solanaceous crops; tomato, tobacco, potato, and eggplant, mainly in the Mediterranean basin, North Africa, and Asia (Qasem, 1998; Zehhar et al., 2002; Boai et al., 2003). The broomrape (*Orobanche spp*) influence tomato's qualitative and quantitative production and damage can reach 75% (Hodosy, 1981). In Palestine, broomrape is consistently listed among the worst weeds and posses a major constraint to crop production (Al-Hamdi, 2000). The economic impact of broomrape can be attributed to its parasitic lifestyle, which has a direct detrimental effect on the host plant, and to its specialized biology that resist conventional weed control strategies.

Broomrape (*Orobanche*) also posseses a regional problem since its seeds respect no political boundaries and there is continuous flow of seeds via wind, water, human activities, animal manures and farm machinery (Jacobsohn, 1986; Ruso et al., 1996).

1. 2. Distribution and host range

Broomrapes are widely spread parasitic weeds decimating yields on almost all vegetables, grain legumes, and sunflowers in the Southern of Europe,

the Balkans and Russia, around the Middle East and North Africa (Sauerborn, 1991; Al-Hamdi, 2000). Broomrapes had a wide distribution in the world except in the tropics because of high temperatures and humidity (Parker & Riches, 1993; Burnhard, 1995). The majority of broomrapes are found in the warm and temperate parts of the northern hemisphere, especially the Mediterranean region but some species have spread to many other parts of the world. For example *O. aegyptiaca* occurs mainly in southeastern Europe, northeastern Africa, and the Middle East, whereas *O. ramosa*, which is closely related to *O. aegyptiaca*, is mostly found in the Middle East. *O. cernua* and *O. cumana* are primarily distributed in the Middle East, Southern and Eastern Europe, and Northern Africa. *O. crenata* is restricted to the Middle East (Sauerborn 1991).

Parker (1993) reported that five economic important broomrape species, *O. aegyptiaca, O. cernua, O. crenata, O. ramosa*, and *Orobanche minor*, are widely spread and parasitize crops including vegetables, field, food, flower, and spice crops from several botanical families (i.e., *Fabaceae, Solanaceae, Compositae, Cruciferae, Cucurbitaceae,* and *Umbelliferae*) (Parker& Riches, 1993). Many important crops are greatly affected by broomrape such as tomato, potato, eggplant, carrot, parsley, broadbean, chickpea, pea, peanut, lentil, vetch, sunflower, cole crops, cucumber, and squash. In Palestine, there are many *Orobanche* species including the most economically important, Egyptian broomrape (*O. aegyptiaca*), tobacco broomrape (*O. ramosa*), sunflower broomrape (*O. cumana*), fababean broomrape (*O. crenata*), clover broomrape (*O. minor*) in addition to Palestine broomrape (*O. palastina*) (Al-Hamdi, 2000; Abu- Irmaileh, unpublished).

1. 3. Morphology and ecology

Broomrape is a fleshy herbaceous, annual, parasitic plant 15 to 56 cm tall; the stems are simple and yellow to straw-colored; leaves are small triangular flaps; alternate to the stem, the roots are short, unbranched, fleshy, and attached to the roots of broadleaf hosts. The self pollinated flowers of *Orobanche* are borne in an elongated terminal cluster. Petals are about 1.2 cm long and are snapdragon like. Flowers coloration is off-white to yellowish with violet markings. The flowering period is short, starting about one week following emergence, with seed release beginning about one month following emergence. The broomrape seeds are minute (dust like seeds) easily dispersed, prolifically produced and long-lived; some remaining viable for many years, (Parker and Riches, 1993; Holm et al., 1997). *Orobanche* is an obligate parasite, lacking chlorophyll; thus plants must obtain all their nutrients and water on the expense of a host plant causing severe damage, reducing yield quantity and quality (Musselman, 1994; Parker and Riches, 1993). The optimum temperature for the *O. aegyptiaca* germination and radicle elongation ranging between 23-25°C (Nandula et al., 1996). The seed germination and development of the broomrape are greatly influenced by soil moisture through its effect on the host physiology and host development (Sauerborn, 1991). The broomrapes are widely distributed in alkaline soils and *O. aegyptiaca* is reduced at low pH (Parker and Riches, 1993). The calcareous fields are highly infested with *Orobanche* (Haider and Bibi, 1995).The soil salinity has adverse effect on the broomrape dry matter and number of attachment to the host (tomato) roots (Abu –Irmaileh 1998). Broomrapes reacts differently towards light, for example *O. crenata* germinates better in dark and still has germination in light while *O. aegyptiaca* completely inhibited by light (Sauerborn, 1991; Parker and Riches,

1993). The soil fertility has specific effect on broomrapes development since it prefers the poor unfertilized soils (Parker and Riches, 1993).

1. 4. Growth and development

1. 4. 1. Seeds

Broomrapes are annuals that reproduce by seeds. Seeds are usually dark brown, oval shaped; they are 0.35 mm long, 0.25 mm wide and 0.1 thick (Kadry & Tawfic 1956), and weigh 3 to 6 µg (Parker & Riches 1993). The number of seeds produced per plant varies from 10^5 to $5x10^5$ depending on the species. *Orobanche crenata* for example, produce more than 4000 seeds per capsule (Sauarborn, 1991) while *Orobanche cernua* may produce 340,000 seeds per plant (Jacobsohn, 1989). The seeds have a pattern of raised ridges on their surface. There is a hardened testa, surrounding a fatty endosperm that has an undifferentiated embryo at one end (Kadry & Tawfic 1956); the embryo lacking cotyledons and root cap (Jacopsohn, 1989).

1. 4. 2. Broomrape seed germination

Several factors influence germination of broomrapes in the soil including temperature, moisture, pH, nutrients, soil type, and stimulants produced by host plants. Broomrape seed germination requires exposure to biological exudates produced by the roots of the host plant with suitable temperature and moisture conditions (Joel et al, 1995b). Reports of inhibitory effects of nitrogen on the growth of broomrapes, including germination have been common in the literature for many years (Parker & Riches, 1993; Sauerborn, 1991). Osmotic stress has been implicated as a possible reason for inhibition of broomrape germination by nitrogen (Ernst, 1986). Wegmann (1986) observed that broomrape has a lower osmotic potential compared to the host caused by mannitol synthesis, and hence its ability to retain water and nutrients. Germination tests at different osmotic potentials demonstrated an adaptation of

the germ tube of *O. ramosa* to dry habitats (Linke, 1987). Optimum temperatures for germination and tubercles development are different among broomrape species. Studies on the effect of temperature on germination of *O. aegyptiaca, O. crenata,* and *O. cumana* indicated that every species had a specific optimum temperature range for germination and development which generally reflected its geographical distribution. The optimum temperatures for both conditioning and germination were about 18°C for *O. crenata,* and about 23 °C for *O. ramosa* (Sauerborn, 1991).

1. 4. 3. Radicle elongation and attachment to host

Upon germination, broomrape seed develops a small radicle which penetrates a fine rootlet of the host and becomes firmly connected with it; and the radicle immediately begins using nutrients from the host, and stores these as a starchy reserve in the upper part of the radicle causing it to become distended. The radicle gradually forms a nodule on the host root (Brenchy, 1920; Kuijt, 1969; Joel & Portnoy, 1998). As the nodule increase in size, small prominences emerge on its surface. The prominences develop into roots, which surround the nodule closely, and penetrate the host roots in other places, thus becoming attached at several points. The large swelling develops into a shoot, which ultimately elongates to form the above ground portion of the plant. The greater part of the broomrape's life is spent below ground, as nodule developed into a fair sized bulb-like structure, necessary for the development of aerial shoots, requires several weeks. The radicle elongates by cell division and extension (Brenchy, 1920; Parker & Riches 1993), and attaches to host roots mainly in the region of root elongation and absorption (Foy et al., 1989). The part of the broomrape radicle outside the host root swells to form tubercle which develops after 1 to 2 weeks of growth to a shoot bud on the tubercle producing a

flowering spike which elongates, and emerges above the soil (Dörr & Kollman, 1995).

1. 5. Control of broomrape

Control of broomrapes is often difficult due to several reasons. These include the high amount of seed production, long seed viability (Cubero & Moreno, 1979; Linke & Saxena, 1991; and Puzzilli, 1983), lack of seed germination in the absence of a chemical stimulant from a suitable host, parasite vigorous growth, wide host range, close association with the host crop and the seeds posses a seed coat which has some chemicals that inhibits germination (Sauerborn, 1991). Numerous control strategies have been tested over the years, to control broomrapes with limited effectiveness (Al-Hamdi, 2000).

1. 5. 1. Mechanical and physical methods

1. 5. 1. 1. Hand weeding

Hand weeding of broomrape floral spikes is still a common practice. Since the inflorescence appears continuously throughout the growing season, this labor must be repeated periodically, because the broomrape is well developed by the time it is not visible, and most of the damage to the crop is already done; hand weeding only prevents dispersal of new seeds (Parker & Riches, 1993). It was reported that three years of hand weeding could control *O. cernua* in tobacco in India but this method still time consuming and labor intensive (Krishnamurthy & Rao, 1976; Jacobson, 1986; Ransom, 2000).

1. 5. 1. 2. Tillage

Tillage will not be effective control method against broomrape since its seeds are extremely small, produced by large numbers (500,000 seed / fruit) and can remain viable in the soil or in storage for many years (3-12 years or more) (Jacobson, 1986). Therefore, in the absence of a host it is difficult for any tillage to be efficient (Sauerborn, 1991; Parker& Riches, 1993).

1. 5. 1. 3. Deep inversion plowing and fire

Several strategies, which physically affect broomrape seeds, such as deep inversion plowing, fire, and soil solarization have been tested. Placement of seeds at 20 cm deep was observed to cause little emergence of *O. cernua* (Krishnamurthy et al., 1987). Also trench ploughing 45-50 cm deep with a moldboard plough reduced *O. ramosa* by 80-90% in tobacco fields of Eastern Europe (Parker& Riches, 1993). A well-timed burn of broomrape effectively limits seed production, but does not prevent below ground growth or damage to the host plant and did not significantly reduce the amount of viable seeds in the soil(Parker& Riches, 1993).

1. 5. 1. 4. Prevention of seed spreading

Avoiding spread of contaminated farmyard manure, planting materials as well as contaminated implements and vehicles, in addition to controlling the secondary wild hosts of *Orobanche* species is of value to prevent proliferation in the coming season (Sauerborhn, 1991; Jacobson, 1986; Parker & Ritches, 1993; and Burnhard, 1995).

1. 5. 1. 5. Crop rotation

Crop rotation has a very limited effect as a controlling method against *Orobanche* (Jacobson, 1986). *Orobanche* seeds are extremely small, produced by large numbers and can remain viable in the soil or in storage for many years in the absence of a host (Saurborhn, 1991; Parker & Riches, 1993), these characters making it difficult for any crop rotation to be efficient.

1. 5. 1. 6. Soil solarization

Solarization is a method for disinfecting wet soil by heating it using a cover of a clear or black polyethylene (PE) sheets and heating the soil by solar radiation. The temperature of the soil is increased, and the polyethylene cover preserves moisture and at the same time prevents temperature loss. This process

can increase the temperature of covered soil by more than 10°C compared to uncovered soil. Abu-Irmaileh (1991) found that using black or clear PE sheets in soil solarization reduces *O. ramosa, O. aegyptiaca* and *O. cernua* by 100% in tomato and squash farms at different locations in Jordan. *O. aegyptiaca* (Jacobsohn et al., 1980; Sauerborn & Saxena, 1987), *O. crenata* (Sauerborn and Saxena 1987), and *O. ramosa* infestations have been reduced by using solarization. Soil solarization completely controlled branched broomrape (*O. ramosa*) and improved plant growth and fruit yield of tomato grown under greenhouse conditions (Mauromicale et al., 2005). Soil solarization is simple, non hazardous and does not employ toxic materials and suitable to the organic agriculture (Jacobsohn et al., 1980 and Mauromicale et al., 2005). However the biggest limitation to this method, is the high cost of the polyethylene sheets (Foy et al., 1989), making, it not practical in open large areas.

1. 5. 1. 7. Fertilization

Orobanche prefers the non-fertilized lands, the lower the fertilizer use the higher the *Orobanche* infestation (Parker & Riches, 1993). A good suppression of *O. ramosa* in tomato and tobacco was accomplished with ammonium sulfate at the rate of 4 g kg-1 soil. However, the yields of tomato were reduced if both phosphorus and potassium were not added (Abu-Irmaileh, 1979, 1981). Ammonium nitrate at 1 g N kg-1 soil reduced *O. aegyptiaca* emergence (Jain & Foy, 1987 and 1992). Also, ammonium nitrate reduced germination and radicle length of *O. ramosa*, grown in association with host crop seedlings (Abu-Irmaileh, 1994). Westwood and Foy (1999) reported that ammonium is more effective than nitrate in reducing *Orobanche* seeds germination suggesting that direct radicle elongation inhibition belongs to ammonium ions. The germination and growth of *O. crenata* were severely affected when exposed to nitrogen in the form of urea or ammonium (Pieterse, 1991). In addition, Ghosheh et al.

(1999) reported that amendment of soil with olive Jift greatly reduces either germination or attachment by *Orobanche* which may be due to specific inhibitory chemicals in the Jift or to indirect effect on the soil micro flora. Also, the use of fresh manure containing viable seeds in fields is believed to spread *Orobanche*. However, if the manure is processed into granules or pellets, the high temperatures of this process kills high percentages of the seeds (Joel et al., 1988). The use of chicken manure (20 t ha^{-1}) and elementary sulfur (8 and 12 t ha^{-1}) mixture reduced *Orobanche* infestation by 75% (Haidar & Sidahmed, 2006).

1. 5. 1. 8. Sowing date and cropping density

The degree of infestation by broomrape is closely related to the sowing date of the host crop. Delay of the sowing date has resulted in reduced parasitism of broad bean (*Vicia faba*) and lentil (*Lens culinaris*) by *O. aegyptiaca* (Sauerborn, 1991). Late sowing of winter crops like broadbean in Syria reduced the *Orobanche* biomass (Parker & Riches, 1993). High infestation of *O. aegyptiaca* and *O. crenata* parasitized carrots when planted in the field in September, October, and November while no infestation is found when planted in April to August (Jacobsohn, 1986 and Burnhard, 1995). This strategy takes advantage of the optimum seasonal temperatures of broomrape seed germination, it is useful only when early maturing varieties are available to compensate for the loss in yield due to the short vegetation period of a conventional variety under late sowing conditions. However, increase in other inputs such as seeds, cultivation, fertilizer, and pesticides may result in higher production costs. Increasing density of broad bean reduced competition from *O. crenata* (Pieters & Aalders, 1986) and number of attachments (Manschadi et al., 1997).

1. 5. 2. Trap and catch crops

Trap crops, plants that stimulate parasite seed germination but are tolerant to the parasite, and catch crops, plants that are parasitized and destroyed before parasite seed development were tested by several researchers (Musselman, 1980, Sauerborn, 1991, Parker & riches, 1993, and Kleifeld et al.,1994). Catch crops were mentioned as constituents of a crop rotation to decrease broomrape seed populations in contaminated fields (Foy et al.,1989, and Parker & Riches, 1993). A good examples of trap crops for *Orobanche* species are flax and pepper (Hershenhorn et al., 1996); the pepper roots stimulates the seeds of *Orobanche aegyptiaca* and *O. cernua* without formation of parasitic attachments while Flax (*Linum usitatissimum*) and sweet pepper (*Capsicum frutescens*) are suggested to be a trap crops for *O. ramosa*, but not for *O. crenata* and *O. aegyptiaca* (Abu-Irmaileh, 1982). Clover (*Trifolium alexandrium*) is considered as a catch crop while fababean (*Vicia faba*) heavily infested by *Orobanche* was considered as a trap crop (khalaf, 1992). Cabbage (*Brassica campestris*) has been used as a catch crop in Nepal, it reduced *O. aegyptiaca* seed bank by 30% (Acharya et al., 2002).

1. 5. 3. Resistant cultivars

The use of resistant cultivars of some crops including sunflower (*Helianthus annuns*), fababean (*V. faba*), and common vetch (*V. sativa*) is effective against broomrape (Gil et al., 1987 and Cubero, 1991). Resistance to parasitic weeds has been proposed to occur through a variety of mechanisms such as deposition of lignin and cellulose to block parasite entery into the root, or accumulation of phytoalexins to poison the developing parasite (Olivier et al., 1991 and Goldwasser et al., 1999). Death of host root tissues around the haustorium (common mechanism in legumes); host tissue necrosis occurred around the sites of attachments of *O. aegyptiaca* on resistant vetch (Gold wasser

et al., 1996). Cubero (1991) has summarized the work done in Italy, Spain, and Egypt which showed various degrees of susceptibility in broad bean to broomrape. Only one broad bean variety resistance to *O. crenata* (F-402) that was identified in Egypt and has been successfully used in breeding programs (Nassib et al., 1979, 1982 and 1984). But resistant varieties is not available for all crops.

1. 5. 4. Genetically engineered herbicide-resistant crops

Development of a specific herbicide would be difficult due to the complex interaction between broomrape and its hosts. Under these circumstances, availability of genetically engineered herbicide-resistant crops has provided an effective broomrape control method. Foy et al. (1989) addressed the first applications of genetically engineered herbicide-resistant crops for broomrape control. Complete control of *O. aegyptiaca* was achieved when transgenic tobacco was treated with chlorsulfuron (Joel, 1992). Typically, two kinds of herbicide resistance can be genetically engineered into crop plants (Gressel et al. 1994). First, target-site resistance where the herbicide binding to the target enzyme is prevented. This permits movement of the herbicide through the treated host crop to parasitic attachments on the host root. The second resistance mechanism involves metabolism or breakdown of the herbicide by treating a crop with harmless compounds. Metabolic resistance, therefore, is not suitable for systemic herbicides, but applicable only for herbicides applied directly to the parasite below the soil (Joel et al. 1995b). In addition, Noureddine (2005) reported that genetic engineering strategy for inhancing resistance in the crops against parasitic plants is effective. The mechanism for resistance to the parasitic *Orobanche* was based on expression of sarcotoxin IA in transgenic tobacco. Sarcotoxin IA is a 40-residue peptide with antibiotic activity isolated from *Sacophaga peregrina* fly. The sarcotoxin

IA was fused to an *Orobanche* –inducible promoter HMG2, which induced in the host root at the point of parasite contact, used to transform tobacco. The transgenic plants (plants expressing sarcotoxin IA) showed enhanced resistance to *O. aegyptiaca* obvious by abnormal parasite development and higher parasite mortality after attachment as compared to control (Noureddine et al., 2005).

1. 5. 5. Chemical control

Chemical control of broomrapes includes soil fumigation, germination stimulants, and herbicides.

1. 5. 5.1. Soil fumigation

Soil fumigation with volatile, toxic chemicals such as methyl bromide, metham sodium and dasomet are the most effective in controlling broomrapes. The chemicals penetrate the soil and kill all soil-borne pathogens including bacteria, fungi, nematodes, and physiologically active weed seeds (Jacobson, 1986, Burnhard, 1995). Methyl bromide has been recognized as an effective soil fumigant. Zahran (1970) has demonstrated the use of methyl bromide for controlling *O. crenata* and *O. minor* in Egypt before planting tobacco and broad bean, respectively. There are several limitations that restrict the use of methyl bromide over a large scale mainly the cost of the chemical as well as the polyethylene sheet needed to cover the treated soil, fumigant application difficulty and its hazardous effect to human and environment (Parker & Riches, 1993). Metham-sodium provided good control of broomrape in broad bean (Zahran 1970) and tobacco (James 1976, James & Frater 1977). Application of metham-sodium through irrigation water (chemigation) to dry soil and covering with polyethylene sheet provide consistent control of broomrape (Kleifeld et al, 1991). Metham sodium release the active ingredient (methyl isothiosulphate) applied as a liquid product is the preferred soil fumigation method in U. S. A. and kills 50% of *Orobanche* seeds (Goldwasser et al., 1994).

1. 5. 5. 2. Germination stimulants

The broomrape seeds must attach to a host root shortly after germination to survive, accordingly stimulating seed germination in the absence of a suitable host 'suicidal germination' has a potential as a control strategy (Eplee, 1975). Application of strigol or its synthetic analogs did not provide practical control of broomrape due to their short stability in the soil. Both the activity and stability of the germination stimulants is dependent on the soil pH and moisture conditions. Ethylene was used for stimulation of broomrape seed germination with limited success (Parker and Wilson 1986). Several other compounds including herbicides have been used to stimulate as well as inhibit germination in broomrape seeds (Foy et al. 1989). Germination stimulants, both natural and synthetic, have promising potential as effective tools of management of broomrape, but much remains to be learned about their structure, activity, and stability in the soil. GR_{24} (*3-methyl-4-(2-oxo-3, 3a, 6, 6a- tetrahydro- 2H- cyclo penta (b) furan-3- y lidenemethoxy) but-2-ene- 4-olide*) is strigol analogue, synthetic compound closely related to strigol. It showed high efficacy on germinating *Orobanche* seeds and striga (Mangnus et al., 1992). Al-Hamdi (2000) investigated the influence of tomato root extracts on the germination of *O. cernua* and showed reduction of the developed spikes.

1. 5. 5. 3. Herbicides

Researchers have suggested the use of translocated herbicides for the purpose of broomrape control, but selectivity to the host plant remains the main obstacle. Crop selectivity derived from decomposition binding or limited transportation of the herbicide in the host plant, results in no effect on the parasite and vise versa, since it was found that effective chemicals on the parasite are not selective enough to the host plant. Few investigators have reported effective and selective control of *Orobanche* by herbicides and even

fewer have suggested application of soil residual herbicides (Foy et al., 1989, Garcia-Torres and Lopez-Granados, 1991, and Parker & Riches, 1993). Control of broomrape by chemical herbicides have been limited because very few herbicides are available that can selectively kill broomrape without injury to the host plant. The herbicide chlorsulfuron applied at 5.0g a.i/ha gave 100% control of the number and dry weight of emerged broomrape shoots and underground attachments, showing that under certain conditions, this herbicide may completely prevent parasite development. The results of pot experiments indicated that chlorsulfuron applied at 5 g a.i/ha was the most effective herbicide for broomrape control and the least toxic to the crop. Under field conditions, chlorsulfuron applied at 10g a.i/ha controlled broomrape emergence by 88%. When the herbicide was applied twice, (5 and 10g a.i/ha) it gave complete control of broomrape but delayed crop maturity (Kotoula-Syca, 2002). The herbicides rimsulfuron and sulfonylurea selective to tomatoes effectively controlled *O. aegyptiaca* in pots, but in field applications in drip-irrigated tomatoes, *O. aegyptiaca* control was poor (Reinke et al., 1991). The effective control of *O. aegyptiaca* and *O. ramosa* in potato fields can be achieved by split foliar applications of imazapic at 3.0g/ha repeated three times and rimsulfuron at 12.5g/ha repeated three times, applied into the potato root zone by sprinkler irrigation. The best results for *Orobanche* control in potato was obtained by application of rimsulfuron at 12.5g a.i/ha followed by sequential foliar application (Three times) of glyphosate at 100g a.i/ha (Haider et al., 2005). Split application of low rates of imazapic applied on tomato foliage or chemigated via sprinkler irrigation achieved excellent *O. aegyptiaca* control through out the growing season, but caused premature loss of flowers and early ripening of fruits (Kleifeld et al., 1998). During the last few years, new herbicidal groups are showing promising results in broomrape control;

sulfonylurea, imidazolinone, and other inhibitors of the enzyme acetolactate synthase (ALS) or acytohydroxy acid synthase (AHAS) are some examples. Members of these groups showed some degree of selectivity to broomrape host plants (Gracia-Torrez & Lopez-Granados, 1991, Gracia-Torrez et al., 1995, 1996, and Plakhine et al.,1996). Chlorsulfuron, pronamide and pendimethalin at 2.44g a.i/ha effectively controlled *O. ramosa* by >98% and was the least phytotoxic to tomato plants (Qasem, 1998). Control of *O. crenata* in broad bean have been achieved by using twelve herbicides belonging to the imidazolinone, sulphonylurea, glyphosate analogue and substituted amids families. In general, the effectiveness of any herbicide treatment was largely dependent on the duration of infestation (Garcia- Torrez & Lopez- Granados, 1991). Crop pre-emergence treatments of imazethapyr at 75-100g/ha and imazapyr at 12.5-25g/ha resulted in efficient control without damaging the crop. Imazaquin at about 80g/ha and chlorsulfuron at about 6g /ha applied as pre-emergence treatment were also active, but their performance was likely to be affected by soil conditions. Applied after crop emergence, imazethapyr at 40g/ha and Mon-8000® at 60g/ha gave similar results to be standard treatments of glyphosate at 60g/ha (Garcia- Torrez & F. Lopez- Granados, 1991). Glyphosate, controlled *Orobanche* in Cyprus and Israel when applied at a low rate of 50 g a.i/ ha in carrot and celery (Jacobsohn & Levy 1986.

1. 5. 6. Biological control

Traditional control methods of *Orobanche* have been tested by various investigators on different crops but none of them was completely effective (Amsellem et al., 2001b). Biological controls of broomrape are limited but they could contribute in reduction of broomrape seeds through an integrated approach. Biological control includes the use of insects and fungi.

1. 5. 6.1. The insect (*Phytomyza orobanchia*)

The most investigated insect is *P. orobanchia* (Diptera, Agromyzidae) used to feed on broomrape plants since its host range is restricted to *Orobanche* spp. The adult insects have the ability to detect *Orobanche* plants triggered by the alkaloid orobanchamin with the help of chemoreceptors, which are located in the first two segments of the antennae (Bronstejn, 1972).

The insect deposit eggs on shoots and flowers; after hatching, larvae bore into the stem or the pericarp to the ovule. Seeds are the insect preferred food, but in the absence of food in seed capsules, the larvae mine under the epidermis or the parenchyma of the shoots (Tawfik et al., 1976, Linke et al., 1990). The damage is either caused by adults feeding on shoots, flowers, seeds and capsules, or by larvae which mine in the shoots or in the seed capsules and destroy immature seeds. Secondary infections by fungi may cause early death of shoots or limit the development of flowers and ovules (Klein et al., 1999). A heavy infection often causes wilting and a degeneration of the root tips (Mihajlovic, 1986). The main impact is the reduction of the *Orobanche* seed production, resulting in the prevention of supplementary infestation and seed dissemination, leading to a reduction of seed bank in the soil. The natural impact of *P. orobanchia* on the reduction of *Orobanche* seed production has been between 10% and 80% (Klein et al., 1999). It has been calculated that a biocontrol agent (*P. orobanchia*e) would have to lower seed output by more than 95% to have 50% reduction of parasite density (Smith & Webb, 1996). The efficiency of *P. orobanchia* is mainly limited by low temperature, cultural practices: soil preparation, crop rotation and irrigation (Trenchev, 1981), insecticides and natural enemies: bacteria, fungi, parasitoids and predatores (Okazova, 1973).

1. 5. 6. 2. Fungi

Many isolates of promising pathgenic organisms that could be useful for *Orobanche* control has not been developed to the point of widespread use, due to many problems with formulation, application, and pathogen virulence preservation. Hodosy (1981) obtained excellent results in field experiments in Hungary with *Fusarium solani* isolates. A strain of *Rhizoctonia solani* was mentioned to be a biocontrol agent of *Orobanche* but has not been seriously considered for mycoherbicidal applications because they normally do not produce spores (Boyette et al., 1991, 1996). Bozoukov & Kouzmanova (1994) found that applying conidiospores of *Fusarium lateritium* to tobacco fields during irrigation cause significant control levels of *Orobanche*.

Two very promising isolates of *Fusarium oxysporum* strain E1d (CNCM I-1622) and *Fusarium arthrosporioides* strain E4a (CNCM I-164) were specifically pathogenic to *Orobanche* which had been isolated from diseased shoots and their potential for the biocontrol of *O. aegyptiaca* has been studied (Amsellem et al., 1999 & 2001a&b). Both *Fusarium* strains infected *O. aegyptiaca, O. cernua,* and *O. ramosa,* but not *O. Cumana.*

These mycoherbicides strongly affected broomrapes growing on tomato roots, killing 50-100% of the broomrape tubercles; these pathogens had no visible effect on any of these commonly cultivated vegetables (melons, potatoes, tomatoes, peppers, carrots, and celery), grain legumes (chickpeas) and sunflower (Amsellem & Gressel, 2001a). Mechanisms of biocontrol in *Fusarium* spp. often involve phytotoxins (low molecular weight compounds from the plant metabolism, which are only synthesized upon the attack by pathogens and which enable the plant to defend it self against the attaching pathogen) such as fumonisins (Abbas & Boyette, 1992), fusaric acid (Bacon et al., 1996) and protein toxins (Bailey et al., 2000) that assist in overcoming host

defenses, allowing establishment of the pathogen. Thomas et al. (1999) reported that conidial suspensions of *F. oxysporum* f.sp. *Orthoceras* colonized and infected the seeds of *O. cumana*, a species specific to sun flowers. The fungus was effective in penetrating seed testa. The cell walls of the endosperm were dissolved, cytoplasm degraded, and lipid body membranes were damaged in the infected seeds. The lipid and protein rich endosperm was presumably used by the fungus as a nutrient source.

Transgenically, enhancing auxin indole acetic acid (IAA) levels, increasing *Fusarium* spp. virulence and reducing the severity of *Orobanche* infestation on the host plants are all reported (Cohen et al., 2002). IAA control plant growth, differentiation, structural organization, and the shift from vegetative to reproductive growth (Gaudin, et al., 1994). Exogenous IAA can lead cellulase-catalyzed cleavage of hemicellulose (Gamliel & Katan, 1992), resulting in wall loosening (Taguchi et al., 1999; Vanderhoef & Dute, 1981) and membrane leakage, which stimulate water and nutrient loss (Brandle et al., 1996; Brandle & Lindow, 1998) leading to enhancing the pathogens virulence and effective suppression in the number and size of *Orobanche* shoots.

The time of pathogen application, however, is crucial to obtain a satisfactory level of control, because the pathogen must colonize the soil or host rhizosphere (Boari & Vurro, 2004).

1. 6. Objectives

The objective of this study involved:

1. Identifying the local (Baladi) plant cultivars of tomato (*Lycopersicon esculentum*), broad bean (*Vicia faba*), soybean (*Glycine max*), cowpea (*Vigna unguiculata*), chick peas (*Cicer aritinum*), common vetch (*Vicia sativa*), lentil (*Lens culinaris*), bean (*Phaseolus vulgaris*), peas (*Pisum sativum*), sunflower (*Helianthus annus*), lupine (*Lupinus termis*), wooly vetch (*Vicia villosa*), bitter vetch (*Lathyrus cicera*), and clover (*Trifolium pratense*) that are resistant / tolerant to broomrape.
2. Evaluating the effect of chemical method to control broomrape by using the herbicides, Chlorsulfuron 75% WG (Glean®), DU PONT DE NEMOURS CO., Triasulfuron 75% WG (Amber®) CTS Co., and Imazaquin 180g/L SL (Scepter®), AGAN.
3. Testing biological method to control broomrape (*O. aegeptiaca*) on tomato plants by using native *Fusarium* species isolates and the two identified bioagent strains, *F. oxysporum* strain EId (CNCM-I-1622) (Foxy) and *F. arthrosporioides* strain E4a (CNCM-I-1621) (Farth).

Chapter 2
Materials and Methods

2.1. Screening for tolerance/ resistance to broomrape

Local varieties of tomato (*L. esculentum*), broad bean (*V. faba*), soybean (*G. max*), cowpea (*V. unguiculata*), chick peas (*C. aritinum*), common vetch (*V. sativa*), lentil (*L. culinaris*), bean (*P. vulgaris*), peas (*P. sativum*), sunflower (*H. annus*), lupine (*L. termis*), wooly vetch (*V. villosa*), bitter vetch (*V. ervilia*), and clover (*T. pratense*) were screened for resistance/ tolerance to *O. aegyptiaca* in pots. Clay soil was infested with *Orobanche* seeds by mixing 40 mg of seed per 1 kg air-dried soil in a cement mixer (2500 seeds/kg). From each crop five seeds were sown, in each of the five pot (2 L) containing broomrape infested soil; five seeds in broomrape seed free soil pots were sown for control. Plants were grown in greenhouse at 25C^{o} and fertigated as necessary. After 70 days , soil was washed from roots by water and separated from parasite parts and the number of spikes above and under ground were counted. In addition, the fresh and dry weights for both roots and spikes were measured. The experimental design was completely randomized with five replicates (pots) for each crop.

2. 2. Herbicides

Three herbicides, Chlorsulfuron 75% WG (Glean®), Triasulfuron 75% WG (Amber®), and Imazaquin 180g/L SL (Scepter®), were used for evaluating their efficiency in controlling broomrape in tomato fields (Table 1). The evaluation was carried out at three conditions (on tomato plants in pots, on irrigated open tomato field and under greenhouse conditions).

Table 2.1. The herbicides used in the study

Herbicides	Common name	Producer	Composition
Chlorsulfuron	Glean® (75%WG)	Du Pont. / Wilmington, U.S.A.	(*RS*)-5-ethyl-2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2-yl)nicotinic acid.
Triasulfuron	Amber® (75%WG)	Syngenta / U.S.A.	1-[2-(2-chloroethoxy)phynyl sulfonyl]-3- (4-methoxy-6-methyl-1,3,5-triazin-2-yl)orea.
Imazaquin	Scepter® (180/L SL)	Agan / Israel	(RS)-2- (4-isopropyl-4- methyl-5-oxo-2-imidazolin-2-yl) quinoline-3-carboxylic acid.

2. 2. 1. Effect of herbicides on tomatoe's broomrape in pots

A prelimenary experiment was first conducted, where forty days old tomato (var. Niamey 1684) seedlings were transplanted in 4L pots. The pots were filled with *O. aegyptiaca* infested clay soil prepared by mixing 40 mg of *Orobanche* seeds per 1 kg air-dried soil in a cement mixer (2500 seeds/kg). Plants were grown in greenhouse at 25C°, and fertigated as necessary. After 60 days from transplantation and when the spikes were observed above soil level, the tomato plants were sprayed with the herbicides, Triasulfuron, Chlorsulfuron and Imazaquin each at the concentrations of 0, 10, 20, 30, 40 and 50 ($\mu g\ ml^{-1}$). According to the results of the prelimenary experiment, a new experiment was conducted, considering the lowest herbicide concentrations effective against broomrape. The experiment was repeated using the low concentrations of 0, 1, 3, and 5

$\mu g\ ml^{-1}$. The experimental design was completely randomized with five replicates per each concentration for each herbicide. Plants were sprayed with herbicides suspension (50 ml/plant) while the control treatment plants were sprayed with water; the spraying process was repeated after two weeks. The number and weight of total spikes, dead spikes, and viable spikes were recorded after 4 weeks from last spray. In addition, the tomatoe's foliage fresh and dry weight was determined. The experiment was repeated using further lower herbicide concentrations (0, 0.5, 1, 3, and 5 $\mu g\ ml^{-1}$) to confirm the lowest herbicide concentration effective against the parasite and at the same time safe for tomato plants.

2.2.2. Effect of herbicides on broomrape in tomatoe's open field

Experiments were carried out at two sites in the Dura area. The open field at the first site has naturally uniform, heavy infestation of *O. aegyptiaca*. The field was transplanted with tomato (var. Niamey 1684). The plants were grown in rows and drip irrigated (140X50 cm). The completely randomized design of the experiment included the herbicides, Triasulfuron, Chlorsulfuron and Imazaquin with three replicates (Plots) per each of the concentrations (0, 1, 3, and 5$\mu g\ ml^{-1}$) (Fig. 1.1). Each plot has 3 rows X 4 plants, and one row was dedicated as a border for each plot. After two months from transplanting and when the parasite spikes appeared above the soil surface, the tomato plants were sprayed with the herbicides by 100 ml/plant at the concentrations of 0, 1, 3, and 5$\mu g\ ml^{-1}$; the control treatment plants were sprayed with tap water (100 ml/plant). The number and weight of total spikes, dead spikes, and viable spikes were recorded after two weeks of treatment.

2.2.3. Effect of herbicides on broomrape in tomatoe's in greenhouse

The experiment of the second site, was carried out in a commercial greenhouse which has naturally uniform and heavy infestation with

Orobanche aegyptiaca. The greenhouse was transplanted with tomato (var. FA 144). The plants were grown in rows and drip irrigated (120X50 cm). The completely randomized design was used in the experiment with three replicates (Plots) per each concentration (0, 5, and 10 µg ml^{-1}) of the herbicides, Triasulfuron, Chlorsulfuron and Imazaquin (Fig. 1.2). Each plot has 3 rows X 7 plants, and one row was dedicated as a border for each plot. After 12 weeks from transplanting and when the parasite spikes uniformly appeared above the soil surface, the tomato plants were sprayed with 200 ml/plant of the above mentioned concentrations. The number and weight of total spikes, dead spikes, viable spikes of the parasite and tomato plant heights were recorded after three weeks from treatments.

Figure 2.1. The experimental layout of the irrigated open field tomato experiment at the Dura site (2005).

I*5**,R3	CK,R3 3 rowsX4 plants	C5,R3	C5,R1	CK,R1
T5,R1	C1,R1	I5,R1	T1,R3	T1,R1
	I1,R3	C1,R3	I1,R2	C1,R2
	C3,R2	T1,R2	I3,R3	T3,R1
	T3,R3	C3,R3	T3,R2	I3,R1
	I3,R2	T5,R3	C3,R1	I5,R2
	C5,R2	I1,R1	CK,R2	T5,R2

* Herbicides, T - Triasulfuron, C - Chlorsulfuron, I - Imazaquin , and CK- control .
** Herbicide concentration (µg ml^{-1}) and R, replicate.

Figure 2.1. The experimental layout for the greenhouse tomato experiment at the Dura site (2005).

I*5**, R1	I10, R2	C10, R3	C5, R1	CK, R1 (3 rows X 7 plants)
T5, R1	C10, R1	I5, R2	T10, R1	T20,R1
CK,R2	I10, R1	C5, R3	I5, R3	C20,R1
C5, R2	C20, R2	T20, R2	I10, R3	T5, R2
I20, R1	T10, R3	C20, R3	T20, R3	I20, R1
T5, R3	I20, R2	CK, R3	C10, R2	T10,R2

* Herbicides, T - Triasulfuron, C - Chlorsulfuron, I - Imazaquin , and CK- control
** Herbicide concentration ($\mu g\ ml^{-1}$) and R, replicate.

2. 3. Biological control

2. 3. 1. Isolation of broomrape pathogens

In summer 2003 and 2004 during field survey of broomrape, 135 samples of the diseased broomrape spikes were collected from 137 fields in Hebron agricultural areas. Forty four of the samples were collected from fields of naturally-infested tomato plants growing under rain fed conditions, sixty from drip irrigated fields and thirty one from eggplant, cabbage, cauliflower, salvia, sunflower and chickpeas fields (Table 2). Diseased tubercles and broomrape spikes were cut into 3-4 mm pieces, surface–sterilized by immersion in 1% sodium hypochlorite solution for 4 min, and rinsed three times with sterile distilled water. Three pieces were placed in each 90 mm plastic petri dish containing original Martin's medium (Rechcigl, 1978) supplemented with 250 mg/l chloramphenicol, the petri dishes were incubated at 25°C. Original Martin's medium contains (g/l): Difco peptone, 5; Glucose, 10; KH2PO4, 1; MgSO4.7H2O, 0.5; Difco agar, 20; Rose-bengal, 0.03; and PCNB, 0.005. Ten days later, single isolates growing as fungal hyphae out of the broomrape pieces were sub-cultured on potato dextrose agar medium amended with 250 mg/l chloramphenicol (Rechcigal, 1978). Single spore cultures for the isolates were sub cultured, and one of the growing colonies was used to inoculate several dishes. The isolates were taxonomically identified as *Fusarium* spp., (Burgess et al 1994) (Table 2).

Table 2.2. Fungul isolates collected from naturally infested *Orobanche aegyptiaca* spikes.

Isolate No.	Fungus spp.	Location	Host	Irrigation method
Fu 1	*Fusarium* spp	Beitolla	Salvia	Drip
Fu 2	*Fusarium* spp	Beitolla	Salvia	Drip
Fu 3	*Fusarium* spp	Beitolla	Sunflower	Rain fed
Fu 4	*Fusarium* spp	Beitolla	Sunflower	Rainfed
Fu 5	*Fusarium* spp	Dura	Cauliflower	Drip
Fu 6	*Fusarium* spp	Dura	Cauliflower	Drip
Fu 7	*Fusarium* spp	Dura	Cauliflower	Drip
Fu 8	*Fusarium* spp	Dura	Tomato	Drip
Fu 9	*Fusarium* spp	Dura	Tomato	Drip
Fu 10	*Fusarium* spp	Halhol	Tomato	Drip
Fu 11	*Fusarium* spp	Beit kahel	Tomato	Drip
Fu 10	*Fusarium* spp	Halhol	Tomato	Drip
Fu 11	*Fusarium* spp	Beit kahel	Tomato	Drip
Fu 12	*Fusarium* spp	Henena	Tomato	Rainfed
Fu 13	*Fusarium* spp	Henena	Tomato	Rainfed
Fu 14	*Fusarium* spp	Henena	Tomato	Rainfed
Fu 15	*Fusarium* spp	Henena	Tomato	Rainfed
Fu 16	*Fusarium* spp	Wad-alshajna	Tomato	Rainfed
Fu 17	*Fusarium* spp	Wad-alshajna	Tomato	Rainfed
Fu 18	*Fusarium* spp	Wad-alshajna	Tomato	Rainfed
Fu 19	*Fusarium* spp	Wad-alshajna	Tomato	Rainfed
Fu 20	*Fusarium* spp	Wad-alshajna	Tomato	Drip
Fu 21	*Fusarium* spp	Wad-alshajna	Tomato	Drip
Fu 22	*Fusarium* spp	Wad-alshajna	Tomato	Drip
Fu 23	*Fusarium* spp	Wad-alshajna	Tomato	Rainfed
Fu 24	*Fusarium* spp	Wad-alshajna	Tomato	Drip
Fu 25	*Fusarium* spp	Wad-alshajna	Tomato	Drip
Fu 26	*Fusarium* spp	Wad-alshajna	Tomato	Drip
Fu 27	*Fusarium* spp	Dura	Cabbage	Drip
Fu 28	*Fusarium* spp	Dura	Cabbage	Drip
Fu 29	*Fusarium* spp	Halhol	Tomato	Rainfed
Fu 30	*Fusarium* spp	Halhol	Tomato	Rainfed

Continue Table 2.2.

Isolate No.	Fungus spp.	Location	Host	Irrigation method
Fu 31	*Fusarium* spp	Trama	Tomato	Rainfed
Fu 32	*Fusarium* spp	Halhol	Tomato	Rainfed
Fu 32	*Fusarium* spp	Haska	Tomato	Rainfed
Fu 33	*Fusarium* spp	Haska	Tomato	Rainfed
Fu 34	*Fusarium* spp	Abda	Eggplant	Drip
Fu 35	*Fusarium* spp	Khursa	Tomato	Rainfed
Fu 36	*Fusarium* spp	Wad-abda	Tomato	Rainfed
Fu 37	*Fusarium* spp	Khursa	Tomato	Rainfed
Fu 38	*Fusarium* spp	Khursa	Tomato	Rainfed
Fu 39	*Fusarium* spp	Khursa	Tomato	Rainfed
Fu 40	*Fusarium* spp	Trama	Eggplant	Drip
Fu 41	*Fusarium* spp	Trama	Eggplant	Drip
Fu 42	*Fusarium* spp	Trama	Tomato	Drip
Fu 43	*Fusarium* spp	Trama	Tomato	Drip
Fu 43	*Fusarium* spp	Almajour	Tomato	Rainfed
Fu 44	*Fusarium* spp	Almajour	Tomato	Rainfed
Fu 45	*Fusarium* spp	Almajour	Tomato	Rainfed
Fu 46	*Fusarium* spp	Almajour	Tomato	Rainfed
Fu 47	*Fusarium* spp	Henena	Tomato	Rainfed
Fu 48	*Fusarium* spp	Almajour	Tomato	Drip
Fu 49	*Fusarium* spp	Almajour	Tomato	Drip
Fu 50	*Fusarium* spp	Almajour	Tomato	Drip
Fu 51	*Fusarium* spp	Almajour	Tomato	Drip
Fu 52	*Fusarium* spp	Almajour	Tomato	Rainfed
Fu 53	*Fusarium* spp	Khursa	Tomato	Drip
Fu 54	*Fusarium* spp	Khursa	Tomato	Drip
Fu 55	*Fusarium* spp	Almajour	Tomato	Rainfed
Fu 56	*Fusarium* spp	Almajour	Tomato	Rainfed
Fu 57	*Fusarium* spp	Almajour	Tomato	Rainfed
Fu 58	*Fusarium* spp	Almajour	Tomato	Rainfed
Fu 59	*Fusarium* spp	Almajour	Tomato	Rainfed
Fu 60	*Fusarium* spp	Almajour	Tomato	Rainfed

Continue Table 2.2.

Isolate No.	Fungus spp.	Location	Host	Irrigation method
Fu 61	*Fusarium* spp	Saer-alduwareh	Tomato	Drip
Fu 62	*Fusarium* spp	Saer-alduwareh	Tomato	Drip
Fu 63	*Fusarium* spp	Saer-alduwareh	Tomato	Drip
Fu 64	*Fusarium* spp	Saer-alduwareh	Tomato	Drip
Fu 65	*Fusarium* spp	Saer- alras	Tomato	Drip
Fu 66	*Fusarium* spp	Saer-alras	Tomato	Drip
Fu 67	*Fusarium* spp	Saer-duwareh	Tomato	Drip
Fu 68	*Fusarium* spp	Saer-alduwareh	Tomato	Drip
Fu 69	*Fusarium* spp	Saer-wad chames	Tomato	Drip
Fu 69	*Fusarium spp*	Saer-wad chames	Tomato	Drip
Fu 70	*Fusarium* spp	Saer-wad chames	Tomato	Drip
Fu 71	*Fusarium* spp	Saer-ras alarouth	Tomato	Drip
Fu 72	*Fusarium* spp	Saer-ras alarouth	Tomato	Drip
Fu 73	*Fusarium* spp	Saer-ras alarouth	Tomato	Drip
Fu 74	*Fusarium* spp	Saer-ras alarouth	Eggplant	Drip
Fu 75	*Fusarium* spp	Saer-ras alarouth	Eggplant	Drip
Fu 76	*Fusarium* spp	Dura	Tomato	Rainfed
Fu 77	*Fusarium* spp	Dura-abda	Tomato	Rainfed
Fu 78	*Fusarium* spp	Dura-abda	Tomato	Rainfed
Fu 79	*Fusarium* spp	Dura-albiareh	Tomato	Drip
Fu 80	*Fusarium* spp	Halhul-wad alshunar	Tomato	Rainfed
Fu 81	*Fusarium* spp	Halhul-althurwa	Tomato	Rainfed
Fu 82	*Fusarium* spp	Halhul-althurwa	Tomato	Rainfed
Fu 83	*Fusarium* spp	Halhul-althurwa	Tomato	Rainfed
Fu 84	*Fusarium* spp	Halhul-althurwa	Tomato	Rainfed
Fu 85	*Fusarium* spp	Dura-albiareh	Eggplant	Drip
Fu 86	*Fusarium* spp	Halhul-wad alshunar	Eggplant	Drip
Fu 87	*Fusarium* spp	Dura-albiareh	Cabbage	Drip
Fu 88	*Fusarium* spp	Dura-ein emran	Eggplant	Drip
Fu 89	*Fusarium* spp	Dura-albiareh	Cabbage	Drip
Fu 90	*Fusarium* spp	Dura-ein emran	Tomato	Drip

Continue Table 2.2.

Isolate No.	Fungus spp.	Location	Host	Irrigation method
Fu 91	*Fusarium* spp	Halhul-wad alshunar	Cauliflower	Drip
Fu 92	*Fusarium* spp	Halhul-wad alshunar	Cauliflower	Drip
Fu 93	*Fusarium* spp	Halhul-wad alshunar	Cauliflower	Drip
Fu 94	*Fusarium* spp	Halhul-wad alshunar	Tomato	Drip
Fu 95	*Fusarium* spp	Halhul-wad alshunar	Tomato	Drip
Fu 96	*Fusarium* spp	Dura-albiareh	Tomato	Drip
Fu 97	*Fusarium* spp	Halhul-wad alshunar	Tomato	Drip
Fu 98	*Fusarium* spp	Halhul-wad alshunar	Tomato	Drip
Fu 99	*Fusarium* spp	Halhul-althurwa	Tomato	Rainfed
Fu 100	*Fusarium* spp	Halhul-kirbet isha	Tomato+Chickpeas	Drip
Fu 101	*Fusarium* spp	Halhul-kirbet isha	Tomato+Chickpeas	Drip
Fu 102	*Fusarium* spp	Halhul-althurwa	Tomato	Drip
Fu 103	*Fusarium* spp	Halhul-althurwa	Tomato	Rainfed
Fu 104	*Fusarium* spp	Halhul-althurwa	Tomato	Rainfed
Fu 105	*Fusarium* spp	Halhul	Tomato	Drip
Fu 106	*Fusarium* spp	Halhul	Tomato	Drip
Fu 107	*Fusarium* spp	Halhul	Tomato	Drip
Fu 108	*Fusarium* spp	Jenein-alzababdeh	Tobacco	Rainfed
Fu 109	*Fusarium* spp	Jenein-alzababdeh	Tobacco	Rainfed
Fu 110	*Fusarium* spp	Hebron- dwerban	Tomato	Drip
Fu 111	*Fusarium* spp	Hebron- dwerban	Tomato	Drip
Fu 112	*Fusarium* spp	Halhul-kirbet isha	Tomato	Drip
Fu 113	*Fusarium* spp	Halhul-zaboud	Tomato	Rainfed
Fu 114	*Fusarium* spp	Halhul-zaboud	Tomato	Rainfed
Fu 115	*Fusarium* spp	Halhul-haska	Tomato	Drip
Fu 116	*Fusarium* spp	Halhul-haska	Tomato	Drip
Fu 117	*Fusarium* spp	Halhul-haska	Tomato	Drip
Fu 118	*Fusarium* spp	Halhul-arnaba	Tomato	Drip
Fu 119	*Fusarium* spp	Beit-omar	Tomato	Drip
Fu 120	*Fusarium* spp	Beit-omar	Tomato	Drip
Fu 121	*Fusarium* spp	Beit-omar	Tomato	Drip
Fu 122	*Fusarium* spp	Halhul-arnaba	Tomato	Drip
Fu 123	*Fusarium* spp	Halhul-arnaba	Tomato	Drip
Fu 124	*Fusarium* spp	Hebron- azoun	Tomato	Drip
Fu 125	*Fusarium* spp	Hebron- azoun	Tomato	Drip

2. 3. 2. Pathogenicity bioassays

According to preliminary results of mycelium growth rate and conidial production, the *Fusarium* isolates which produced the highest mycelium weight and conidial number were selected (Fu 8-04, Fu 12, Fu 12-04, Fu 20, Fu 25, Fu 52, Fu 59, Fu 87, Fu 100, Fu 116, Fu 2, Fu 2-4, Fu 4/2, Fu 5, Fu 5-04, Fu 6, Fu 14, Fu 16, Fu 23, Fu 30, Fu 45, Fu 53, Fu 75, Fu 115, Fu 119, Fu 123), in addition to the two bioagent strains to broomrape, *F. oxysporum* strain EId (CNCM-I-1622) and *F. arthrosporioides* strain E4a (CNCM-I-1621) which were isolated previously from a melon field heavily infested with broomrape (Gressel, 2000) and deposited with the Collection Nationale de Cultures de Microorganisms (CNCM), Institute Pasteur, Paris. Both strains were reported to be pathogenic to *O. aegyptiaca* (Amsellem et al., 2001), and non-pathogenic to any of commonly cultivated vegetables (melons, potatoes, tomatoes, peppers, carrots, and celery), grain legumes (chickpeas) and sunflowers. Fungal biomass was prepared by gently transferring 5mm mycelium plug from two-weeks old fungal colonies of *Fusarium* isolates to each of three autoclaved (200 ml flasks) containing 100 ml potato dextrose broth amended with 250 mg/l Chloramphenicol . The flasks were shaken for 14 days at 25°C and 14h photoperiod/ day. Spores from cultures were washed out free of mycelia through Miracloth® (Calbiochem), centrifuged (6000 rpm for 20 min), and the supernatant was decanted. The spores were resuspended in sterile water, centrifuged again, decanted, and resuspended as above. Spore concentrations were determined with haemocytometer. The mycelia pads were washed three times with sterilized, deionized and distilled water, and weighed to obtain fresh weight (FW). The inoculum of each isolate was conidial-mycelium suspension (CMS) prepared by incorporating both fungal mycelium and fungal conidia which were separately suspended in DW. The mixture of 5 g fungal mycelium

with $5*10^8$ conidia was then suspended in 15 ml 1M sucrose. The suspension was homogenized by Ultra-Turrax® homogenizer for 60 seconds at the speed of (5000 rpm). The experiment was conducted in completely randomized design and included four replicates (4 plants in 4 pots), per treatment. Treatments included growing tomato plants in broomrape's free soil (To), in broomrape's infested soil (T1) at the inoculum concentration of 40 mg broomrape seeds/ kg soil and in broomrape's infested soil along with one of the *Fusarium* isolates (T 2). The inoculum of each *Fusarium* isolate was calibrated to have the concentration of 10^8 conidia and 0.5 mg mycelium per 1 g soil. Four - week old tomato plants growing in 4 L pots were irrigated with 50 ml of each isolate inoculum suspension (CMS) followed by 100 ml of water for the T2 treatment.

The plants in To and T1 were irrigated with 150 ml water for each pot. Plants were then incubated under greenhouse at 25°C and fertigated as necessary. After 8 weeks, the number and weight of total spikes, dead spikes, and viable spikes were recorded. In addition, the tomato plant's disease rate, fresh and dry weight were recorded. The pathogenicity bioassays of the *Fusarium* isolates were carried out in two patches; the first patch included the *Fusarium* isolates: Foxy, Farth, Fu 8-04, Fu 12, Fu 12-04, Fu 20, Fu 25, Fu 52, Fu 59, Fu 87, Fu 100, Fu 116, while the second patch included the *Fusarium* isolates: Foxy, Farth, Fu 2, Fu 2-4, Fu 4/2, Fu 5, Fu 5-04, Fu 6, Fu 14, Fu 16, Fu 23, Fu 30, Fu 45, Fu 53, Fu 75, Fu 115, Fu 119, Fu 123. Another experiment of the most promising *Fusarium* isolates (Foxy, Farth, Fu 12-04, Fu 20, Fu 25, Fu 30, Fu 45, Fu 52, Fu 59, Fu 87, Fu 112, and Fu 119) were repeated and the same previous parameters were measured and recorded.

2.3.3. Identification of *Fusarium* isolates

The most promising *Fusarium* isolates (Fu 30, Fu 119, Fu 59, Fu 20, Fu 52, Fu 12-04, Fu 87 and Fu 25) were identifed according to Burgess et al., 1994. The technique started by preparing a suspension of conidia by taking a disc of one week old *Fusarium* isolate culture in 10 ml sterile distilled water in a test tube; the suspension was vortexed for 60 seconds at the speed of (5000 rpm). The conidia were separated by filtering the suspension through Miracloth. Conidial suspension (200) µm were seeded over the surface of 9 mm PDA plates where three replicates were used for each isolate. The plates were incubated at 25ºC; after 3 days, a single germinated conidium was removed using a sterile knife and transfered to a new PDA plate. Inoculated plates were incubated at 25ºC and after three days the plates were exposed to natural light at 25ºC. After one week, conidial suspensions were prepared and three plates were inculated centrally with single conidia for each isolate. The plates were incubated at 25ºC and colony diameters were measered after three days. These plates were then transferred and incubated under natural light for three weeks. The following parameters were evaluated and studied for taxonomic differentiation:

1- Mycelium diameter.
2- Pigmentation and color produced by the isolate on PDA medium.
3- Shape of micro and macroconidia.
4- Presence or abcence of clamydospore

2.3.4. Statistical Analysis

The data of all experiments were analysed statistically by using Fisher LSD test, using SigmaStat® Programe 1997.

Chapter 3

Results

3. 1. Screening for tolerance/ resistance to broomrape

The results indicated that the varieties of soybean, cowpea, bean and pea tested were immune to *O. aegyptiaca* since seeds of the broomrape have not germinated. Wooly vetch, bitter vetch, lupine and clover were resistant. Tomato, broad bean, chickpea, sunflower, common vetch and lentils were susceptible to *O. aegyptiaca*; seeds of the parasite germinated, attached to the host roots and established the spikes over the soil. Broomrape (*O. aegyptiaca*) produced the highest numbers of spikes on broadbean (14.5), chickpea (14), and tomato (13.3) plants, respectively (Fig. 3.1) but with no significant differences among them at $p \leq 0.05$ (Table 3.1). Other crops induced lower numbers of spikes, sunflower (10.6), common vetch (8.8), and lentil (6.8), respectively (Fig. 3.1). Furthermore, *O. aegyptiaca* recorded the highest spikes weights on tomato (2.1), chickpea (1.8), and common vetch (1.7), respectively but with no significant differences among them at $p \leq 0.05$ (Table 3.1) . Other crops induced lower spikes weights, sunflower (1.5), lentil (1.4), and broad bean (1.3), respectively (Fig. 3.1 & 3.2). The effect of broomrape on the fresh and dry weight of the hosts depends on its susceptibility/tolerance or resistance. The reductions in fresh weight of susceptible crops compared to the control were 65.8%, 48.6%, 83.1%, 58.6%, 55.8%, and 63.1 for tomato, broad bean, chickpea, common vetch, lentil, and sunflower, respectively (Fig. 3.3 and Table 3.1). The reductions in dry weight were 76.4%, 48.6%, 81.8%, 70.5%, 55.6% and 63.1, respectively. On the other hand, soybean, cowpea, bean, pea, wooly vetch, bitter vetch, lupine and clover showed no significant difference compared to the control in respect to fresh and dry weights (Fig. 3.3 & Table 3.1).

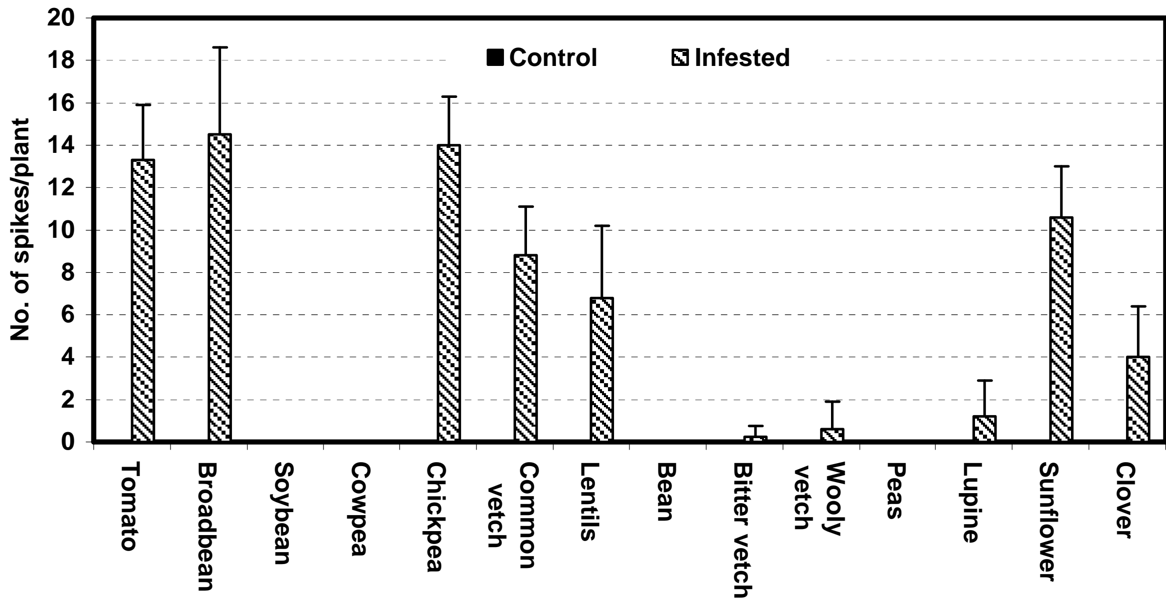


Figure3.1. The number of broomrape spikes attached to the roots of various plants.

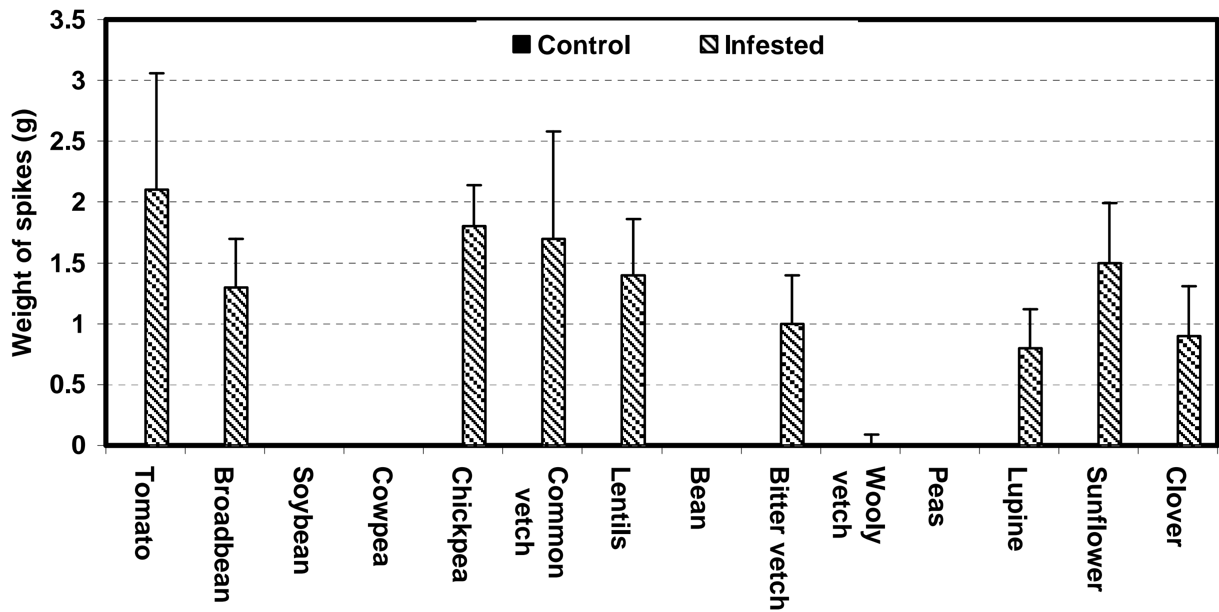


Figure 3.2. The broomrape spikes weights of those attached to the roots of various plants.

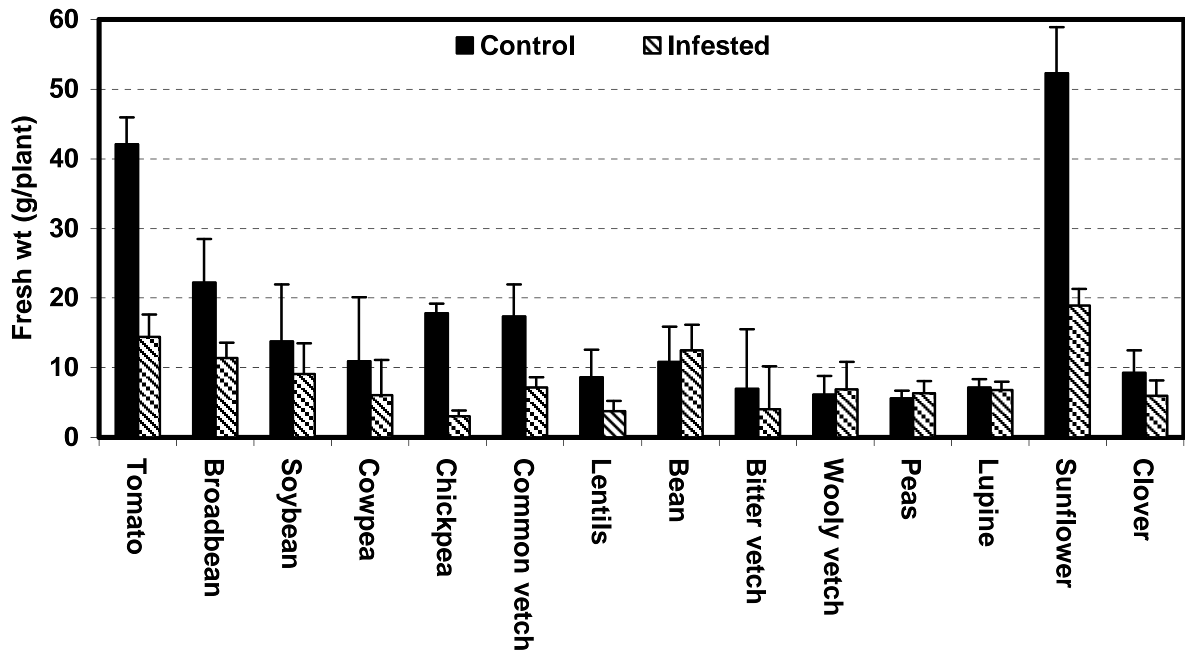


Figure 3.3. Effect of broomrape's infestation on plant's fresh weight.

Table 3.1. Numbers and weights of broomrape spikes attached to the roots of various plants and the effect of broomrape's infestation on fresh and dry weights of plants.

	Broomrape / plant				Plant			
	Spikes No.		Spikes weight (g)		Fresh weight (g/plant)		Dry weight (g/plant)	
Crop	Control	Infested	Control	Infested	Control	Infested	Control	Infested
Tomato	0 d	13.3** a	0 d	2.1** a	42.1	14.4*	8.9	2.1*
Broad bean	0 d	14.5 a	0 d	1.3 b	22.2	11.4*	3.7	1.9 *
Soybean	0 d	0 d	0 d	0 d	13.8	9.1	5.6	3.1
Cowpea	0 d	0 d	0 d	0 d	10.9	6.1	4.6	2.7
Chickpea	0 d	14 a	0 d	1.8 a	17.8	3*	4.4	0.8 *
Common vetch	0 d	8.8 b e	0 d	1.7 a	17.4	7.2*	4.4	1.3 *
Bitter vetch	0 d	0.25d	0d	1.0 C	7	4	3.1	3
Lentils	0 d	6.8 c e	0 d	1.4 b	8.6	3.8*	1.8	0.8*
bean	0 d	0 d	0 d	0 d	10.8	12.5	2.4	3.4
Wooly vetch	0 d	0.6 d	0 d	0.03 d	6.2	6.9	1.4	1.3
Peas	0 d	0 d	0 d	0 d	5.6	6.3	1.9	2.5
Lupine	0 d	1 d	0 d	0.2 d	7.2	6.8	2.5	1*
Sunflower	0 d	10.6 be	0 d	1.5 b	52.1	18.9*	5.2	4*
Clover	0 d	4 c	0 d	0.9 c	9.3	6	1.6	1.1

* There was significant difference compared to the control of the same species ($p \leq 0.05$).
** Means followed by the same letters within column are not significantly different ($p \leq 0.05$).

Figure 3.4. Effect of broomrape on growth of chickpea (A) and common vetch (B). (Left: control, Right: infested).

Figure 3.5. Effect of broomrape on growth of lentil (Left: control, Right: infested).

3. 2. Herbicides

Preliminary results showed that the herbicides, chlorsulfuron, triasulfuron and imazaquin were effective against broomrape of tomato plants at the concentrations (≤ 10 *ppm* a.i; 1 g/dunum) used as a foliar spray**.** In addition, herbicides at these concentrations had no effect on the tomato plants. However tomato plant's fresh weights were higher when plants were treated with herbicides before the flowering stage compared to treatment after the flowering stage (Fig.3.6). The three herbicides controlled broomrape of tomato plants growing in pots, under irrigated open field and greenhouse conditions.

The three herbicides significantly ($p \leq 0.05$) controlled broomrape of tomato plants grown in pots at the concentrations (0.5- 5 *ppm*; 0.05 g/dunum). Triasulfuron increased the dead spikes (%) over the control by 79, 77, 84 and 84; Chlorsulfuron by 59, 51, 84 and 84 and Imazaquin by 52, 59, 66 and 84 at the concentrations 0.5, 1, 3 and 5 *ppm* (0.05, 0.1, 0.3 and 0.5 g/dunum) (Fig.3.7 & Table 3.2), respectively.

Herbicides significantly ($p \leq 0.05$) increased the dead spikes (%) under irrigated open field tomato over the control (Fig. 3.8 & Table 3.3); Triasulfuron increased the dead spikes (%) by 10.5, 13.5, and 26.6; Chlorsulfuron by 13.6, 20.1 and 29.1 and Imazaquin by 13.1, 22.2, and 28.9 at the concentrations 1, 3, and 5 *ppm* (0.1, 0.3 and 0.5g/dunum), respectively, over the control. In addition, the three herbicides controlled broomrape of tomato plants under greenhouse conditions; Triasulfuron, Chlorsulfuron, and Imazaquin increased the dead spikes (%) by 30 and 51; 60 and 68; 30 and 61 at the concentrations 5 and 10 *ppm* (0.5 and 1.0 g/dunum), (Fig. 3.9 & Table 3.4), respectively.

In a nutshell, results revealed that the tested herbicides can be used effectively in the control of tomato broomrape in the open field, in pots, and greenhouse at the concentrations 3-5 *ppm* as a foliar spray without visible effect

on the plants; there were high correlations between dead spikes and herbicide concentrations.

In respect to the effect of herbicides on plant's growth, the three herbicides have not significantly affected fresh weights of tomatoes grown in pots (Fig.3.10A). Triasulfuron and Chlorsulfuron, however, was noted to slightly increase the tomato root's fresh weight compared to the control (Fig.3.10B). Furthermore, the herbicides Triasulfuron and Imazaquin did not affect the tomato plant growth under greenhouse condition; Chlorsulfuron reduced plant's growth (height by 8% and the leaves curled at the concentrations above 5 *ppm*) (Fig. 3.11 & Table 3.5).

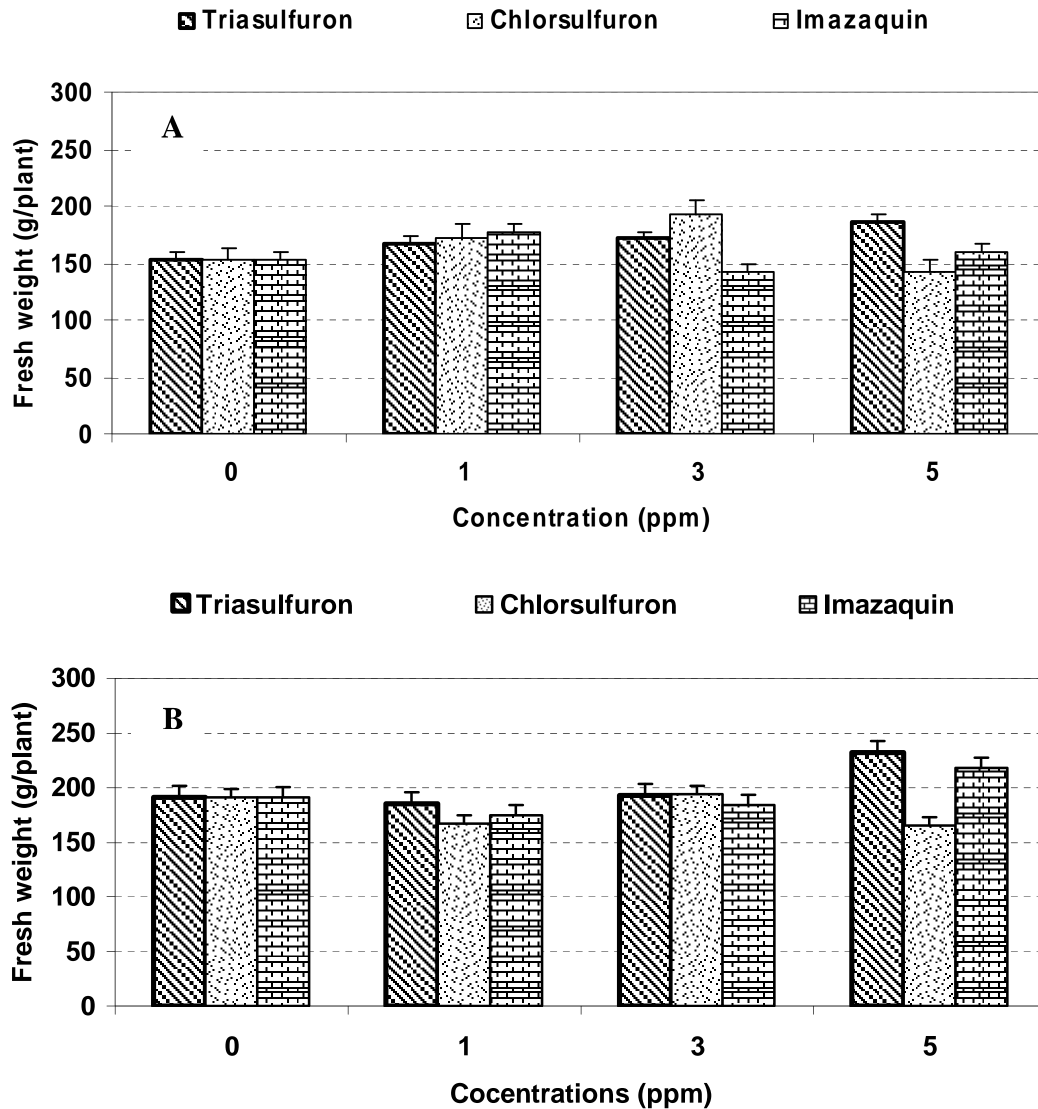


Figure 3.6. Effect of different concentrations of herbicides on fresh weights of tomato plants grown in pots treated before (A) and after (B) the flowering stage.

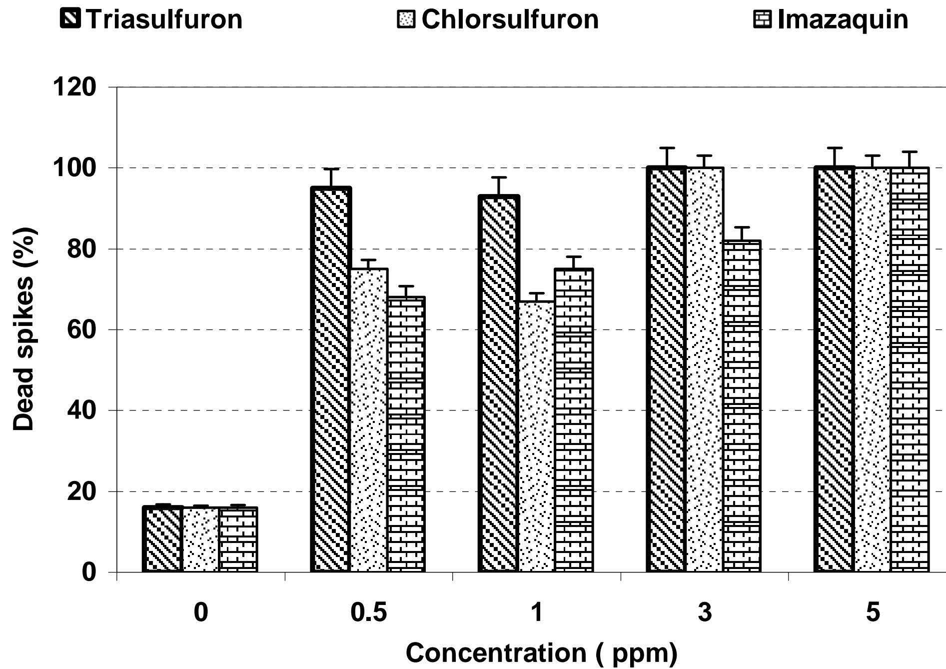


Figure 3.7. Effect of different concentrations of herbicides on broomrape of tomato plants growing in pots.

Table 3.2. Effect of different concentrations of herbicides on broomrape of tomato plants growing in pots.

Herbicides	Concentration (ppm)				
	0	0.5	1	3	5
Triasulfuron	16* c	95 a	93 a	100 a	100 a
Chlorsulfuron	16 c	75 b	67 b	100 a	100 a
Imazaquin	16 c	68 b	75 b	82 b	100 a

* Data represent broomrape's dead spikes percentages.

*Means followed by the same letter in a column or row are not significantly different according to Fisher LSD test, (LSD =2.77), ($P \leq 0.05$).

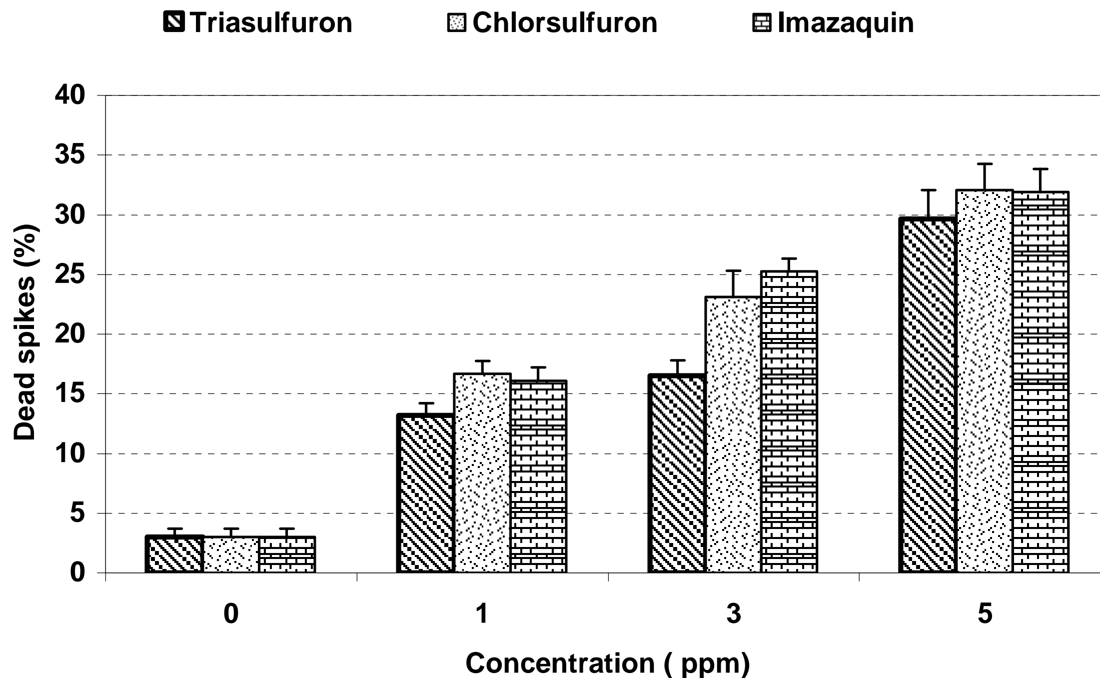


Figure 3.8. Effect of different concentrations of herbicides on broomrape of tomato plants growing under irrigated open field.

Table 3.3. Effect of different concentrations of herbicides on broomrape of tomato plants growing in irrigated open field.

Herbicides	Concentration (ppm)			
	0	1	3	5
Triasulfuron	3* d	13.5 c	16.5 c	29.6 a
Chlorsulfuron	3 d	16.6 c	23.1 b	32.1 a
Imazaquin	3 d	16.1 c	25.2 b	31.9 a

* Data represent broomrape's dead spikes percentages.

*Means followed by the same letter in a column or row are not significantly different according to Fisher LSD test, (LSD =2.77), ($P \leq 0.05$).

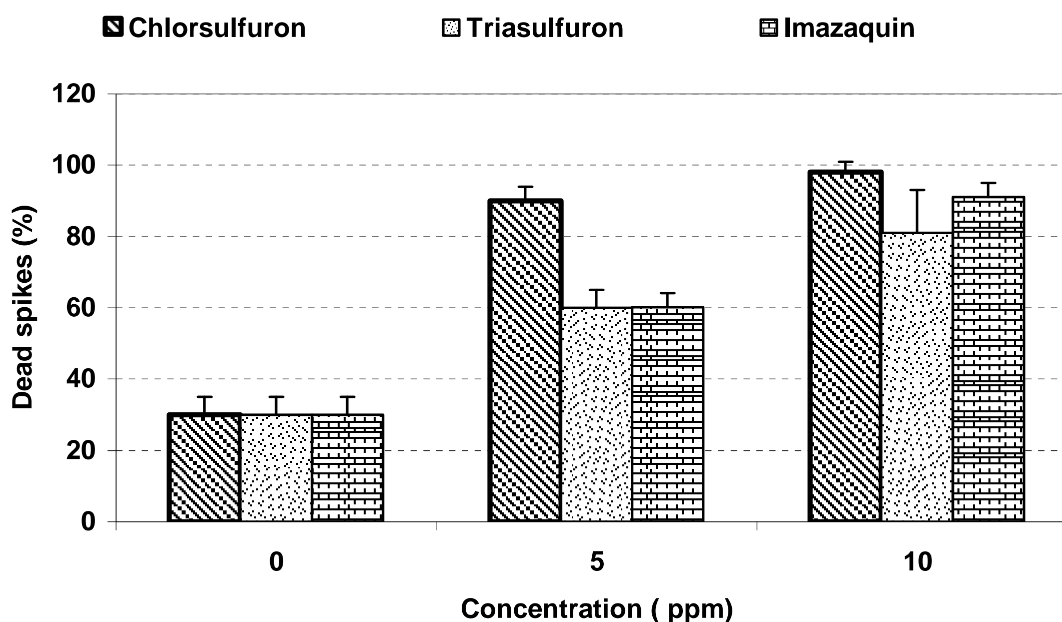


Figure 3.9. Effect of different concentrations of herbicides on broomrape of tomato plants growing under greenhouse conditions.

Table 3.4. Effect of different concentrations of herbicides on broomrape of tomato plants growing under greenhouse conditions.

Herbicides	Concentration (ppm)		
	0	5	10
Triasulfuron	30* d	60 c	81 b
Chlorsulfuron	30 d	90 a	98 a
Imazaquin	30 d	60 c	91 a

* Data represent broomrape's dead spikes percentages.

*Means followed by the same letter in a column or row are not significantly different according to Fisher LSD test, (LSD = 10), ($P \leq 0.05$).

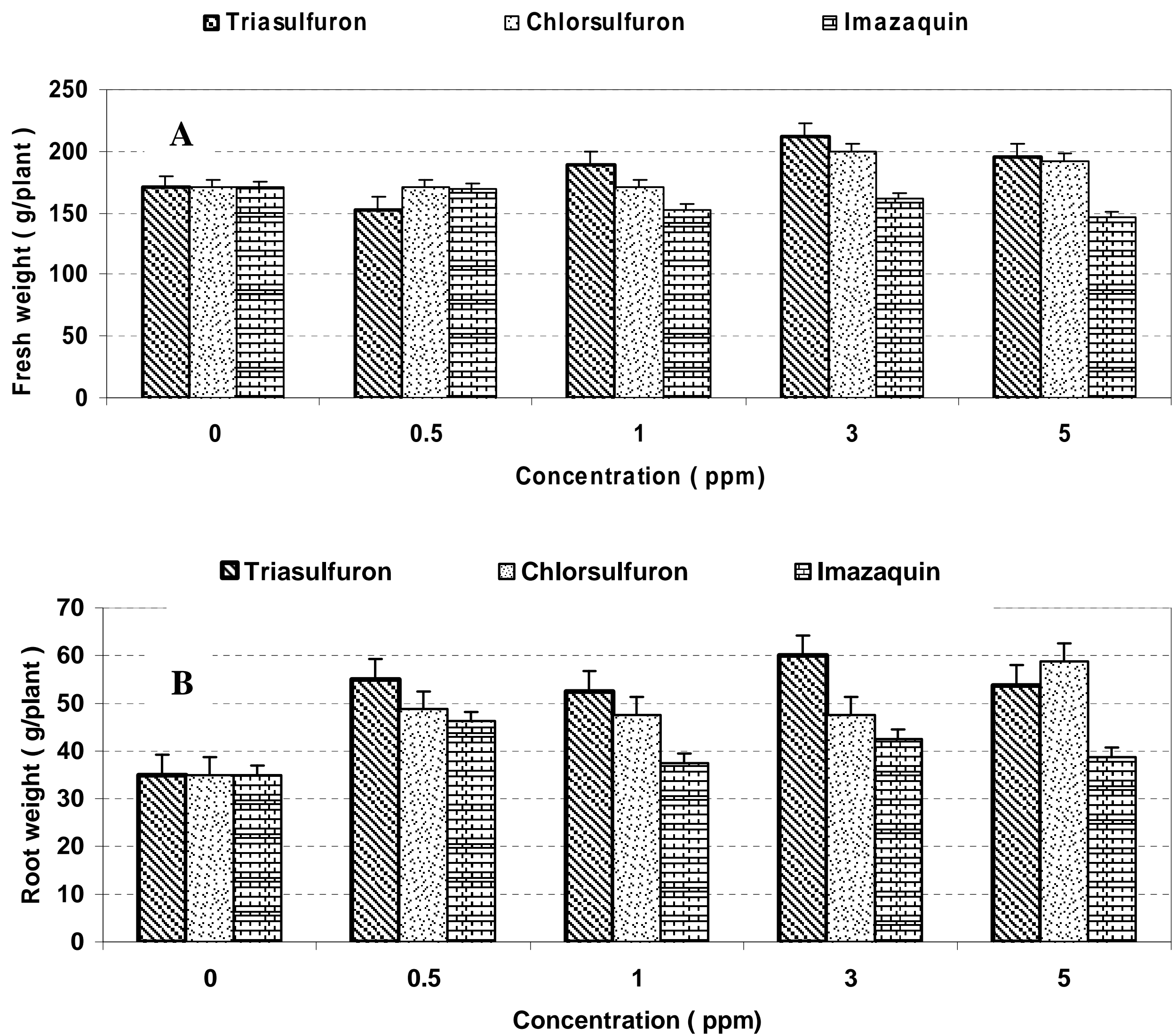


Figure 3.10. Effect Effect of different concentrations of herbicides on foliage fresh weights (A) and root's fresh weight (B) of tomato plants grown in pots.

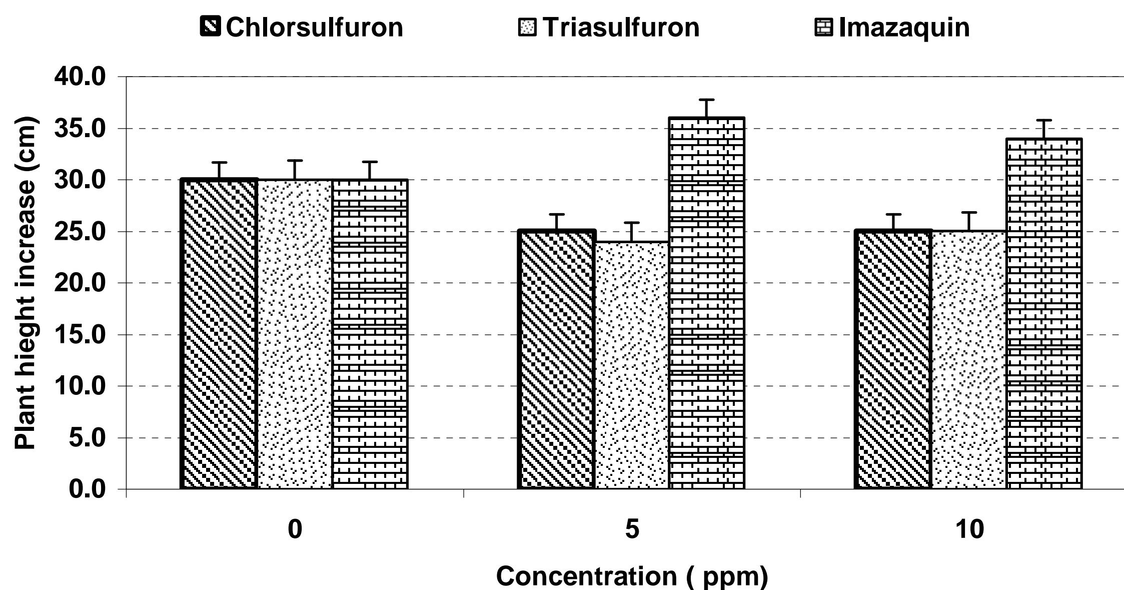


Figure 3.11. Effect of different concentrations of herbicides on plant heights of tomatoes grown in greenhouse.

Table 3.5. Effect of different concentrations of herbicides on plant heights of tomatoes grown in greenhouse.

	Concentration (ppm)		
Herbicides	0	5	10
Triasulfuron	30* a	25a	25 a
Chlorsulfuron	30 a	24 a	25 a
Imazaquin	30 a	36 a	34 a

* Data represent tomato plant heights in cm.

*Means followed by the same letter in a column or row are not significantly different according to Fisher LSD test ($P \leq 0.05$).

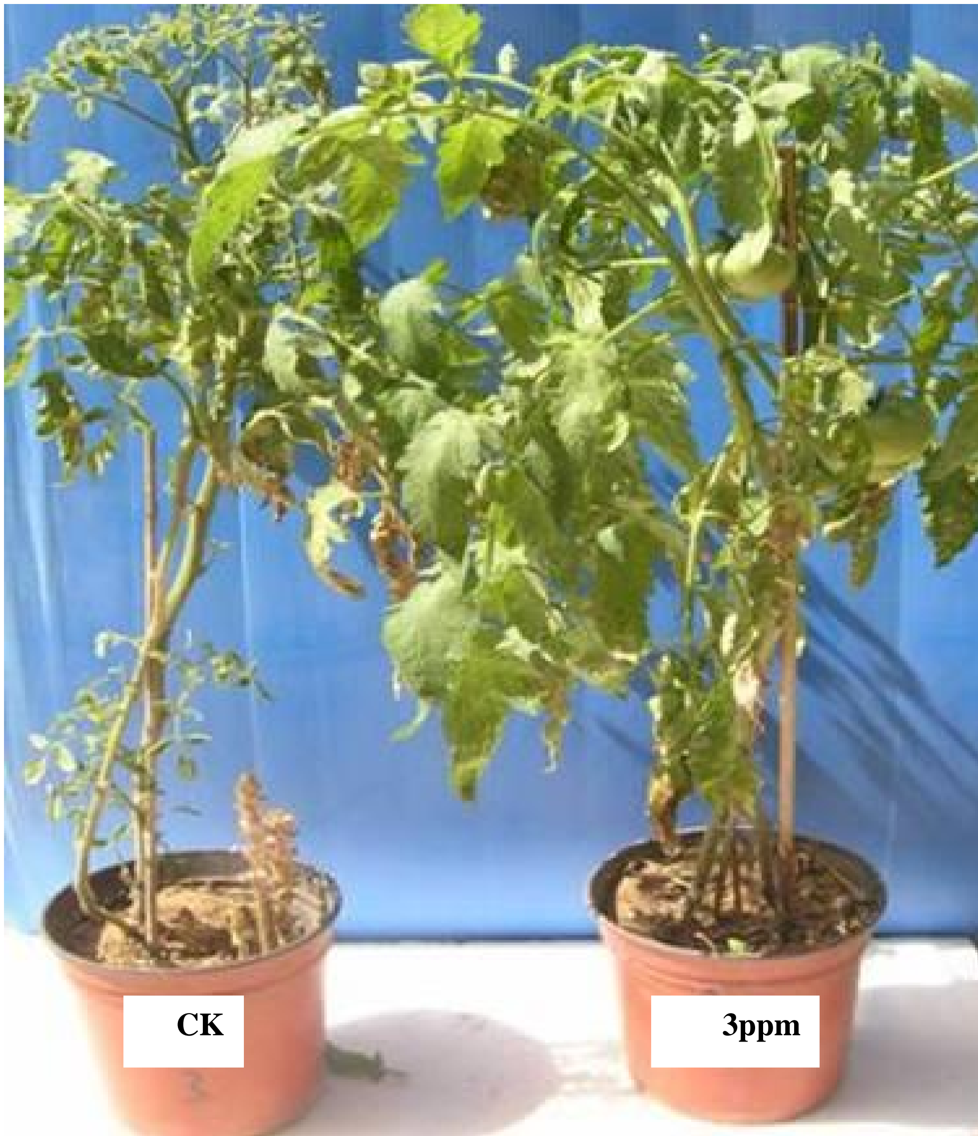


Figure 3.12. Effect of the herbicide Triasulfuron at the concentration (3ppm) on broomrape of tomato plants growing in pots.

Figure 3.13. Effect chlorsulfuron at the concentration (3ppm) on broomrape of tomato plants growing in pots .

3. 3. Biological control

3.3.1. Pathogenicity Bioassays

The results of the first preliminary experiment on *Fusarium* spp. isolates pathogenicity to broomrape of tomato plants grown in pots showed that the isolates, Fu 20, Fu 52, Fu 12-04, Fu 59, Fu 87, Fu 112, Fu 25, *Fusarium oxysporum* strain EId (CNCM-I-1622) (Foxy), *Fusarium arthrosporioides* strain E4a (CNCM-I-1621) (Farth) and Fu 100, significantly ($P \leq 0.05$) controlled broomrape of tomato plants at inoculum concentrations of 10^8 conidia & 0.5 mg mycelium g^{-1} soil by increasing broomrape's dead spikes (%) over the control by 56.9, 55.5, 51.5, 46.8, 46.73, 43.5, 42.6, 42.0, 40.8 and 37.6, respectively (Fig 3.15 & Table 3.6 Patch.1). In the second preliminary experiment, the isolates, Fu 30, Foxy, Fu 5, Fu 45 and Fu 119, significantly ($P \leq 0.05$) controlled broomrape of tomato plants at inoculum concentrations of 10^8 conidia & 0.5 mg mycelium g^{-1} soil over the control by 100, 81.6, 75, 67.3, and 59.1, respectively (Fig 3.14 & Table 3.6 Patch 2).

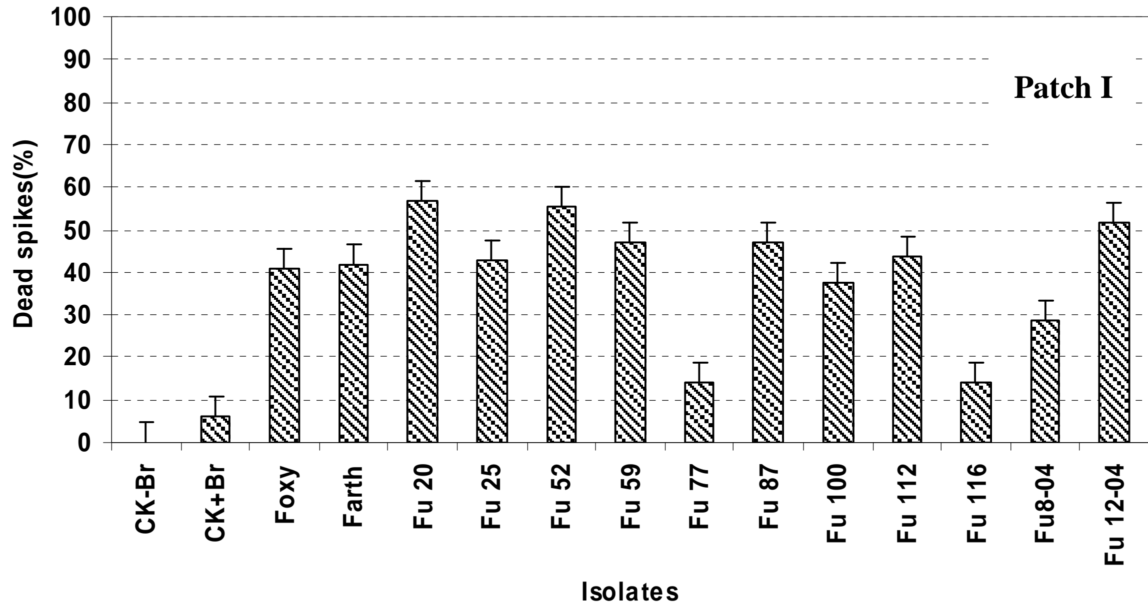
The evaluation of the most promising *Fusarium* spp. isolates was repeated twice and the results further confirmed that the isolates Fu 30, Fu 87, Fu 20, Farth, Foxy, Fu 119, Fu 59 and Fu 52, significantly increased the broomrape of tomato dead spikes (%) over to the control by 72.7, 61.5, 57.1, 51.6, 50.0, 44.3, 35.2 and 33.6, respectively (Fig 3.15 & Table 3.7).

The tested isolates have no negative effect on the tomato plants, fresh and dry weights compared to the control (CK + broomrape). Infact, the isolates (Fu 119, Fu 30, Fu 52, and Fu 87) significantly increased fresh and dry weights of tomato plants (%) compared to the control (CK + broomrape) by 236, 159, 150 and 114%, respectively (Table 3.6).

3.3.2. Identification of *Fusarium* isolates.

According to the taxonomic keys used (Burgess et al, 1994), the isolates (Fu 20, Fu 25 and Fu 119) were identified as *Fusarium solani*; the diameter of their colonies after three days of single conidial culturing were 1.8 mm, 2.4 mm and 2.1 mm, respectively; the color of the colonies growing on PDA medium were pinky for Fu 20, dark pinky for Fu 25, but red for Fu 119. The mycelium for all isolates was segmented**;** microconidia were oval for Fu 20, obovoid or oval for Fu 25, and oval for Fu 119; macroconidia dorsal side more curved than the ventral side in the three isolates, and chlamydospore**s** were found in both Fu 20 and Fu 25, but no chlamydospore**s** for Fu 119 (Table 3.8 & Figs 3.17, 18 & 3.24).

The isolates Fu 30, Fu 52, Fu 59, Fu 87 and Fu 12-04 were identified as *Fusarium oxysporum*. The diameter of their colonies after three days of single conidial culturing were 2.1, 1.0, 2.1, 2.1, and 2.2 mm, respectively. The color of the colonies growing on PDA medium were peach or pale orange for Fu 30, rose red for Fu 52, pinky dark for Fu 59, red for Fu 87 and rose red for Fu 12-04. The mycelium of all isolates was segmented; microconidia were obovoid with truncate base for Fu 30, oval for Fu 52, obovoid with truncate base for Fu 59, but obovoid for Fu 87 and Fu 12-04. Fu 30 and Fu 87 macroconidia were slender straight while Fu 52, Fu 59, and Fu 12-04 macroconidia dorsal side was more curved than the ventral side. Chlamydospore was found in Fu 52, Fu 59, and Fu 12-04, but no chlamydospores were found in Fu 30 (Table 3.8 & Figs 3.19, 3.20, 3.21, 3.22 & 3.23).



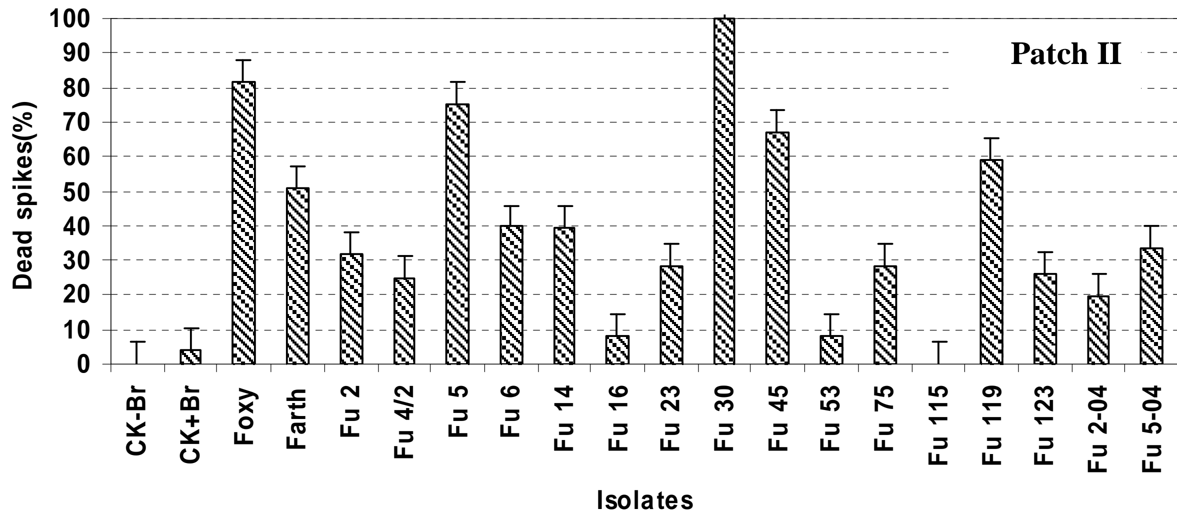


Figure 3.14. Effect of *Fusarium* spp. isolates on broomrape of tomato plants grown in pots.

Table 3.6. Effect of *Fusarium* spp. isolates on the number and weight of broomrape spikes, and tomato fresh and dry weight.

Isolate	Dead spikes (%)	Dead spikes Weight	Plant fresh weight	Plant dry weight
First experiment				
CK+Br	1.3* b	0.0 b	55 c	8.25 c
CK-Br	0.0 b	0.0 b	149 a	22.35 a
Fu 20	56.9 a	37 a	61 c	9.15 c
Fu 52	55.5 a	28 a	105 b	15.75 b
Fu 12-04	51.5 a	34 a	79 c	11.85 c
Fu 59	46.8 a	41 a	89 b	13.35 b
Fu 87	46.73 a	34 a	118 a	17.7 a
Fu 112	43.5 a	25 a	84 c	12.6 c
Fu 25	42.6 a	26 a	63 c	9.45 c
Farth	42.0 a	16 a	87 b	13.05 b
Foxy	40.8 a	34.4 a	81 c	12.15 c
Fu 100	37.6 a	26 a	62 c	9.3 c
Fu 8-04	28.8 b	22 a	94 b	14.1 b
Fu 116	14.2 b	16 b	79 c	11.8 c
Fu 77	13.99 b	5.4 b	62 c	9.3 c
Second experiment				
CK+Br	0.0* b	0.0 b	112 c	19.04 c
CK-Br	0.0 b	0 b	238 b	40.46 b
Fu 30	100.0 a	48 a	291 a	49.47 a
Foxy	81.6 a	76 a	259 b	44.03 b
Fu 5	75.0 a	13 b	300 a	51 a
Fu 45	67.3 a	39 b	258 b	43.86 b
Fu 119	59.1 a	20 b	377 a	64.1 a
Farth	51.0 b	0 b	232 b	39.44 b
Fu 6	39.6 b	27 b	266 b	45.22 b
Fu 14	39.1 b	68 a	115 c	19.55 c
Fu 5-04	33.3 b	7.1 b	276 a	46.9 a
Fu 2	31.7 b	11 b	308 a	52.36 a
Fu 23	28.6 b	57 a	126 c	21.42 c
Fu 75	28.3 b	37 b	203 b	34.5 b
Fu123	26.3 b	42 a	167 b	28.39 b
Fu 4/2	24.9 b	46 a	193 b	32.8 b
Fu 2-04	19.7 b	23 b	238 b	40.46 b
Fu 53	8.3 b	6 b	281 a	47.8 a
Fu 16	7.9 b	12 b	115 c	19.55 c
Fu 115	0.00 b	0 b	214 b	36.38 b

* Data represent broomrape's dead spikes percentages.

***Means followed by the same letter in columns are not significantly different according to Fisher LSD test ($P \leq 0.05$).**

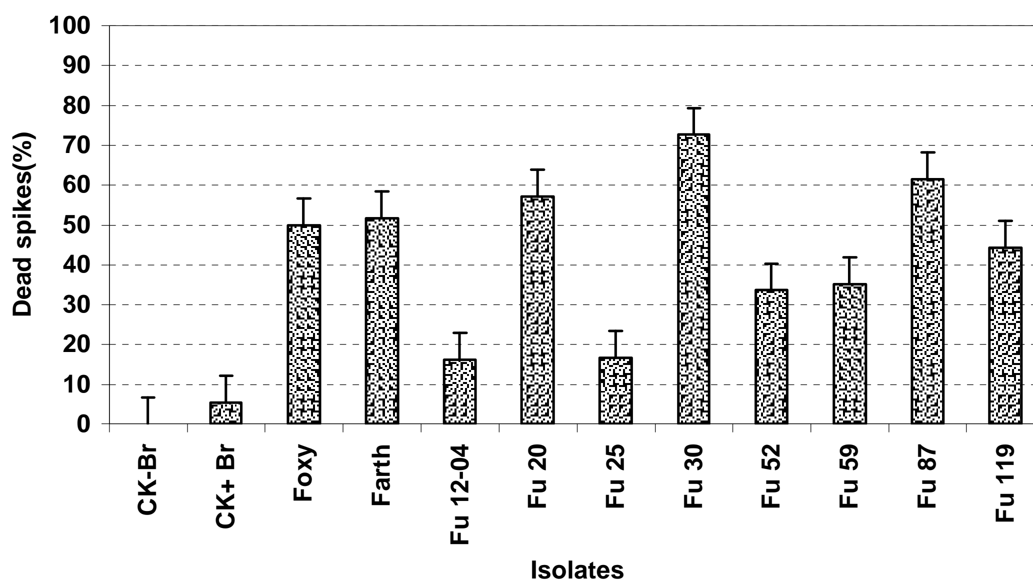


Figure 3.15. Effect of the most promising *Fusarium* spp. isolates on broomrape of tomato plants grown in infested soil pots.

Table 3.7. Effect of the most promising *Fusarium* spp. isolates on the number and weight of broomrape dead spikes, tomato fresh and dry weight.

Isolates	Broomrape dead spikes		Tomato plants weight (g)	
	Dead (%)	Weight (g)	Fresh	Dry
CK-Br	0.0 b	0 b	300 a	45 a
CK+ Br	5.4 b	0 b	168 b	25.2 b
Fu 12-04	16.2 b	1.3 b	206 b	30.9 b
Fu 20	57.1 a	28 a	123 c	18.45 c
Fu 25	16.6 b	1.3 b	165 b	24.75 b
Fu 30	72.7 a	9.3 b	208 b	31.2 b
Fu 52	33.6 a	9.3 b	140 b	21 b
Fu 59	35.2 a	6.3 b	114 c	17.1 c
Fu 87	61.5 a	18 a	104 c	15.6 c
Fu 119	44.3 a	6.3 b	221 a	33.15 a
Foxy	50.0 a	3.8 b	189 b	28.35 b
Farth	51.6 a	14 a	159 b	23.85 b

*Means followed by the same letter in a column or row are not significantly different according to the Fisher LSD test ($P \leq 0.05$).

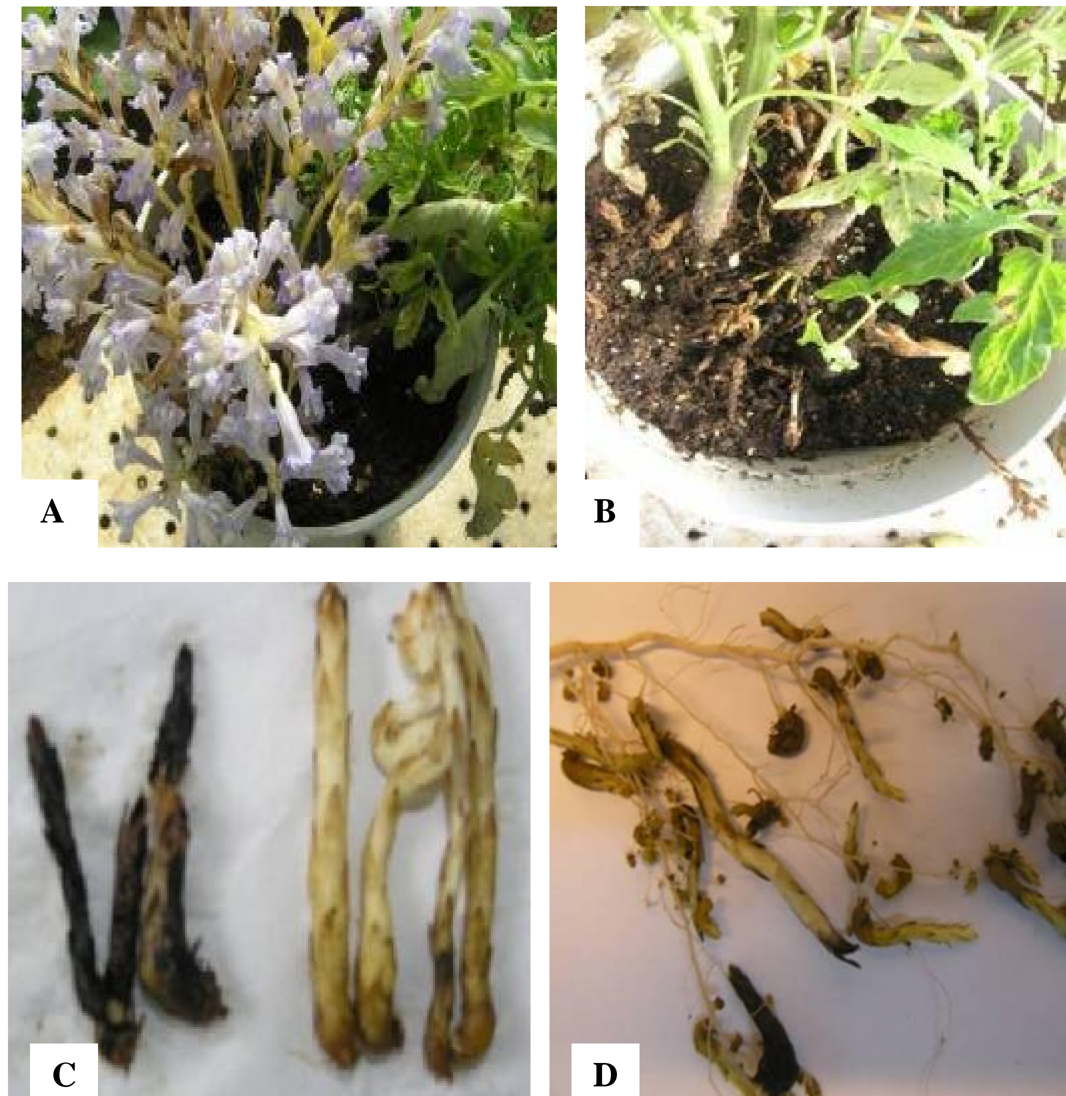


Figure 3.16. Effect of *Fusarium* isolate (Fu 52) on broomrape of tomato (A) Br-Fu 52; (B) Br+Fu 52; (C +D) Healthy and diseased tubercles.

Table 3.8. Native *Fusarium* isolates identification.

Isolate No.	Colony Diameter (cm)	Color	Conidia Shape		Chlamydo-spore	Speices
			Micro	Macro		
Fu 30	2.1	Peach or Pale orange	Obovoid With truncate base	Slender straight	-	*Fusarium oxysporium*
Fu 87	2.1	Rose	Obovoid		+	
Fu 20	1.8	Pink	Oval	Dorsal side more curved than the ventral side	+	*Fusarium solani*
Fu 119	2.1	Red	Oval		-	*Fusarium solani*
Fu 59	2.1	Pink	Obovoid with truncate base		+	*Fusarium oxysporium*
Fu 52	1	Rose red	Oval	Dorsal side more curved than the ventral side	+	*Fusarium oxysporium*
Fu 25	2.4	Pink	Obovoid or oval		+	*Fusarium solani*
Fu 12-04	2.2	Rose red	Obovoid		+	*Fusarium oxysporium*

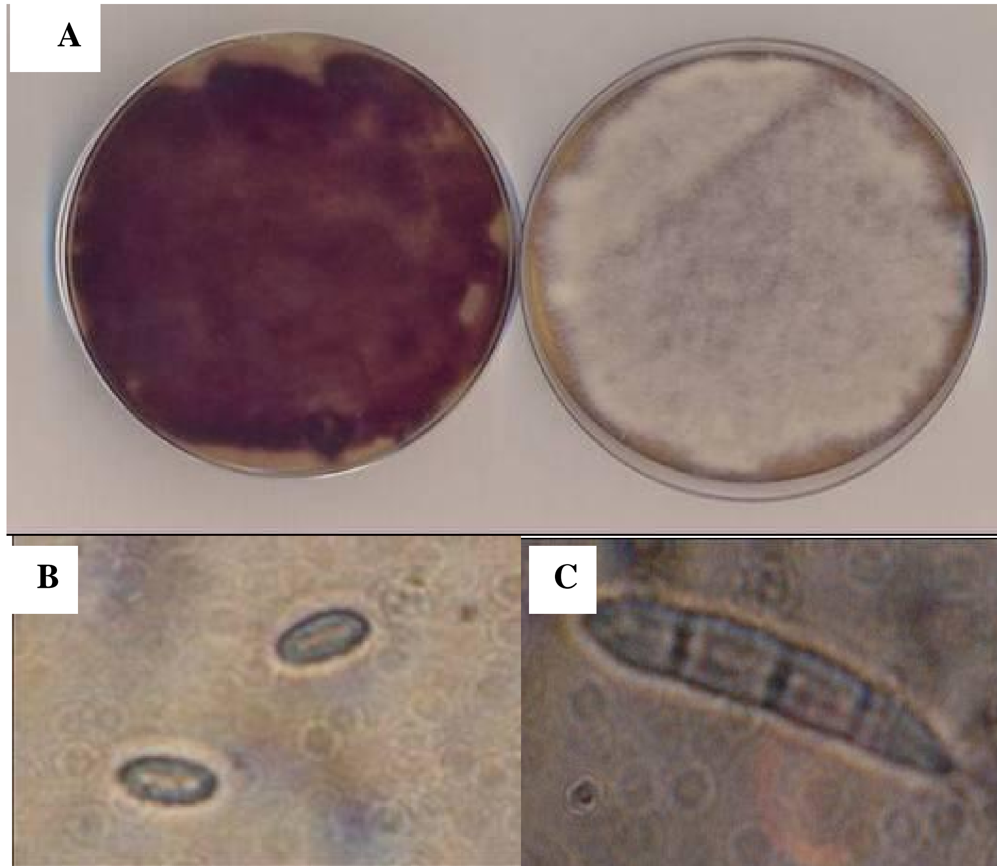


Figure 3.17. ***Fusarium*** **isolate 20, mycelium color on PDA (A) and conidial shape (B&C).**

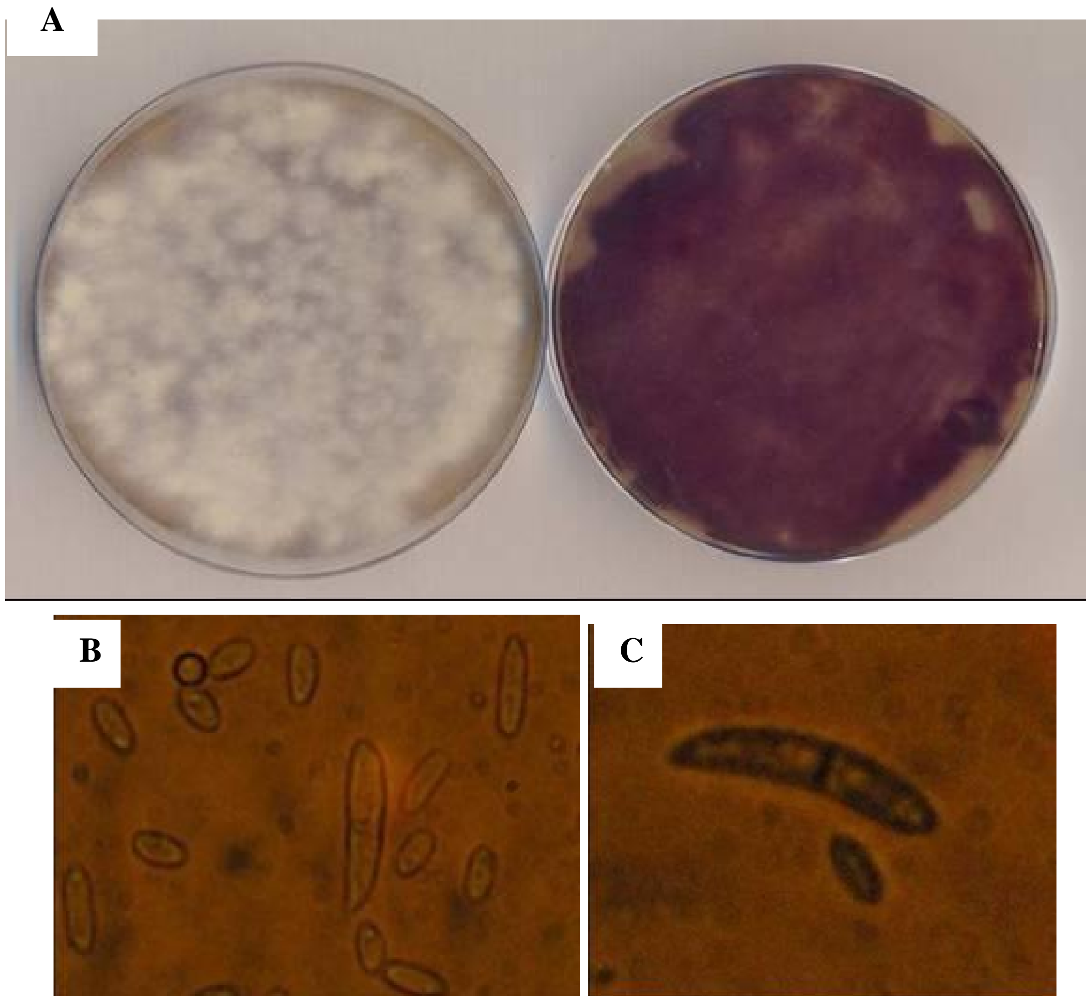


Figure 3.18. ***Fusarium*** **isolate 25; mycelium color on PDA (A) and conidial shape (B&C).**

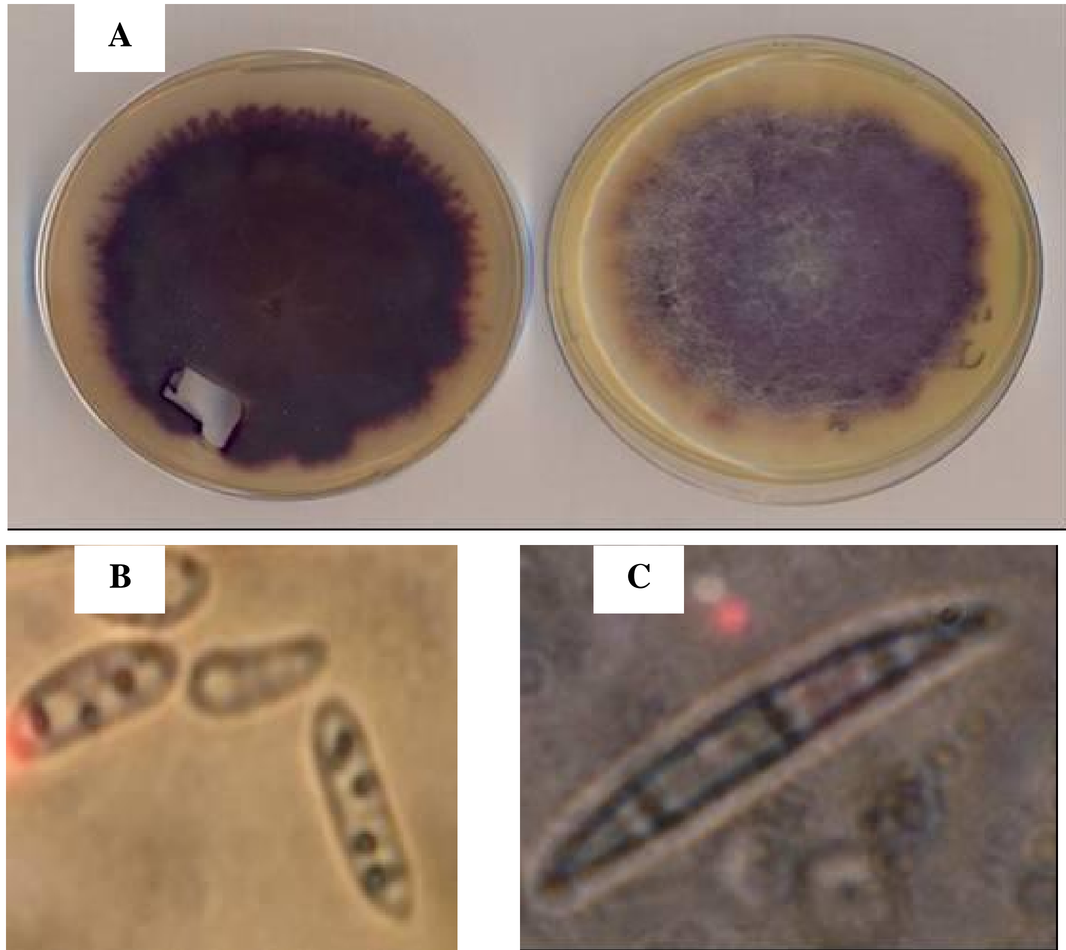


Figure 3.19. ***Fusarium*** **isolate 59; mycelium color on PDA (A) and conidial shape (B&C).**

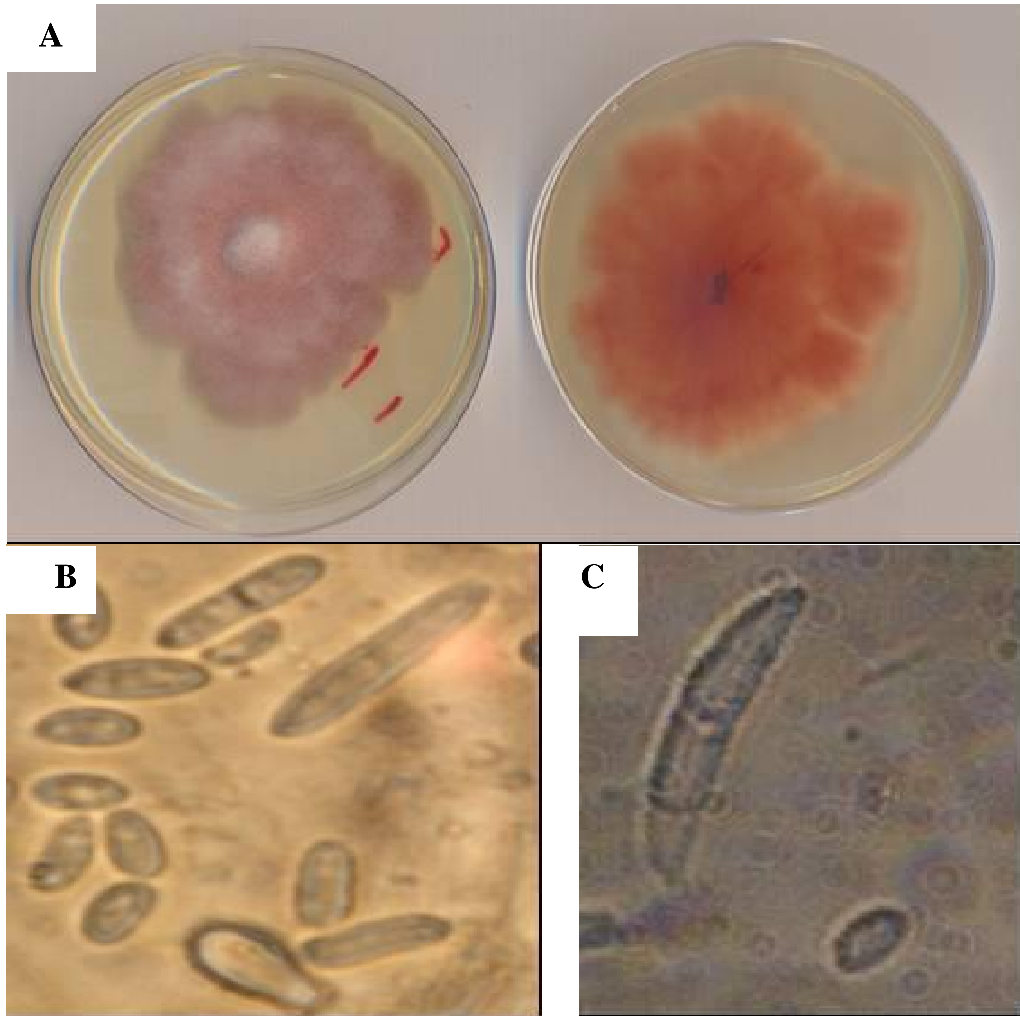


Figure 3.20. ***Fusarium*** **isolate 52; mycelium color on PDA (A) and conidial shape (B&C).**

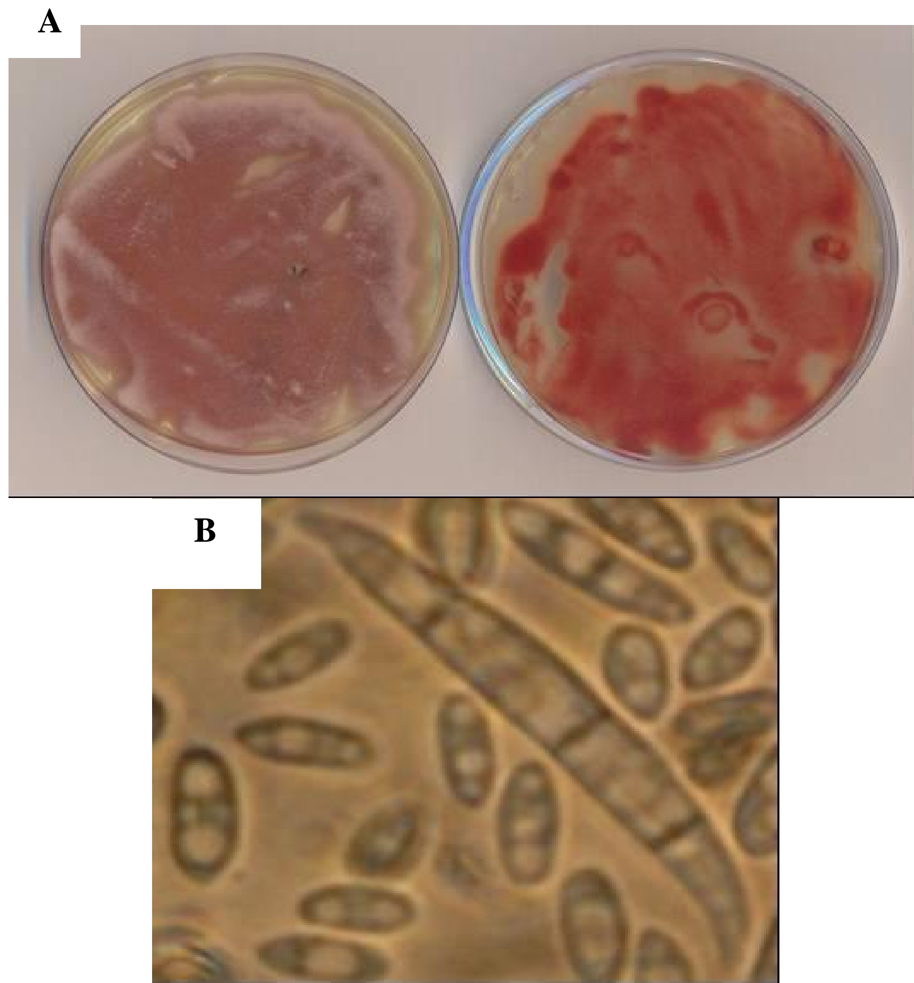


Figure 3.21. ***Fusarium*** **isolate 12-04; mycelium color on PDA (A) and conidial shape (B).**

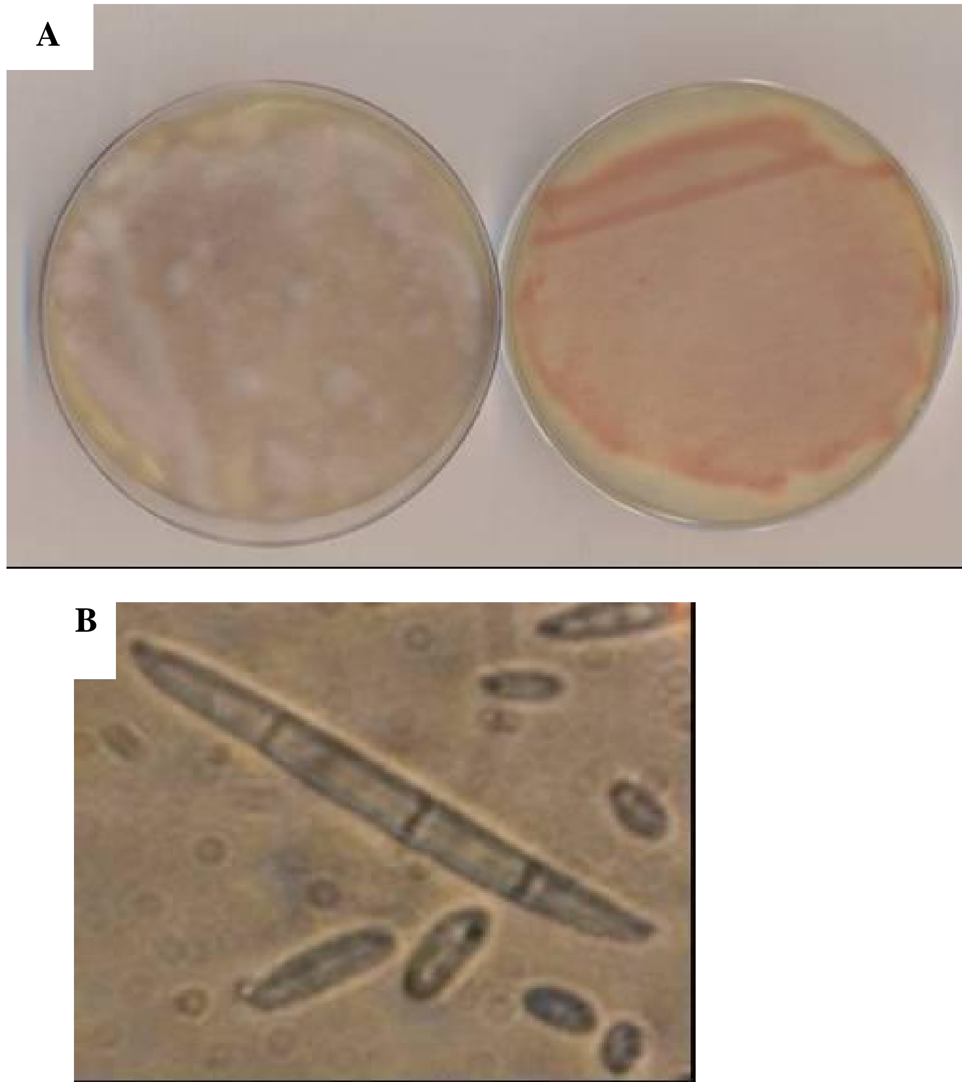


Figure 3.22. ***Fusarium*** **isolate 30; mycelium color on PDA (A) and conidial shape (B).**

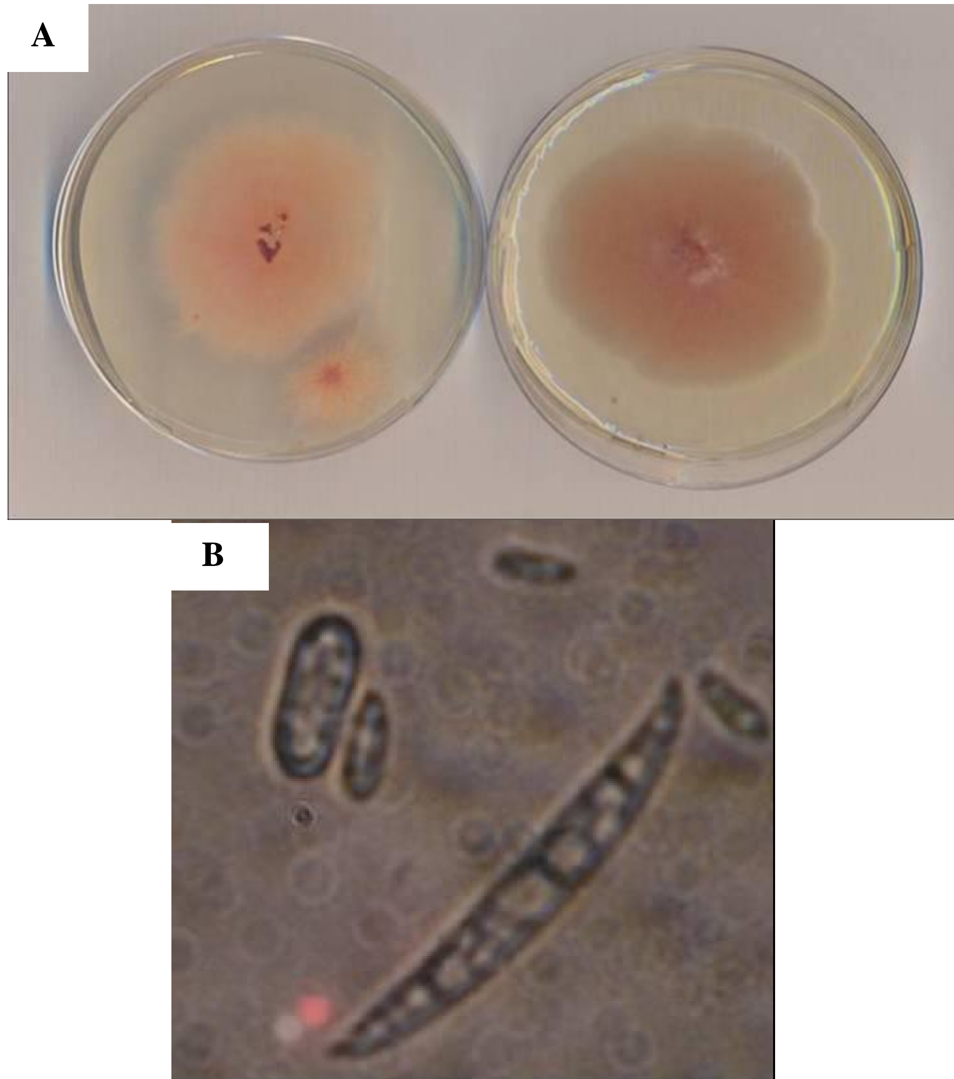


Figure 3.23. ***Fusarium*** **isolate 87; mycelium color on PDA (A) and conidial shape (B).**

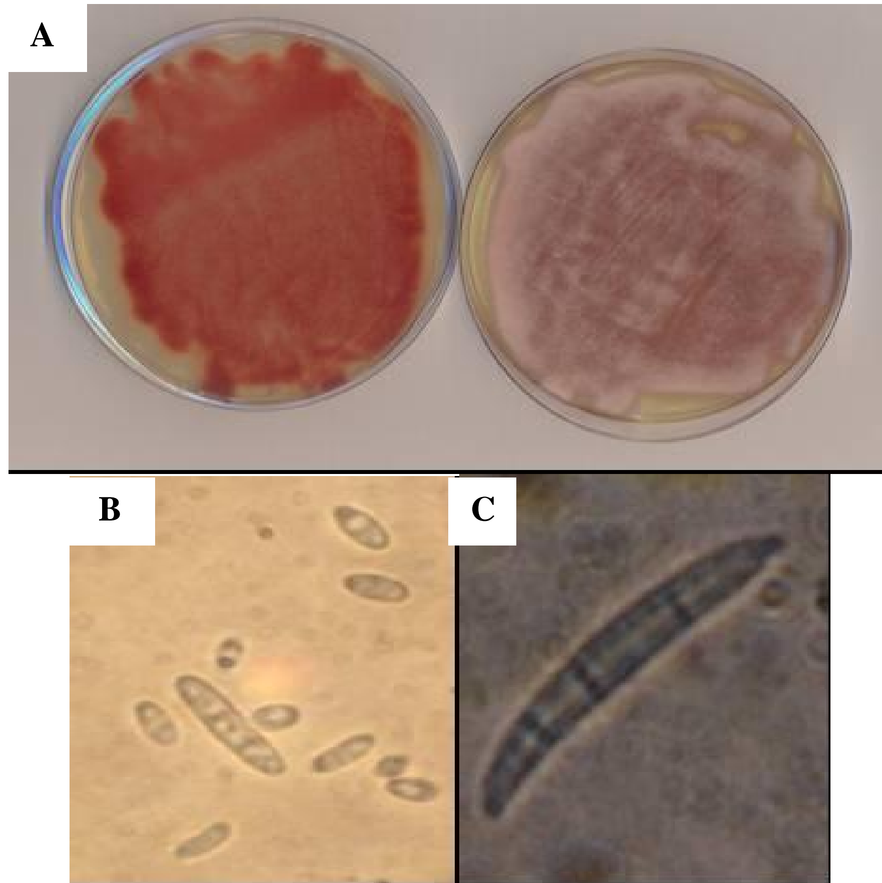


Figure 3.24. ***Fusarium*** **isolate 119; mycelium color on PDA (A) and conidial shape (B&C).**

Chapter 4

Discussion

4. 1. Tolerance/ resistance to broomrape

The results of the present study revealed that soybean, cowpea, bean and peas were immune to *O. aegyptiaca* since broomrape did not parasitize the plants and no attachments on the roots or shoots were developed. Similar results mensioned that cowpea was resistant to broomrape (Lane and Bailey, 1992). They explained that the mechanism behind cowpea resistance was hypersensitive reactions including necrosis of the host cells that block haustorial penetration of the parasite. Similar results by Krishnamurthy and Chandwani, (1975) revealed that some species of *Phaseolus* were resistant to *Orobanche minor* by their ability to stimulate the germination of *Orobanche minor* seeds; broomrapes were attached to the roots, but didn't develop tubercles, then shriveled and died. Parker, (1994) on the other hand, reported that some broomrape species such as *Orobanche crenata* caused considerable yield losses in legumes, particularly in broad beans, peas and lentils.

Results of the present study also showed that wooly vetch, bitter vetch, lupine and clover were relatively resistant to *O. aegyptiaca*. Similar results were found by Goldwasser et al., (1997) who demonstrated that there were a distinct polymorphism among vetch genotypes in response to *O. aegyptiaca*; common vetch genotypes were sensitive to the parasite, while purple vetch genotypes exhibited high resistance. In additions, Goldwasser et al., (2000) reported that woolly vetch showed resistant response to both *O. aegyptiaca* and *O. crenata* while the purple vetch (*V. atropurpurea*) showed resistance to *O. aegyptiaca* but not to *O. crenata* which is a similar *Orobanche* species. However Foy (1989) reported that vetches were highly

susceptible to *O. aegyptiaca* and *O. crenata*, where both cause severe yield losses in the Middle East and Mediterranean region.

Results of the present study also showed that soybean, lupine and bitter vetch showed as well relative resistance to broomrape and their resistace/susceptibility to broomrape has not been documented before. In addition, tomato, broad bean, chickpea, sunflower, common vetch, and lentil, however, were susceptible to *O. aegyptiaca*. Seeds of the parasite germinated, attached to the host roots and established the spikes over the soil (Fig. 3.5 & 3.6). Cubero (1991) has summarized the work done in Italy, Spain, and Egypt which showed various degrees of susceptibility in broad bean to broomrape. Nassib et al., (1979, 1982 and 1984) found that, only one broad bean variety was resistant to *O. crenata* (F-402) which was identified in Egypt and has been successfully used in breeding programs. In addition, Ruiales et al., (2002) reported that *Orobanche crenata* is a major parasite of fababean, pea, lentil, and various forage legumes and their resistance is scarce and complex in nature. Similarly, Saurborn (1991), Parker and Riches (1993), and Musselman (1994), reported that *O. aegyptiaca* parasitized sunflower, and *O. cumana* is a specific parasitic weed in the root system of sunflower. In addition, chickpea in this investigation was susceptible to *O. aegyptiaca* similar to what Saurborn (1991), Parker and Riches (1993) Burnhard (1995) and Colguhoun et al., (2001) reported. However, Ruiales et al., (2003) reported that there were two chickpea accessions (CA 2065 and P 2245) resistant to *O. crenata* due to a combination of low stimulant production and the darkening of the host cell tissues in contact with the broomrape radicle, leading to a failure of attachment.

In respect to mechanisms of resistance, Tiburzy and Reisener, (1990), Lane and Baily, (1992) and Dörr et al., (1994) reported that mechanisms of host resistance involved hypersensitive reaction characterized by the

development of necrotic lesions around the penetrating parasite radicle or death of the penetrating radicle before shoot development.

4. 2. Herbicides

All three herbicides, chlorsulfuron, triasulfuron and imazaquin had significant effects on broomrape of tomato plants growing in pots at the concentrations (0.5- 5 *ppm* a.i) used as a foliar spray. Furthermore, the herbicides at these concentrations had no effect on tomato plant's fresh weights in pots, however triasulfuron and chlorsulfuron, however was noted to increase tomato root's fresh weight compared to the control. In addition, tomato plants fresh weights increased when plants were treated before the flowering stage of the plants, compared to those treated after the flowering stage, since the parasite became developed and well established and consegnently affected the plants development. Significant variation between the herbicides effect on broomrape in pot experiment were recorded; the herbicides, triasulfuron, chlorsulfuron and imazaquin significantly controlled the broomrape at 0.5- 5 *ppm.* The herbicide triasulfuron was the most efficient in controlling broomrape. In addition, there was a high correlation between the broomrape dead spike % and the herbicides concentrations. A similar results by Qasem (1998) revealed that the herbicide chlorsulfuron application at 2.44g ha^{-1} (a.i.) completely prevented the broomrape infestation when thoroughly mixed with the soil prior to tomato transplanting and a single application of chlorsulfuron at 9.75g ha^{-1} (a.i.) 3-4 weeks after emergence, significantly reduced broomrape infestation and increased tomato growth compared with the control. In addition, Hershinhorn et al. (1998) reported that the herbicide chlorsulfuron applied at 5.0 g ha^{-1} (a.i.) gave 100% control of the emerged broomrape spikes and underground attachments, showing that under certain conditions this herbicide may completely prevent parasite development.

In the irrigated tomato open field, the three herbicides showed significant control effect on broomrape of tomato at the concentrations 1-5 *ppm* applied as foliar spray without visible impact on the plants. It was

noted, however, that the percentages of dead spikes in the open field were much lower than in pots. This might be due to the wide distribution of roots in the open field soil, plant's vigor, and herbicide mobility in soil. In addition, percentages of dead spikes were highly correlated with herbicides increased concentrations; triasulfuron (r^2 =0.95) , chlorsulfuron (r^2 =0.98) , and imazaquin (r^2 =0.98) (Appendix 3). Similar results were obtained by E. Kotoula-Syca, (2002) who reported that under field conditions chlorsulfuron applied at 10g ha^{-1} (a.i.), reduced broomrape emergence by 88% and when the herbicide was applied twice, (5 and 10 g ha^{-1} (a.i.) it gave complete control of broomrape but delayed crop maturity. In addition Garcia-Torres et al., (1991) showed that imazaquin and chlorsulfuron applied to broad bean open field at 40-80 g ha^{-1} (a.i.) and 6g ha^{-1} (a.i.), respectively, considerably reduced the number of broomrape spikes and their dry weights. Additionally, Hershinhorn et al. (1998) found that the herbicides chlorsulfuron and triasulfuron applied directly to soil planted with tomato seedlings by sprinkler chemigation, controlled broomrape and showed no phytotoxic effect on tomato plants. Goldwasser et al. (2001) mentioned that a single application of triasulfuron at 7.5 g ha^{-1} (a.i.) sprayed on potato foliage, effectively controlled broomrape but at the same time, severely damaged the crop.

Other investigators, Reinke et al. (1991) reported that other herbicides such as rimsulfuron and sulfonylurea selective to tomatoes, effectively controlled *O. aegyptiaca* in pots, but in drip-irrigated tomato fields, *O. aegyptiaca* control was poor. Kleifeld et al. (1998) showed that split application of low rates of imazapic applied on tomato foliage or chemigated via sprinkler irrigation achieved excellent *O. aegyptiaca* control through out

the growing season, but caused premature loss of flowers and early ripening of fruits. In addition, Haider et al, (2005) reported that *O. aegyptiaca* and *O. ramosa* were controlled on potato by split foliar applications of imazapic at 3.0g ha^{-1} and rimsulfuron at 12.5g ha^{-1} repeated three time, and by a combination of rimsulfuron at 12.5g ha^{-1} and glyphosate at 100g ha^{-1} applied three time via sprinkler irrigation.

At the greenhouse level, the three herbicides (Triasulfuron, Chlorsulfuron, and Imazaquin) controlled broomrape of tomato plants at the concentrations 5 and 10 *ppm*. Furthermore, the herbicides Triasulfuron and Imazaquin had no impact effect on tomato plants. On the other hand, Chlorsulfuron reduced tomato plant's growth (height by 8% and the leaves curled at the concentrations above 5 *ppm*). The effect of the herbicides, chlorsulfuron, triasulfuron and imazaquin against broomrape in greenhouse production has not been previously investigated.

Its worth mentioning, however, that the percentages of dead spikes in treated greenhouse were higher than those in the open field. This might be due to the higher concentrations of herbicides applied, greenhouse growth factors and broomrape sensitivity under such growth conditions.

4. 3. Biological control

The native *Fusarium* spp. isolates Fu 20, Fu 30, Fu 52, Fu 59, Fu 87, Fu 119 in addition to Farth and Foxy, significantly reduced broomrape of tomato plants grown in pots from 67.3 % for the isolate Fu 30 to 10.8 % for the isolate Fu 12-04 over the control. The variation between the isolates were observed and the most effective isolates were Fu 30, Fu 87, Fu 20, Farth, Foxy and Fu 119. The infected broomrape tubercules and germ tubes infected by the *Fusarium* isolates showed black rot and necrotic black lesions (Fig. 3.16). In addition *O. aegyptiaca* seed germination was inhibited and the number of broomrape attachments on tomato roots decreased. However the isolates, Fu 30, Fu 52, Fu 87 and Fu 119

significantly increased fresh and dry weight of tomato plants probably as a result of broomrape control or competed with other minor soilborne pathogens on available nutrients. The native *Fusarium* spp. isolates have no negative effect on the tomato plants.

In this direction, Bedi and Donchev (1991) reported that *Fusarium oxysporum* f. sp. *orthoceras*, is a potential agent for biological control of the root- parasitic weed *O. cumana* in sunflower. They mentioned that the germ tube of the *Fusarium* microconidia penetrated and colonized the *Orobanche* dormant seeds and destroyed it. *Fusarium* hyphae dissolved the endosperm cell walls and degraded the cytoplasm damaging lipid body membranes in the infested seeds; finally the lipid and protein rich endosperm was utilized by the fungus as a nutrient source.

Additionally, Thomas et al. (1998 & 1999) found that some isolates of *Fusarium oxysporum* inhibited *O. cumana* seed germination; the germ tube of the germinated seed became necrotic and the number of broomrape attachments on sunflower roots decreased. They explained that the conidia of *F. oxysporum* developed long germ tubes which penetrated all parts of the seed, producing pectin methylestrase and pectin transeleminase which might be responsible for dissolving the pectin-rich endosperm cell walls, destroying cytoplasm compartmentation, protein bodies' disappearance and lipids merging through the cell contents. In addition, the protein-rich endosperm was effectively utilized by the fungus as nutrient substrates. Other investigators reported that *Fusarium oxysporum* often produce phytotoxins such as fumonisins (Abbas & Boyette, 1992), fusaric acid (Bacon et al., 1996) and protein toxins (Bailey et al., 2000) that assist in over coming host defenses, allowing establishment of the pathogen.

Similarly, many investigators (Amsellem et al., 1999 & 2001; Gressel et al., 2001 & 2002; Cohen et al., 2002) reported that the strains (Farth and Foxy) are pathogenic to *O. aegyptiaca, O. crenata,* and *O. ramosa*

parasitizing cultivated vegetables (melon, potatoes, tomatoes, peppers, carrots, and celery), grain legumes (chick peas), and sunflower. Furthermore, Amsellem et al. (2001) reported that Foxy and Farth strains may be effective as seed, transplants and soil drench treatments of high-value vegetables and other crops. They further explained that the two *Fusarium* strains mode of action may include, depletion of starch out of the infected *Orobanche* and the sugars derived from starch are possibly used by *Orobanche* as energy to mobilize a rapid response to infection, including the synthesis of lignin-like materials or other defense responses (fumonisin like ceramide synthase inhibitors) (Gressel, 2001). However, Cohen et al. (2002) reported that fusaric acid was produced only by Foxy in liquid culture. In addition, Desjardins and Hohn (1997) reported that these strains produce toxins such as fusaric acid, fuminisins, beauvericin, enniatin, moniliformin and trichothecenes which many of them has phytotoxic or herbicidal effect.

4. 4. Identification of *Fusarium* isolates.

The native *Fusarium* spp. isolates (Fu 20, Fu 25 and Fu 119) were identified as *F. solani*, while the isolates Fu 30, Fu 52, Fu 59, Fu 87 and Fu 12-04 were identified as *F. oxysporum*. In this direction, many investigators (Amsellem et al., 1999 & 2001; Gressel et al., 2001 & 2002; Cohen et al., 2002) reported that *Fusarium* species (Farth and Foxy) are pathogenic to *O. aegyptiaca*. Boari and Abouzied (2002) found that a strain of *F. oxysporum* and a strain of *F. solani* being able to strongly reduce the number and weight of emerging broomrape. Thomas et al., (1998 & 1999) found that some isolates of *F. oxysporum* inhibited *O. cumana* seed germination and the number of broomrape attachments on sunflower roots decreased. Furthermore, Panchenko (1981) indicated that strains of the fungi *F. oxysporum* var. *orthoceras* gave some control of *O. aegyptiaca*

while Bedi and Donchev (1991) clarified that *F. oxysporum* var. *orthoceras* controlled *O. cernua.*

4. 5. Conclusions

This study revealed that screening for tolerance/ resistance to broomrape experiments revealed that the local varieties of cowpea, bean, peas and soybean were immune to *O. aegyptiaca* while wooly vetch, bitter vetch, lupine and clover were relatively resistant to *O. aegyptiaca* but tomato, broad bean, chickpea, sunflower, common vetch, and lentil, were susceptible.

Chemical control of broomrape *Orobanche aegyptiaca* can be achieved by using the herbicides, Chlorsulfuron 75% WG (Glean®), Triasulfuron 75% WG (Amber®), and Imazaquin 180g/L SL (Scepter®). They have effectively controlled broomrape's of tomato plants growing in pots, open field and greenhouse at the concentrations (3-10 *ppm* a.i = 0.3-1.0g/ dunum) used as foliar spray without visible effect on tomato plants.

In addition, *Fusarium* native isolates (Fu 20, Fu 30, Fu 52, Fu 59, Fu 87 and Fu 119) and *Fusarium oxysporum* strain EId (CNCM-I-1622) (Foxy) and *Fusarium arthrosporioides* strain E4a (CNCM-I-1621) (Farth) at inoculum concentrations of 10^8 conidia & 0.5 mg mycelium g^{-1} soil, provided promising results in the biological control studies; future field trials are needed to confirm efficacy under common agricultural practices.

Further studies on higher concentrations of herbicides in tomato open field, mode of action of herbicides, the effect of herbicices on different *Orobanche* species, bioagents mode of action and their effect on other *Orobanche* species are necessary.

4.6. Abstract in arabic

الهالوك نبات مزهر إجباري التطفل يحصل على الغذاء والماء من العائل لانه لا يحتـــوي علـــى الكلوروفيل ، ينتمي الهالوك الى العائلة الهالوكية ، ويؤدي الى أضرار اقتصادية بالغة في العديد من المحاصيل الخضريه ،المحاصيل البقولية وعباد الشمس في مناطق ومواسم زراعية مختلفـــة. تم إستخدام العديد من الطرق لمكافحة الهالوك على عدد كبير من المحاصـــيل الإقتـــصادية منهـــا الطرق الميكانيكية، الفيزيائية، الكيميائية، والبيولوجية، إلا ان اساليب المكافحة في مجملها لم تكـــن كافية.

خلال هذه الدراسة، تم دراسة حساسية الأصناف المحلية لمحاصـــيل فـــول الـــصويا، اللوبيـــاء، الفاصولياء، البازيلاء، الكرسنة، الكرسنة ذات القرون الـــصوفية، التـــرمس، البرســـيم، الفـــول، الحمص، البيقياء، العدس، عباد الشمس والبندورة لطفيل الهالوك المصري. اظهرت النتائج ان كل من محاصيل فول الصويا، اللوبياء، الفاصولياء و البازيلاء منيعة ولا تـــصاب بالهـــالوك ، امـــا محاصيل الكرسنة، الكرسنة ذات القرون الصوفية، الترمس و البرسيم فهـــي مقاومـــة بـــدرجات مختلفة ، أ ما محاصيل الفول، الحمص، البيقياء، العدس، عباد الشمس والبندورة فاثبتت انهـــا ذات حساسية عالية وتتاثر كثيرا بالهالوك .

ومن خلال هذه الدراسة ايضا، تم إستخدام بعض مبيدات الأعشاب (ترياســـلفرون triasulfuron الكلورسلفرون chlorsulfuron، الإمازاكوين imazaquin) في مكافحة الهالوك المصري علـــى البندورة في القواوير، الحقل المكشوف وفي البيت البلاستيكي. اظهرت النتائج ان استخدام مبيـــد ترياسلفرون زاد نسبة الهالوك الميت (%) في تجربة القواوير ب 79 ، 77 ، 84 ، 84 ؛ مبيـــد الكلورسلفرون ب 59 ،51 ، 84 ، 84 و مبيد الإمازاكوين ب 52، 59، 66 ، 84 عند إســـتخدام التراكيز (0.5 ،1، 3، 5 جزء من المليون) على التوالي. اما في تجربة الحقل المفتوح فمبيـــد ترياسلفرون زاد نسبة (%) الهالوك الميت ب 10.5، 13.5، 26.6، الكلورســـلفرون ب 13.6 ، 20.1 و 29.1 والإمازاكوين ب 13.1، 22.2 ، 28.9 بإستخدام التراكيز(1، 3، 5 جزء مـــن المليون) على التوالي . وعند إستخدام هذه المبيدات في البيت البلاستيكي فإن مبيد ترياسلفرون زاد نسبة الهالوك الميت (%) ب 30 و51 ؛ مبيد الكلورسلفرون ب 60 و 68 و مبيد الإمازاكوين ب 30 و 61 عند إستخدام التراكيز(5 و 10 جزءمن المليون) على التوالي . ولم يلاحظ اثـــار سلبيه على نباتات البندورة عند استخدام المبيدات بالتراكيز المذكوره.

اما فيما يتعلق بالمكافحة الحيوية، فقد تم عزل 125عزلة من فطر الفيوزاريوم المستوطن في التربة من سيقان الهالوك المصري المريض المتطفل على البندورة وبعض المحاصيل الاخرى من حقول زراعية مختلفة في منطقة الخليل . تم دراسة وبائية هذه العزلات وقدرتها على مكافحة الهالوك المتطفل على البندورة؛ تبين من النتائج ان العزلات (Fu 87, Fu 59, Fu 52, Fu 30, Fu 119 Fu 20) زادت نسبة الهالوك الميت (%) ب (57.1، 72.7، 33.6، 35.2، 61.5، 44.3) على التوالي . كما ادت العزلات Fu 112 و Fu 115 ادت إلى منع إنبات بذور الهالوك. اما عزلات الفيوزاريوم *F. oxysporum* strain EId (CNCM-I-1622) (Foxy)و (Farth) *F. arthrosporioides* strain E4a (CNCM-I-1621) والتي تم تعريفها من قبل معهد باستور لتجميع الكائنات الدقيقة في باريس فقد زادت نسبة الهالوك الميت (%) ب 50 و 51.6 % على التوالي. وتم تعريف عزلات الفيوزاريوم حيث تبين ان كل من العزلات Fu 12-04, Fu 87, Fu 59, Fu 52, Fu 20 تنتمي إلى نوع *F. oxysporum* اما العزلات Fu 30, Fu 25 و Fu 119 فتنتمي إلى نوع *F. solany*.

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Appendix 6. Component of modified Hoagland's solution

Item	**Concentration (mM)**
$NH_4H_2PO_4$	**0.5**
KNO_3	**3**
$Ca(NO_3)_2$	**2**
Mg SO_4	**1**
Fe EDTA	**90**
H_3BO_3	**46**
Mn Cl_2	**9**
Zn SO_4	**0.8**
Cu SO_4	**0.32**
$(NH_4)_6$ Mo_7O_{24}	**0.016**
PH	**5.6**

Appendix 7. Component of modified Martin media.

Item	**Weight(g)**
Difco pepton	5
Glucose	10
KH_2PO_4	1
$MgSO_4.7H_2O$	0.5
Difco agar	20
Chloramphenicol	0.25
Rose-bengal	0.13
PCNB	0.005

Appendix 8. The linear regression analyses of the herbicides experiments in pots, open field, and greenhouse (Y- dead spikes (%), X- concentration (*ppm*)).

Herbicide	Regression equation	r^2
Pots planting		
Amber®	Y= 17.3x +28.9	0.57
Glean®	Y= 19.3x + 13.7	0.79
Scepter®	Y= 18.2x + 13.6	0.83
Irrigated open field		
Amber®	Y= 8.33x -5.23	0.95
Glean®	Y= 9.36x -4.70	0.98
Scepter®	Y= 9.60x -4.90	0.98
Greenhouse		
Amber®	Y= 34 X+ 4.67	0.84
Glean®	Y= 25.5 X + 6	0.98
Scepter®	Y= 30.5 X - 0.6	0.99

Appendix 9. The susceptibility of various economic crops to *Orobanche* species.

Crop	*O.aegyptiaca*	*O.ramosa*	*O.crenata*	*O. cernua*	*O.cumana*	*O. minor*	*O.palastia*	References
Tomato	S*	S	R	S	R	R	S	Saurborn 1991; Parker and Riches, 1993; Burnhard, 1995. Colguhoun et al., 2001
Broad bean	S	R	S	R	R	S	_	
Pea	R	R	S	R	R	R	_	
Common vetch	S	R	S	R	R	R	_	
Bitter vetch	R	_	_	_	_	_	_	
Wooly vetch	R	_	R	_	_	_	_	Goldwasser et al., 2000
Chickpea	S	R	S	R	R	R	_	Saurborn 1991; Parker and Riches, 1993; Burnhard, 1995. Colguhoun et al., 2001
Red clover	R	R	R	R	R	S	_	
Lentils	S	R	S	R	R	S	_	
Sunflower	S	S	S	R	S	S	_	
Lupine	R	_	_	_	_	_	_	
Bean	R	R	S	_	_	R	_	Krishnamurthy and Chandwani, 1975
Soy bean	R	_	_	_	_	_	_	
Cowpea	R	_	_	_	_	_	_	

S- Susceptible to broomrape species.
R - Resistant to broomrape species.
*- The results of susceptibility/ resistant to *O. aegyptiaca* accoding to current investigation.

Appendix 10. Statistical analysis of data.

Experiment	SS of treatment	SS of residual	MS treatment	MS of residual	F	LSD
Local crops resistance to *Orobanche* (No. of spikes)	2965.3	708.0	228.1	10.9	20.9	3.8
Local crops resistance to *Orobanche* (Weight of spikes)	50.6	12.9	3.9	0.2	19.6	0.5
Effect of herbicide on *Orobanche* in irrigated tomato open field (No. of dead spikes)	2360	52.9	262.2	2.6	99.0	2.77
Effect of herbicide on *Orobanche* under green house conditions (No. of dead spikes)	10515.6	456.7	1752.6	32.6	53.7	10.0
Pathogenicity of *Fusarium* isolates to *Orobanche* (No of spikes) (Patch 1)	18221.4	31672.7	1401.6	575.8	2.4	30.4
Pathogenicity of *Fusarium* isolates to *Orobanche* (Weight of spikes) (Patch 1)	8805.8	16779.1	677.3	310.7	2.2	22.3
Pathogenicity of *Fusarium* isolates to *tomato* (Weight of plants) (Patch 1)	38395.0	32498.7	3199.6	637.2	5.0	32.0
Pathogenicity of *Fusarium* isolates to *Orobanche* (No of spikes) (Patch 2)	62247.3	65622.5	3276.2	1093.7	2.99	46.8
Pathogenicity of *Fusarium* isolates to *Orobanche* (Weight of spikes) (Patch 2)	74010.9	71899.2	3524.3	1089.4	3.2	46.6
Pathogenicity of *Fusarium* isolates to *tomato* (Weight of plants) (Patch 2)	374149.7	409032.2	17006.8	5928.0	2.9	108.6
Pathogenicity of *Fusarium* isolates to *Orobanche* (No of spikes) (Repeated)	33775.1	33885.7	2598.0	806.8	3.2	40.5
Pathogenicity of *Fusarium* isolates to *Orobanche* (Weight of spikes) (Repeated)	4492.3	6172.2	374.3	158.2	2.3	18.0
Pathogenicity of *Fusarium* isolates to *tomato* (Weight of plants) (Repeated)	166289.7	137506.2	12791.5	3273.9	3.9	81.6