AN INVESTIGATION ON THE POSSIBILITIES OF BIOLOGICAL CONTROL OF *OROBANCHE* SPP. IN ETHIOPIA

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In partial Fulfilment
of the Requirements for the Degree
of Master of Science in Biology

BY
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SCHOOL OF GRADUATE STUDIES

AN INVESTIGATION ON THE POSSIBILITIES OF BIOLOGICAL CONTROL OF *OROBANCHE SPP* IN ETHIOPIA

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DEDICATION

To My parent, brothers and sisters, who

always prays for me to succeed

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ABSTRACT

In this study, the possibilities of biological control of *Orobanche* spp. (broomrapes), a serious root parasites of tomato (*Lycopersicon escelentum* Mill.) and other members of the family *Solanaceae* in Ethiopia have been investigated. Intensive surveys were conducted for insect parasites of *Orobanche* spp. in tomato production areas, namely, Nura Era, Merti Jeju and Malima Farms. The surveys showed that, the fly *Phytomyza orobanchia* (Diptera: *Agromyzidae*) occurs only in Malima Farm where it exhibited destructive ability to the seed capsules of *O. ramosa* and *O. cernua* up to 81.4% and 71.7%, respectively.

Surveys were also conducted in Ziway, Nura Era, Merti Jeju and Malima farms for microorganisms pathogenic to *Orobanche* spp. for the purposes of their isolation, identification and exploration of their potential as biological control agents. Twenty fungal isolates were isolated and identified from infected *Orobanche* plants. These were: *Fusarium. gibbosum*; (2) *F. nygamai*; (11) *F. Oxysporum*; (2) *F. proliferatum*; *F. Solani var. Solani* and (3) *Fusarium* sp. All the identified fungal isolates belong to the genus *Fusarium* and have not been reported before in Ethiopia. Two of them *F. nygamai* and *F. proliferatum* were identified for the first time on *Orobanche* plants as parasites.

Preliminary pathogenicity tests showed that *F. gibbosum, F. nygamai, F. oxysporum* and *Fusarium* spp. were promising as pathogens to *O. ramosa*. They significantly reduced the number of emerged shoots when applied at a rate of 10 gkg^{-1} of soil under glasshouse conditions. The same isolates significantly decreased the dry weight of *O. ramosa* and resulted in significant increase of tomato dry weight by 30.3%, 30.3%, 32.1%, and 30.3.%, respectively.

F. oxysporum and *F. nygamai* significantly killed *O. aegyptiaca* tubercles when applied at a rate of 0.5 X 10^6 spore ml^{-1} in the root chamber. *F. oxysporum* killed 81% and 82%, while *F. nygamai* killed 41% and 48% of the tubercles after 10 and 15 days of inoculation, respectively. The colour of the diseased tubercles was changed to dark brown or black. Symptoms like wet rotting were also observed.

Recommendations and suggestions are forwarded for further work on the biological control of *Orobanche* spp. in Ethiopia. This should include comprehensive evaluation of the role played by the *Phytomyza orobanchia* as an effective bioagent.

1. INTRODUCTION

The plant kingdom includes about 20 families to which parasitic flowering plants belong (Kuijt, 1969). Members of the family *Orobanchaceae* include some economically important parasitic weeds (Parker, 1991). This family, consists of 14 genera. The most important genus from economical point of view is *Orobanche*. This genus has more than 100 species, of which five are considered as major parasites of crops (Parker and Riches, 1993).

Orobanche spp. (broomrapes) are noxious root parasites which seriously constrain the production of many crops, particularly members of *Leguminosae* and *Solanaceae* families (Parker and Riches, 1993). *Orobanche* spp. are obligate holoparasitic flowering plants. They penetrate the roots of the host, and withdraw water, minerals and carbohydrates, causing drought stress and wilting of the host. Their main centre of distribution is the Mediterranean region (Saghir, 1986; Linke et al., 1989) where large areas are heavily infested. Heavy infestation also occur in Near East and parts of Europe and in various republics of the former USSR (Schroeder, 1994). The parasite causes devastating damage to field and vegetable crops in semi-arid regions. Yield losses due to *Orobanche* are reported to range from 5-100% (Linke et al., 1989). Due to the complete devastation caused by *Orobanche* in many areas, production methods had to be modified.

Three species of *Orobanche* are known to be wide-spread in Ethiopia (Fig. 1). The most common one is *O. minor* Sm. This species mainly parasitizes wild ornamental plants of the family *Asteraceae*, as well as other economically important crops such as sunflower

(*Helianthus annuus* L.) and nuog (*Guizotia abyssinica* Cass.) (Stroud, 1989). The second species which is also very widespread on a range of different families such as *Solanaceae* and *Cruciferae* is *O. ramosa* L. It is found to cause a serious problem on tomato (*Lycopersicon esculentum* Mill.), tobacco (*Nicotiana tabacum* L.) and rapeseed (*Brassica napus* L.) in Ziway and Upper Awash Valley (Gelma, 1991). The third species which is much more restricted in distribution is *O. cernua* Loefl.. However, this species is an acute problem in tomato, egg plant (*Solanum melongena* L.) and tobacco production in the Upper Awash Valley, especially at Nura Era Horticulture Development Enterprise and around Nazreth (Parker, 1992).

It has been reported that over 1000 ha of irrigated tomato fields were infested by *O. ramosa* and *O. cernua* at the Nura Era Horticulture Development Enterprise (Sherif and Gelma, 1992). Losses from heavy infestation of *O. ramosa* were estimated at 50-60% in tomato (Kassa Admassu[1], 1994 personal communication). Due to the *Orobanche* problem in Ethiopia in tomato production, the Ziway Horticulture Enterprise has totally shifted from tomato to flower production (Sherif and Gelma, 1992).

Orobanche crenata Forsk., is a localized recent problem on faba bean (*Vicia faba* L.) at Kedijo Geter and Kutaber near Dessie town (Northern Ethiopia) (Fig. 1). It has caused complete failure of faba bean in both localities (Admassu and Berche, 1994). More recently

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(1) Kassa Admassu; Manager, Nura Era State Farm, Nura Era Horticulture Development Enterprise, Ethiopia.

(Jurgan Kroschel[1], 1995 personal communication) confirmed the occurrence of *O. crenata* in North Ethiopia (Tigray region).

Compared to non-parasitic weeds, the control of *Orobanche* spp. has proven to be exceptionally difficult. So far, no consistent, effective and economical control method is reported for *Orobanche* control (Saghir, 1979; Saghir et al., 1980; Jacobsohn, 1986; Kropff and Schipper, 1986). The most prominent characteristics of *Orobanche* spp. are their prolific production of minute seeds (up to 200,000 per plant) which remain viable in the soil for more than ten years, and their intimate physiological interaction with their host plants which limits the application of conventional weed control measures.

However, several attempts have been made and in a few instances, complete eradication of the parasite was accomplished (Parker and Riches, 1993). Control measures using herbicides have shown partial success in controlling *Orobanche* (Saghir, 1979; Jacobsohn and Levy, 1986). However, only glyphosate herbicides work to control *O. crenata* in faba bean (Linke, 1992). Herbicides are not usually recommended to small farmers because they are either not economical, not available or difficult and dangerous to use. Solarization could offer a solution but only for small strips of infested fields since it would be impractical to cover large fields with polyethylene sheets. Other means of control such as cultural, mechanical, biological, as well as the use of resistant crop varieties may prove to be of some benefit as part of an integrated control measures.

---

(1) Jurgan Kroschel; Coordinator, Ecology and Management of Parasitic Weeds (gtz UH), University of Hohenheim, Stuttgart, Germany.

In Ethiopia, only hand pulling is practised to control *Orobanche* in the state farms. This incurs high cost and considerable mechanical damage to tomato plants, resulting in significant reduction of yield. However, some preliminary field observations indicated that there is a possibility of using biological control agents to reduce the damage caused by *Orobanche* spp. in Ethiopia (Fessehaie and Parker, 1992).

Biological control of *Orobanche* has been already successfully carried out in the field. The fly *Phytomyza orobanchia* Kalt. (Diptera, *Agromyzidae*), has been used in the former USSR and Eastern Europe for *Orobanche* control (Lekic, 1970; Kurbanov, 1970; Bronshtein, 1971, Kaprolov, 1974; Lekic, 1974; Horvath, 1983; Klyueva and Pamuchki, 1983; Horvath and Wittmann, 1988). This fly occurs widely in Ethiopia but it was too often killed by routine use of insecticides (Fesselaie and Parker, 1992). The larvae of the insect *Psila* sp. (Diptera, *Psilidae*) was also found in the stems of *O. minor* along with *P. orobanchia* (Parker and Riches, 1993). Soil fungi (e.g. *Fusarium* spp., *Alternaria* spp. and *Ulocladium* spp.) have been reported to be pathogenic on *Orobanche* (Linke et al., 1992). However, further research is needed to see which herbivores and microorganisms of *Orobanche* spp. occur in Ethiopia and to evaluate their potential for biological control of these parasites. This study aims at investigating the availability and the effectiveness of potential natural agents in the control of *Orobanche* spp. in Ethiopia.

1.1 Research Objectives

Broomrape control is extremely difficult and it seems that the chance to find a single economical and universal answer is very low. Thus, the development of integrated control measures including biological agents could offer good prospects for *Orobanche* control.

Therefore, the objectives of this study are:

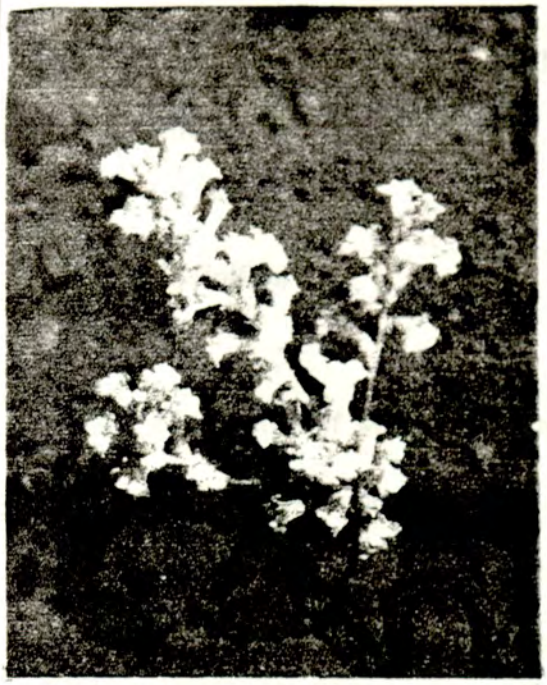
Specific objectives

1/ To identify insects associated with infested *Orobanche* spp. in Ethiopia.

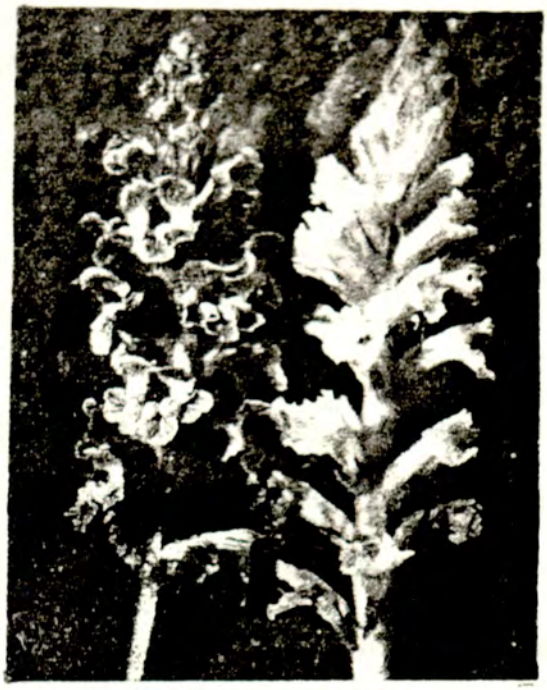
2/ To evaluate the efficacy of such insects as biocontrol agent of *Orobanche* spp.

3/ To isolate and to identify pathogens that have associated with diseased *Orobanche* spp.

4/ To screen for promising pathogens by evaluating their pathogenicity against *Orobanche* under controlled environmental condition in root chamber and glasshouse trials.



Orobanche aegyptiaca/ramosa



Orobanche crenata



Orobanche minor



Orobanche cernua

Fig. 1: The most important *Orobanche* species found in Ethiopia.
Source Linke (1992).

2. LITERATURE REVIEW

2.1 General

2.1.1 Definition

Biological control of weeds means the manipulation of weeds by using other organisms such as higher plants as competitors, microorganisms, invertebrates or vertebrates (Koch and Kunisch, 1989). Three basic types of biological weed control can be distinguished according to Auld et al. (1987) as: (1) classical biological control involving the introduction of a natural enemy (predator or parasite) of a weed from its native origin, into the exotic area where the weed has reached pest status; (2) augmentative control which refers to a situation in which a biological agent is unable to sustain itself in the long-term in all areas of weed infestation without augmentation of its population; (3) inundative biological control which is short-term control achieved by releasing a biocontrol agent in high concentration such as suspensions of fungal pathogens applied in the same way as herbicides (mycoherbicides).

2.1.2 Natural enemies of *Orobanche* spp.

Many natural enemies of *Orobanche* spp. have been reported particularly from Eastern Europe, but few attempts have been made to exploit them for biocontrol. Martelli, 1933 (cited in Schroeder, 1994) was the first to search for biological agents against *Orobanche* spp. Among 14 or more polyphagous species, he found that only, *Phytomyza orobanchia* (*Agromyzidae*), *Chyliza ariseta Mg.* (Diptera: *Psilidae*) and *Smicronyx cyaneus* Gyll. (Coleoptera: *Curculionidae*) caused significant damage to *Orobanche*. In investigations on

insects feeding on *Orobanche* spp. in former Yugoslavia between 1967-1972, six polyphagous species were recorded, out of which only *P. orobanchia* had potential for biological control of *Orobanche* spp. (Mihajlovic, 1986). More recently AL-Khresraji et al., (1987) reported attack of *Orobanche* spp. by larvae of *Eumerus* sp. (Diptera: *Syrphidae*) and an unidentified clear-wing moth (Lepidoptera.: *Sesiidae*) which may be of potential interest for biological control of *Orobanche* spp.

The use of pathogens for *Orobanche* control was first developed in the former USSR. Certain fungi isolated from *Orobanche* were proved to be useful for its biological control (Kott, 1969).

2.2 Insects

Many polyphagous insects were found associated with *Orobanche* spp. (Table 1). Only *Phytomyza orobanchia* was reported by Mihajlvoic (1986) as monophagous (i.e. feeds only on broomrapes and injures their reproductive organs), thus, it is the only species with good perspective for biological control.

Table 1: Insects found associated with *Orobanche* species

Order/Family	Insect species	*O.* host sp.	Country	Author
Diptera				
Psilidae	*Psila* spp.	*O. minor*	Ethiopia	Parker and Riches(1993)
	Chyliza extenuata Rossi	*O.* spp.	Netherlands	Puzzilli, (1983)
	Chyliza ariseta Mg.	*O.* spp.	-	Schroeder, (1994)
Syrphidae	*Eumerus* spp.	*O.* spp.	Iraq	AL-Khesraji, et al., (1987)
Coleoptera				
Curculinoidae	*Smicronyx cyaneus Gyll.*	*O.* spp.	Italy	Manijunath and Nagarkatti, (1977) Mihajlovic, (1986) Sauerborn, (1991)
Elateridae	*Agrotis* spp.	*O.* spp.	Yugoslavia	
Scarabaeidae	*Epicometis* sp.	*O. crenata*	Syria	Mihajlovic, (1986)
Heteroptera				
Miridae	*Lygus pratensis* L.	*O. cumana*	Yugoslavia	Puzzilli, (1983) AL-Kgesraji, et al., (1987)
Homoptera				
Aphidae	*Aphis maidiradicis Forb.*	*O.* spp.	-	Mihajlovic, (1986)
	A. gossypii	*O.* spp.	Iraq	
	A. fabae Scop.	*O.* spp.	Yugoslavia	Manijunath and Nagarkatti, (1977)
Isoptera				
Termitidae	*Microtermes* sp.	*O. cernua*	India	Lekic,(1970)
Lepidoptera				
Noctuidae	*Scotia segetum* (Schiff)	*O. cumana*	Yugoslavia	AL-khesraji, et al., (1987)
Sesiidae	Unidentified sesiid moth	*O.* spp.	Iraq	Mihajlovic, (1986)
Tortricidae	*Agryrotaenia pulchellana* Haw.	*O. cumana*	Yugoslavia	

O: *Orobanche*

2.2.1 *Phytomyza orobanchia* Kalt.

The biological control of *Orobanche* by *P. orobanchia* was considered for the first time at the beginning of this century in Italy (Spencer, 1973). The fly occurs throughout the regions where *Orobanche* species are native (Linke et al., 1990, Parker and Riches, 1993). It is particularly common throughout the Mediterranean region and extends eastward to the Balkan, the Ukraine and Central Asia, and southward to Ethiopia (Bronsthein, 1968; Spencer, 1973). *P. orobanchia* is capable of sensing broomrape over a radius of 3 Km by the use of chemical receptors, which are located on the first two segments of the antenna (Bronsthein, 1972 cited in Pieterse, 1979; Puzzilli, 1983). The larvae of this midge live only in broomrapes, where they feed on all parts of the plant. Their favorite food are young seed capsules, and the tip of the plant. Where they occur in greatest numbers, they invade the whole plant. The tunnels made by the larvae on the stem can also lead to secondary fungal infection. During the first two larval instars, no serious damage occurs and no external symptoms on broomrape can be seen (Tawfik et al., 1976). However, the last (3rd) larval instar causes the greatest damage when it starts to feed.

2.2.1.1 Life history

The fly of *P. orobanchia* is 2.5 mm long, with yellowish-brown fronts and black legs with yellow knees (Spencer, 1973). The females lay eggs singly in slits in flower buds, on flowers, newly formed seed capsules, on the very tip of the plant between flower buds, and on the axilla of rudimentary leaves along the stem. The eggs (0.39 mm long and 0.19 mm wide) are oval in shape, very fine and hardly noticeable (Tawfik et al., 1976). The female can lay 180-200 eggs in its life time (Sauerborn, 1991). After hatching, the larvae

(3-4 mm long) tunnel the part of the plant where the eggs were laid and feed on the soft parts of the plants. In contrast to the stem where many larvae can be found, normally, only one larva occurs per seed capsule, but at low *Orobanche* and high *P. orobanchia* densities up to three larvae per seed capsule can be found (Horvath, 1987). Pupation occurs at the feeding sites. The pupa is of coarctate type, and measures 4.1 mm long and 1.3 mm wide (Tawfik et al., 1976). On *O. cumana* in former Yugoslavia, *P. orobanchia* completes its development between 25-30 days (Horvath, 1983). The insect overwinters as pupae in the lower part of the stem, seldom in the dried seed capsules. After hatching in the spring, the adults mate and lay eggs on the developing inflorescence. Mating takes place at any time during the day, in bright sunshine as well as in shade. The number of generations depends on weather conditions and the length of the growing season. There is a reproductive potential of about 24 larvae per female and 2-4 generations per year (Lekic, 1970). Four to five generations were recorded in N-Africa and W-Asia (Sauerborn, 1991), and 3-4 generations per one vegetation period in Morocco (Klein et al., 1995).

Diapause duration of *P. orobanchia* overwintering generation is a significant phenomenon in the maintenance of its population. Most of the larvae of the last generation of the vegetation period go into diapauses which can last up to 3 years (Puzzilli, 1983), and thus, the fly is able to survive temporary disappearance of its host plant. The greatest number of overwintering individuals (67.7 - 88.1%) complete their development in the following spring (Mihajlovic, 1986, Schroeder, 1994). It has been reported that 2-22% of the pupae of the first generation, 33-86% of the second generation and almost 100% of the third generation stay up to 4 years in diapause (Sauerborn, 1991). Prolonged diapause of a certain proportion of local population is also reported by Klyueva and Pamukchi (1978; 1982)

2.2.1.2. Infestation rates

The natural impact of the broomrape fly *Phytomyza orobanchia* as biological control agent of *Orobanche* spp. was investigated with positive results in the former USSR and Eastern Europe (Kurbanov, 1970; Bronsthein, 1971; Lekic, 1974; Horvath, 1983; KLyueva and Pamuchki, 1983), in Turkey (Giray and Nemli, 1983), in Syria (Linke et al., 1990, Linke, 1992) and in India (Manjunath and Nagarkatti, 1977). Its infestation rates were reported from different countries (Table 2).

Table 2: Infestation rates of *Phytomyza orobanchia* on *Orobanche*

Country	O. species	Host	Infested capsules %	Destr yed seeds %	Author
Bulgaria	*O. cernua*	sunflower	85-100	-	Trenchev, 1981
	O. ramosa	tobacco	20-30	-	Trenchev, 1981
Egypt	*O. crenata*	faba bean	82	89	Hammad et al., 1967
Hungary	*O. cumana*	sunflower	37.5-69	60	Horvath, 1987
Iraq	*O. aegyptiaca*	potato, tomato	41-87	21	AL-Khesraji and Abdel-Wahid, 1988
Italy	*O. crenata*	faba bean	70.4	-	Martelli, 1933
Morocco	*O. crenata*	faba bean	93	96	Klein et al., 1995
Syria	*O. crenata*	faba bean	45.5	30	ICARDA, 1989
	O. crenata	faba bean	32	29	Linke et al., 1990
Turkey	*O. crenata*	faba bean	94	-	Giray and Nemli, 1983
Yugoslavia	*O. cumana*	sunflower	96	-	Lekic, 1970
USSR	*O. cumana*	sunflower	-	80-90	Kapralov, 1974
	O. cumana	sunflower	-	37	Klyueva and Pamuchki, 1978
	O. ramosa	tobacco	-	10.5	Klyueva and Pamuchki, 1978
	O. cumana	sunflower	52.3-69.3	57.3	Klyueva and Bonduryanskaya, 1986
	O. ramosa	tabacco	12.3-20.3	15.7	Klyueva and Bonduryanskaya, 1986

2.2.1.3. Factors limiting *Phytomyza orobanchia* infestation

The effectiveness of *P. orobanchia* in the control of *Orobanche* is greatly reduced by the following factors : (i) the use of intensive agrotechnical measures, and (ii) action of natural enemies. The use of intensive agrotechnical measures includes soil cultivation, crop rotation and pesticide application. These factors affect the mass reproduction of *P. orobanchia* population. In autumn and spring, during preparation of soil for sowing, especially by deep ploughing, hibernating puparia could be destroyed and/or buried which inhibits the emergence of the fly. Another factor which may limit the effect of *P. orobanchia* is crop rotation which leads to disappearance of broomrape in the place where they occurred in the previous year, so that it affect the insect by the absence of the main host plant. A further problem is the use of pesticides (insecticides) against crop pests which can largely eliminate *P. orobanchia* (Mihajlovic, 1986, Parker and Riches, 1993, Schroeder, 1994).

Another limiting factor that reduces the activity of *P. orobanchia* is the occurrence of natural enemies (parasite, predators and diseases) of the fly it-self. Spencer (1973), Lekic (1974), Kovalev (1977), Klyueva et al.(1978), Puzzilli (1983), Mihajlovic (1986), Horvath (1983a, 1987), Sauerborn (1991) and Parker and Riches (1993) reported the high degree of parasitation of *P. orobanchia*. The most important parasites reported are *Crataepiella carlinarum* Szel. (Hymenoptera: *Eulophidae*), *Sphegigaster orobanchiae* Kurd. (Hymenoptera: *Pteromalidae*), *Cyrtogaster vulgaris* Walk. (Hymenoptera: *Pteromalidae*), and *Opius* spp. (Hymenoptera: *Braconidae*) In addition, *P. orobanchia* can be affected by fungus species (e.g. *Fusarium* spp).

2.3 Pathogens

Only a few examples for the use of pathogens for *Orobanche* control are reported in the literature. Many fungi were isolated from *Orobanche* spp. from different countries. The most common fungi identified are *Fusarium* species. Kott (1969) reported that *F. oxysporum* var. *orthoceras* App. & Woll. was propagated on a chopped straw/maize meal medium and remained effective for 80 days and achieved good results on *O. cumana* spp. parasitizing tobacco and water melon. The fungus causes rot at the link between host and parasite, which can stop further development. In different investigations this species showed potential as a promising mycoherbicides for the control of *Orobanche* spp. (Table 3).

The fungus *F. oxysporum* Schecht. has no pathogenic effect on cabbages and tomatoes (Timchenko and Dovgal, 1972), on carrot, faba bean, maize, sunflower, tobacco, tomatoes, flax and wheat (AL-Menoufi, 1986; Mazaheri et al., 1991; Bedi, 1994) and on water melon (Schroeder, 1994). The non-pathogenicity of this fungus to tomato plants was confirmed by Hallmann and Sikora (1994).

Further species referred to as *F. orobanches* had been used by Kabulov and Khalimov (1974) to control *O. aegyptiaca Press.*, and Wan and Wang (1991) also reported successful results using *F. orobanches* against *O. aegyptiaca* in China.

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Table 3: Results of *F. oxysporum* f.sp. *orthoceras* application for *Orobanche* control

Country	*Orobanche* sp.	Host[a]	Inoculum substrates	Control success (%)	Yield increase %	Author
Bulgaria	*O. cumana*	S	maize meal, barley, wheat straw, soil	>84*	-	Bedi and Donchev, 1991
				>90**	-	Bedi and Donchev, 1991
	O. cumana	S	barley grain, wheat straw	>84*	-	Bedi, 1992
			maize meal	74-90**		Bedi, 1992
	O. cumana	S	-	90	-	Bedi, 1994
Egypt	*O. crenata*	F	-	21-85	-	AL-Menoufi, 1986
Hungary	*O. ramosa*	T	-	>90	-	Hodosy, 1981
Iran	*O. aegyptiaca*	To		75	80	Mazaheri et al., 1991
USSR	*O. agyptiaca*	To	chopped straw,	86-100	-	Kott, 1969
	O. aegyptiaca	W	maize meal	84-88	-	Kott, 1969
	O. mutellii	C	-	44-67	-	Timchenko and Dovgal, 1972
	O. aegyptiaca	W	-	90-97**	278***	Panchenko, 1974
	O. aegyptiaca	W	river sand, maize meal	90-95**	-	Panchenko, 1981

*: Results of the glasshouse trail. The fungus was applied at a rate of 10 g/Kg of soil
**: Results of the field trail. The fungus was applied at a rate of 80-100 g/m^2
***: Kg/h
a: Host plants: **C**: cabbage, **F**: faba bean, **S**: sunflower, **T**: tomato, **To**: tobacco, **W**: water melon
-: Not reported.

Other pathogenic fungi which have been recorded to infect *Orobanche* species including *Sclerotium rolfsii* on *O. cernua* (Nagarkatti and Manjunath, 1976), *F. solani* (Mart.) Sacc., *Alternaria* spp. and *Sclerotinia* spp. on rotted *O. crenata* fruits in Egypt (AL-Menoufi, 1986). *F. lateritium* Ness ex Fr., *F. gibbosum* App. & Woll.and *Sesquicillium microsporum* appeared to be pathogenic on *O. mutellii* L. and *F. sambucinum* Fckl. pathogenic on *O. cernua* in Armenia (Taslakh'yan and Grigoryan, 1978). Sixty five percent control of *O. ramosa* and *O. mutellii* parasitizing tobacco was obtained by Bozoukov and Kouzmanava (1994) using *F. lateritium* in Bulgaria. Moreover, *Rhizoctonia solani* Kuhn. was isolated from soil in which *O. ramosa* was apparently diseased (Gold et al., 1979).

A recent survey in Syria, Morocco and France on *O. crenata* and *O. minor* yielded 16 species of fungi that belong to nine genera (Table 4). Of these the common ones are *Alternaria*, *Fusarium* and *Ulocladium* (Linke, 1992). *Ulocladium atrum* Preuss. has promising potential in destroying underground tubercles and emerged shoots of *O. crenata*, provided temperature was around 20°c and the relative humidity between 50% to 80% (Linke et al., 1992).

Table 4: Fungi identified by Linke (1992) which were not reported before from *Orobanche* spp.

Fungus	Not reported before from	
	Orobanche	*O. crenata*
Alternaria alternata (Fr.) Keissler	x	x
Alternaria infectoria E.Simmons	x	x
Aspergillus niger V. Tieghem	x	x
Cladosporium herbarum (Pers.) Link.	x	x
Cochliobolus spicifer Nelson	x	x
Cylindrocarpon sp.	x	x
Fusarium compactum (Wollenw.)Gordon	x	x
Fusarium equiseti (Corda.)Sall.		x
Phomopsis sp.	x	x
Stemphylium botryosum Wallr.		x
Ulocladium atrum Preuss.	x	x
Ulocladium botrytis Preuss.	x	x
Ulocladium consortiale (Thum.)Simmons	x	x

3. BRIEF DESCRIPTION OF SITES

3.1 Nura Era Horticulture Development Enterprise

Nura Era state farm

The Nura Era state farm is one of the several farms in the Upper Awash Valley which have been merged to form the Nura Era Horticulture Development Enterprise (N.E.H.D.E) (Fig. 2). It is located near Metahara, at a distance of about 180 Km east of Addis Ababa. Details about the farm are given in Table 5. Vegetables, mainly tomato, onion, pepper, beans, grapes and various types of fruits are cultivated in irrigated cropping system. In this area, *O. ramosa, O. cernua* and *O. minor* were found. The major crop heavily attacked in this farm is tomato. Wild hosts are also attacked (e.g. *Xanthium* spp.).

Merti Jeju state farm

This farm adjoins the Nura Era state farm and belongs also to the N.E.H.D.E. It is located at a distance of about 163 Km east of Addis Ababa. Details about the farm are given in table 5. Tomato, onion, beans, maize, cotton, teff, melon and orange are grown by irrigation. The tomato/orange processing factory owned and run by to the enterprise is located in Merti Jeju. Only *O. ramosa* and *O. cernua* were found in this area.

Table 5: Characterization of Nura Era and Merti Jeju state farms

Farm	Area (ha)	Elevation (m)	Rainfall mm/annum	Mean temp. (C°) max.	min.	Soil texture
Nura Era	3500	1100	400-700	30-32	14	loamy
Merti Jeju	1700	1200	400-700	30-32	14	loamy

Modified from Sherif and Gelma (1992)

3.2 Ziway state farm

The Ziway state farm is located in the Rift Valley at a distance of about 170 Km south of Addis Ababa, and at an altitude of 1640 m above sea level (Fig. 2). Average annual rainfall during the months of June to September is approximately 600 mm (Hoschle, 1986), . The soil can be described as loamy-clay sand. The main crops grown by irrigation are cutflower and vegetables as well as subtropical types of fruits and grapes. Tomato cultivation was stopped some years ago because of the excessive infestation by *O. ramosa*.

3.3 Malima farm (Eastern Shoa)

The Malima farms are private farms which are located in the Rift Valley at a distance of about 90 Km south of Addis Ababa, and at an altitude of 1620 m above sea level. The data

are about the same as for the Ziway farm. Tomato, egg plant and pepper are the main vegetables grown in the area. In this area, both *O. ramosa* and *O. cernua* were found.

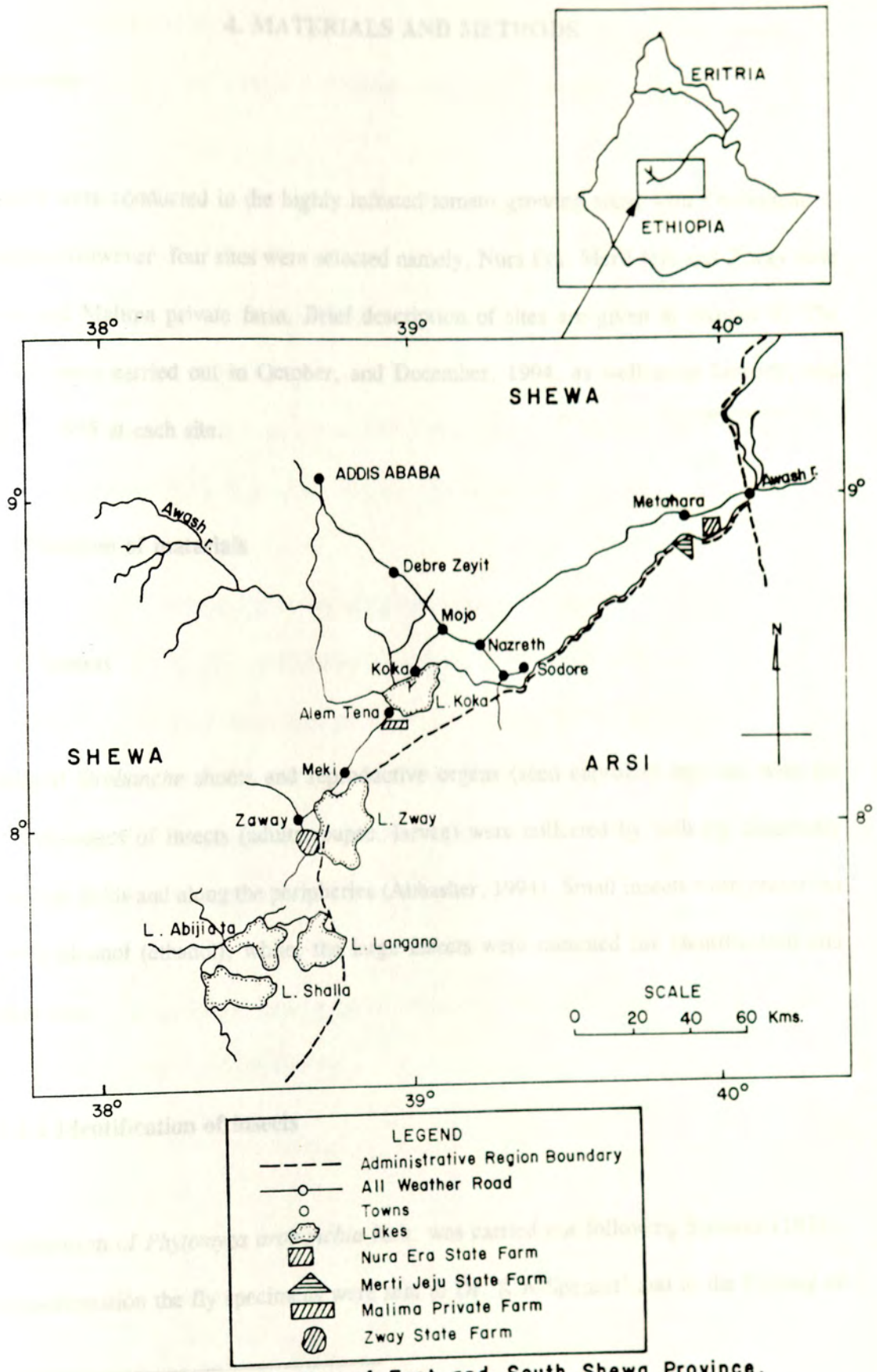


Figure 2: Map of East and South Shewa Province, Showing the areas of the study

Source : modified from Ethiopian Mapping Authority, 1995.

4. MATERIALS AND METHODS

4.1 Survey

Surveys were conducted in the highly infested tomato growing areas with *Orobanche* in Ethiopia. However, four sites were selected namely, Nura Era, Merti Jeju and Ziway state farms and Malima private farm. Brief description of sites are given in chapter 3. The surveys were carried out in October, and December, 1994, as well as in January, and March, 1995 at each site.

4.2 Collection of materials

4.2.1. Insects

Damaged *Orobanche* shoots and reproductive organs (seed capsules) together with the different stages of insects (adults, pupae, larvae) were collected by walking diagonally across the fields and along the peripheries (Abbasher, 1994). Small insects were preserved in 80% alcohol (ethanol), while, the large insects were mounted for identification and further tests.

4.2.1.1 Identification of insects

Identification of *Phytomyza orobanchia* Kalt. was carried out following Spencer (1973). For confirmation the fly specimens were sent to Dr. K.A.Spencer[1] and to the Faculty of

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1: K.A.Spencer, Exwell Farm, Bray Shop, Callington PL17 8QJ, Cornwall, England.

Biology, University of Bielefeld, Germany. Other insects (Lepidoptera) were sent to the Center for Environmental Research, Leipzig, Germany for identification.

4.2.1.2 Preliminary evaluation of *P. orobanchia* for the control of *Orobanche* spp.

In order to estimate the percent of damage caused by *P. orobanchia* to *O. ramosa* and *O. cernua*, three heavily infested locations by broomrape were randomly selected. For this study, a total of 180 broomrape plants (90 plants from each species) were taken from tomato fields of the Nura Era, Merti Jeju and Malima farms.

Thirty broomrape plants at full flowering to early ripening stage were collected randomly per field, with at least one meter between samples. In the laboratory, the percentage of damaged and undamaged broomrape plants by *P. orobanchia* was determined by cutting the *Orobanche* shoot. In addition, fifteen seed capsules were randomly detached from each damaged plant, five from the top, the middle and the bottom of the shoot respectively, to determine the percentage of infested capsules per plant.

The extent of damage on *O. ramosa* and *O. cernua* caused by *P. orobanchia* at Malima, was evaluated using a scale (Table 6).

Table 6: Evaluation of the extent of damage caused by *P. orobanchia* on *Orobanche* plants

Damaged capsule (%)	Class of damage
1-33	slightly
34-67	moderately
68-100	severely

Density of *P. orobanchia* population on *O. ramosa* was determined by counting larval and pupal stages following the dissection of the capsules and stems.

4.2.2 Pathogens

A total of 20 tomato fields (5 per site) were selected based on the severity of *Orobanche* infestation. During the survey period 5-10 *Orobanche* plants were collected by walking diagonally across the fields and along the peripheries. Only diseased broomrape plants which showed symptoms of rotting, wilting, browning, lesions or mycelial growth or dead plants were collected and kept in plastic bags. The bags were sent to the laboratory and stored at room temperature for 1-2 days until the isolation of the fungus was carried out.

4.2.2.1 Isolation of the pathogens

Isolation was carried out at the Biology Department of Addis Ababa University. For sterilization, small bits of infected *Orobanche* tissues were placed in the Petri dish

containing 0.1% mercuric chloride solution for 1-2 minutes and then rinsed three times in three Petri dishes containing sterilized water. Then the pieces of infected tissues were aseptically plated on the solidified media (malt extract agar). The plates were incubated under room temperature for 24-72 hours. Repeated purification was done to obtain pure cultures. Purified isolates were maintained on malt extract agar for further tests.

4.2.2.2 Identification of fungi

Purified isolates were cultured on Potato-Dextrose Agar (PDA) and Special Nutrient poor Agar (SNA) (Nirenberg, 1981) for identification. Fungal isolates were incubated for 48 hours under room temperature (20 °C), and then transferred to the black fluorescent light alternating with darkness (12h/12h) for one week. Morphological identification was done for all isolates based on the colour and shape of the aerial mycelium. Fungal genera and species were identified under the light microscope by using shape, size, colour and manner of arrangement of spores, as well as the shape and the colour of the sporophores or fruiting bodies. Slides from all isolates were prepared by adding one drop of cotton blue. Identification of fungi was carried out following Booth (1971), Nirenberg (1981), and Gerlach and Nirenberg (1982). Purified fungal isolates were sent to the Federal Biological Research Centre for Agriculture and Forestry, Institute for Microbiology, Berlin, Germany for confirmation.

4.2.2.3 Media components

The media were autoclaved for 20 minutes at 15 bar pressure (120 °C). After that the media were cooled down to about 45-50 °C, and dispersed at a rate of 10 ml using 9 cm

Petri dishes under the sterile bank. The following media were used:

A) Malt Extract Agar

This medium was used for isolation of the plant pathogenic fungi (Berecket, 1983). It constituted the following per liter of distilled water:

Malt extract	30.0 g
Mycological peptone	15.0 g
Agar	5.o g

B) Potato-Dextrose Agar (PDA)

Ready-mixture powder of Serra company (Germany) was used which contains 39 g PDA L^{-1} of deionized water:

Potato infusion	4 g
Dextrose	20 g
Agar	15 g

C) Special Nutrient poor Agar (SNA)

KH2PO4	1.0 g
KNO3	1.0 g
MgSO4	0.5 g
KCl	0.5 g
Glucose	0.2 g

Sucrose	0.2 g
Agar	20.0 g
deionized water	1000.0 ml.

PDA and SNA media were used for culturing, maintenance, identification and re-isolation of fungi. Twenty mg/l each of chloramphenicol and streptomycin were added to prevent bacterial growth.

D) Selective Fusarium Agar (SFA)

This medium was used for isolation of *Fusarium* species from plant tissue and debris (Burgess et al., 1988; Tio et al., 1977).

Dextrose	20 g
KH_2PO_4	0.5 g
$NaNO_3$	2 g
$MgSO_4.\ 7H_2O$	0.5 g
Yeast extract	1 g
1% $FeSO_4.\ 7H_2O$	1 ml solution
Agar	20 g
Deionized water	1 l

Pentachloronitrobenzene (1.0 g), streptomycin sulfate (0.1 g), and aureomycin sulfate (0.01 g) were added after autoclaving and cooling of the medium.

4.3 Viability of *Orobanche* seeds

The seeds of *Orobanche* species were first tested for their viability using Tetrazolium test procedure described by Moore (1985), Aalders and Pieters (1985) and modified by Linke, 1987, (cited in Dawoud, 1995). The parasite seeds were placed in small glass tubes to which 5 ml of 2,3,5-triphenyl tetrazolium chloride (TTC) was added. The tubes were firmly closed and incubated for 12 days at 30 °C in the dark. Following the incubation period, the TTC-solution was filtered through a nylon mesh (100 μm), and the seeds were washed with deionized water. In order to remove the dark color of the seed coat, NaOCl (6%) solution was added to the seeds for 5 minutes. Lastly, the seeds were washed with tap water for 3 minutes and dried using filter paper.

Evaluation was carried out under a binocular microscope by taking 4 samples (100 seeds per sample) from the treatment. The embryo of the viable seeds stained red with TTC, whereas the embryo of unviable seeds remained entirely uncolored.

Seed viability of different *Orobanche* spp. was determined from the percentage of seeds with metabolic activity. All *Orobanche* seeds showed a viability of at least 65%. High viability was observed on *O. ramosa* seeds collected from Sudan compared to seeds from Ethiopia and Germany collected in 1985 and 1986 respectively (Table 7). On the other hand, low viable seeds were estimated from newly harvested *O. ramosa* from Ethiopia.

Table 7. Response of *O. ramosa* seeds of different origins to 2.3.5-Tetrazolium Chloride.

Year of harvest	Country (location)	Host	Viability (%)
1985	Ethiopia (Ziway)	Tomato	65
1985	Sudan (Blue Nile Bridge)	-	90.5
1986	Germany (Hohenheim)	Tomato	65
1995	Ethiopia (Nura Era)	Tomato	67.5

4.4 Pathogenicity of different fungal isolates to *Orobanche* spp.

Seeds

For this experiment *O. ramosa* seeds with 65% viability from Ethiopia were used. The parasite seeds were immersed in 1% sodium hypochlorite (NaOCl) for 5 minutes for surface sterilization and then thoroughly washed with tap water, and air dried under room temperature. Tomato seeds from Germany (variety money maker) were used for all experiments.

Soil

A mixture of clay and sand (1:1, v/v) soil was sterilized for 8 hours using steam sterilization apparatus before the soil was used in pot trials. All pot experiments were conducted in plastic pots (13x13x13 cm diameter), with a capacity of 2 kg soil.

Preparation of the inoculum

Ten fungal isolates were tested for their pathogenicity to the seeds and early stages of *Orobanche*. Inocula of the fungi were prepared on wheat grain (Bedi and Donchev, 1991). One third of 2000 ml wide-mouth glass flask was filled with wheat grain and hydrated overnight. Excess water was poured off and the medium was autoclaved at 121 °C and 15 bar pressure for 1 hour. After 24 hours, each flask was inoculated with 20 ml of spore suspension, prepared from 2 weeks old cultures grown on SNA media. The flasks were incubated at room temperature (20 °C) for 14 days (Fig. 3).

Pathogenicity of fungal isolates to the early stages of *O. ramosa*

The fungal inocula were incorporated into the soil at a rate of 20 g (inoculated grain)/pot (10 g/kg of soil). Seeds of *O. ramosa* (140 mg per pot) were mixed together with the inoculum into the soil. *O. ramosa* seeds were preconditioned for one week in the pots, and then 3 tomato plants were sown per pot. Two weeks after sowing, the tomato plants were thinned to one plant per pot, and fertilized with 2% Super Wuxal N-P-K (8-8-6) at a rate of 100 ml per pot. Tomato grown in pots with *O. ramosa* only (C-), pots with *O. ramosa* and autoclaved wheat grain (C-+N), pots with tomato only (C+), and pots with out *O. ramosa* but with autoclaved wheat grain (C++N) were kept as controls. The treatments were replicated four times, and arranged as a completely randomized design. The experiment was conducted in the glass house with temperature ranging between day/night 15 °C and 30 °C and the relative humidity between 50% to 80%. The plants were supplied with light from HQLR-Lamps (1000 W), and irrigated when required.

In order to evaluate the pathogenicity of the fungal isolates, the number of emerged *O. ramosa* shoots were counted weekly, starting with the emergence of the first *Orobanche* shoot. Dry weight of tomato shoots and roots as well as the dry weight of *Orobanche* was also measured as additional parameters.

4.5 Re-isolation of fungi for root chamber experiment

The virulence of *F. oxysporum* and other fungi decreases in the absence of the host. In order to re-gain the virulence of the fungal isolates the following trial was conducted in the vegetation hall. Tomato seeds were sown together with *O. ramosa* seeds in the same

pots described above. After emergence of *Orobanche* shoots, tomato plants were taken out of the pots gently along with a ball of soil. Emerged and underground *Orobanche* shoots were injured vertically (1 cm long) using a sterile scalpel. The wound was inoculated with fungal spores and mycelia, and immediately sealed with petroleum jelly to prevent secondary infection. Each isolate was tested on four plants. After one week, the newly infected tissues were plated on PDA media for re-isolation and re-identification. After culturing the isolates were tested for their pathogenicity in root chamber experiments.

4.6 Root chamber experiment

The aim of this experiment was to determine the effect of fungal isolates on the early developmental stages of *O. aegyptiaca*. It has been reported by Musselman (1986), Linke et al. (1989) and Parker and Riches (1993) that *O. ramosa* and *O. aegyptiaca* are extremely similar species and grouped as *O. aegyptiaca/O. ramosa*. This is the reason why *O. aegyptiaca* seeds with 95% viability were used in the root chamber experiment. The parasite seeds were surface sterilized using 1% NaOCl.

Root chambers as described by Linke and Vogt (1987), Abbasher (1994) and Dawoud (1995) were used. The backs and sides of the chambers were made of non-transparent hard plastic, while the front was closed with clean transparent plexiglass (0.3x6x20 cm). Each chamber has a dimension of 20x6x1 cm in length, width and depth, respectively. The seeds of the parasite were evenly sprinkled on a strip of wet microfiber glass-filter paper (Whatman GF/A) (26X6 cm) and placed in the root chamber facing the plexiglass (Fig. 4D). The cavity of the chamber was filled with sterilized sand. For preconditioning, the sand was kept moist and the chambers were wrapped in black plastic and incubated under

room temperature (20 °C) for 7 days (Fig. 4C). Following this conditioning period, two tomato seeds were sown in each chamber between the filter paper and the lid (Fig. 4C). The chambers were placed at an angle to enhance the growth of tomato roots near the surface of the plexiglass plate. The chambers were transferred to the glass house where the temperature ranged between 15 °C and 30 °C and the relative humidity between 50% and 80%, and irrigated when necessary. Ten ml of mycelium and spore suspension, which was prepared by adding deionized water to 14-days-old fungal cultures, was added after 6 weeks to cover the surface of the tubercles (Linke, 1992). At least 10 tubercles were inoculated per each chamber. Root chamber with *Orobanche* seeds and tomato plant but without inoculum was kept as control. Evaluation was done two weeks after inoculation by calculating the percentage of healthy, diseased and killed tubercles.

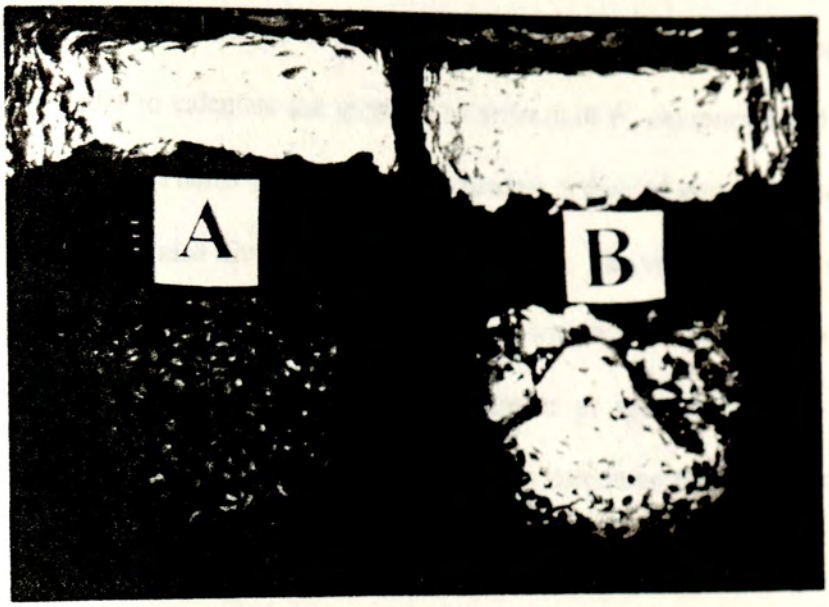


Fig.3 : Inoculum production of *Fusarium* spp. on autoclaved wheat grain (A) uninoculated grain (B) inoculated grain

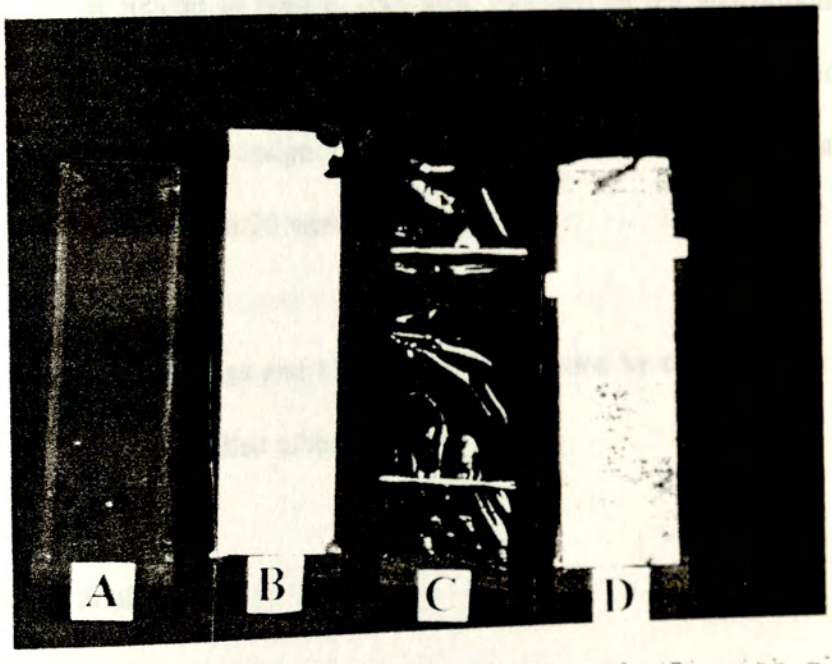


Fig.4 : Root chambers showing (A) the vessel 'B' with glass-filter paper (Whatman GF/A) 'C' wrapped in black plastic 'D' sprinkling of *O. aegyptiaca* seeds on the chamber

In a second root chamber trial, testing of isolates was repeated when pathogenicity was observed. In order to calculate the spore concentration of *F. oxysporum* and *F. nygamai* a counting chamber (Thoma-) was used. The chamber is divided into 16 big squares, each one of these squares also divided into 16 small squares. The volume of one small square is 0.00025 mm^3 (0.05 mm x 0.05 mm x 0.1 mm), with 4 x 10^6 multiplication factor for the number of spores per ml. The average number of spores of 3 big squares was calculated and then multiplied by 4 x 10^6 in order to determine the number of spores per ml.

Spore suspensions of *F. oxysporum* and *F. nygamai* were prepared from 14 days old cultures by adding deionized water with 0.05% Tween 20. Ten ml of spore suspensions with 0.5 x 10^6 spores/ml of both isolates were spreaded on the underground stages of *Orobanche* in the root chamber. The treatments were replicated three times and arranged as a completely randomized design. Uninoculated chambers and inoculated chambers with deionized water with Tween 20 were kept as control.

Evaluation was done 10 days and 15 days after inoculation by estimating the percentage of healthy, diseased and killed tubercles.

4.7 Statistical analysis

In order to evaluate the damage caused by *P. orobanchia* on *Orobanche*, percent of infested seed capsules per plant was calculated using the following formula:

$$PIC = \frac{NIC}{NIC + NHC} 100$$

Where:

PIC = Percentage of infested seed capsules

NIC = Number of infested seed capsules

NHC = Number of healthy seed capsules

The percentage of infested seed capsules per 20 plants was averaged in order to calculate the overall percent of infested capsules per each *Orobanche* species.

All pot and root chamber experiments were arranged in completely randomized designs. The data were first checked for normal distribution using the modified Levene Test (Levene, 1960). The pot and root chambers experiments were subjected to analysis of variance (ANOVA). Where F-values were significant, subsequently Least Significant Difference (LSD) or Duncan's New Multiple-Range Test were used to determine the significant difference in the mean values at p= 0.05 (Steel and Torrie, 1980; Gomez and Gomez, 1984).

Data were analyzed at the university of Hohenheim using IBM microcomputers and SAS System Software (SAS-Institute Irc., 1990).

5. RESULTS

5.1 Insects

Six different insects were observed to associate with *Orobanche* spp. in the area surveyed. However, only *P. orobanchia* was identified. One further unidentified insect belonging to the family *Noctuidae* (Lepidoptera) was found to feed on *Orobanche* as well (Appendix 1).

5.1.1 Preliminary evaluation of *P. orobanchia* for the control of *Orobanche* spp.

Three locations were investigated to evaluate the effectiveness of *P. orobanchia* in controlling *Orobanche* spp. under natural conditions. The fly was found only in Malima with 73.3% and 66.7% infestation of *O. ramosa* and *O. cernua*, respectively (Table 8).

In order to estimate the level of infestation on individual plants, 300 seed capsules of each *Orobanche* species were investigated. Of these, 81.4% and 79.7% of *O. ramosa* and *O. cernua* were damaged respectively (Table 9). Figure (5) shows the extent of damage caused by *P. orobanchia* on *O. ramosa* and *O. cernua* plants. The result indicated that 85% of each *O. ramosa* and *O. cernua* plants were severely damaged.

About 8 larvae and pupae on average were recorded per *O. ramosa* plant, among which 34.5% were found in the capsules and 65.6% were found in the stems (Table 9).

Table 8: Infestation of *O. ramosa* and *O. cernua* plants with *P. orobanchia* at three localities in Ethiopia, December, 1994

Location	*O. ramosa*			*O. cernua*		
	investigated plants	infested plants	%	investigated plants	infested plants	%
Nura Era	30	0	0	30	0	0
MertiJeju	30	0	0	30	0	0
Malima	30	22	73.3	30	20	66.7

Table 9: Percent (average) of damage of *O. ramosa* and *O. cernua* seed capsules caused by *P. orobanchia* at Malima (Ethiopia), December, 1994*

O. species	Infested capsules				Larvae and pupae per plant		
	A	No.	%	B	No.	per capsules (%)	per stems (%)
O. ramosa	15	10.6	81.4	39.6	8	34.5	65.6
O. cernua	15	10.1	79.7	-	-	-	-

*: Twenty plants from each *Orobanche* species were investigated
A: Average No. of seed capsules examined per plant
B: With larvae and pupae (%)
-: Not estimated

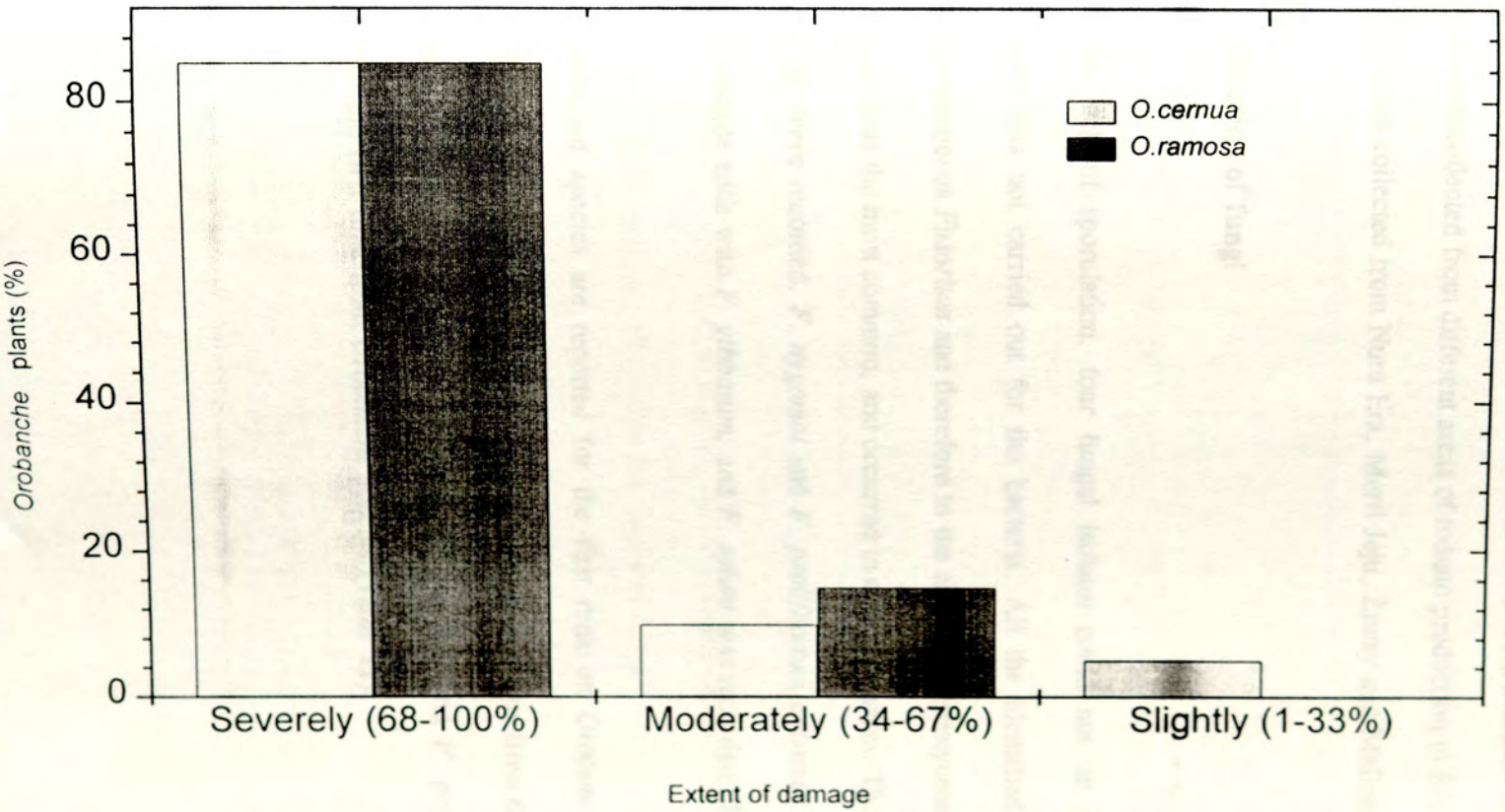


Figure 5: Extent of damage on *O. cernua* and *O. ramosa* plants caused by *P. orobanchia* at Malima (Ethiopia)

Note: twenty plants were investigated from each *Orobanche* species.

5.2 Isolation of fungi and bacteria

A total of 24 fungal isolates and 4 bacteria were isolated from diseased *Orobanche* plants which had been collected from different areas of tomato production in Ethiopia. Six of the fungi each were collected from Nura Era, Merti Jeju, Ziway and Malima (Table 10).

5.3 Identification of fungi

Due to the lack of sporulation, four fungal isolates could not be identified. Also identification was not carried out for the bacteria. All the identified fungal species belonged to the genus *Fusarium* and therefore to the class Deuteromycetes (Appendix 2). *F. oxysporum* was the most common, and occurred in eleven samples. Three samples with *Fusarium* sp. were recorded. *F. nygamai* and *F. proliferatum* occurred in two samples each. One sample each with *F. gibbosum,* and *F. solani* was recorded (Table 11).

All the identified species are reported for the first time on *Orobanche* plants from Ethiopia. Sixteen of them were isolated from *O. ramosa*, and four from *O. cernua* (Table 11). In addition, the results pointed out that, *F. nygamai* and *F. proliferatum* were identified for the first time from *Orobanche* species (Table 12).

Table 10: Fungi isolated from infected *Orobanche* plants samples in areas surveyed in Ethiopia

Fungus/location*	Merti Jeju	Malima	Nura Era	Ziway
F. gibbosum	-	-	7	-
F. nygamai	-	12	4	-
F. oxysporum	6,18,28	9,19	3,11	1,14,15,16
F. proliferatum	5	-	-	23
F. solani	-	-	2	-
Fusarium sp.	13	26	8	-
Unidentified	22	17,21	-	20

*: Figures represent isolates sample number. All *Orobanche* plants were selected randomly by walking diagonally across the field and along the peripheries.

Table 11: Number of *Fusarium* species isolated from diseased *Orobanche* spp. for the first time from Ethiopia.

Sample no.	Fungus	Isolated from[a]	
		O. ramosa	*O. cernua*
4, 12	*F. nygamai*	1	1
1, 3, 6, 9, 11, 14, 15, 16, 18, 19, 28	*F. oxysporum*	9	2
5, 23	*F. proliferatum*	2	-
2	*F. solani* var. *solani*	-	1
8, 13, 26	*Fusarium* sp.	3	-
7	*F. gibbosum*	1	-
Total		16	4

[a]: Figures represent number of samples from which the isolates were identified.

Table 12: Fungi isolated from infected *Orobanche* plants in Ethiopia and their known host range

Fungus	Not reported before from[a]		
	O. cernua	*O. ramosa*	*Orobanche*
F. gibbosum	-	r	r
F. nygamai	-	-	-
F. oxysporum	r	r	r
F. proliferatum	-	-	-
F. solani	-	r	r
Fusarium sp.	r	r	r

[a]-, not reported before; r, reported before

5.4 Pathogenicity of different fungal isolates to *O. ramosa*

Ten fungal isolates were screened for their pathogenicity as soil-borne pathogens to the early stages of *O. ramosa* by incorporating their inocula pre-planting into the soil. All tested isolates significantly reduced the total number of emerged *O. ramosa* plants (Table 13). Of these, four isolates showed significant increase in tomato dry weight compared to the control (C-). *F. oxysporum* (isolate 1), *F. nygamai* (isolate 4), *F. gibbosum* (isolate 7) and *Fusarium* sp. (isolate 8) increased tomato dry weight by 32.1%, 30.3%, 30.3%, and 30.3% respectively (Table 13). The other tested isolates resulted in little or 0% increase in tomato dry weight. No disease symptoms were observed on the emerged *Orobanche* plants due to the presence of all tested isolates.

However, symptoms like wet rotting of the underground seedlings, and change in the tubercles colour to dark brown or black were observed in pots inoculated with *F. oxysporum* (isolate 1), *F. nygamai* (isolate 4), *F. gibbosum* (isolate 7) and *Fusarium* sp (isolate 8).

The effect of the fungal isolates on the emergence of *O. ramosa* was evaluated by counting the number of emerged shoots weekly. Four *Fusarium* spp. caused a delay in the emergence of *O. ramosa* (Fig. 6). However, the first emerged *O. ramosa* shoot was noticed 82 days after sowing of tomato in the control (C-). There was no significant difference in emergence of *O. ramosa* between the *Fusarium* spp. treatment and the control (C-) in the first week of emergence, while *F. oxysporum* (isolate 1) totally inhibited the emergence of *Orobanche* during the same week. *F. gibbosum*, *F. nygamai* (isolate 4) *F. oxysporum* (isolate 1), and *Fusarium* sp (isolate 8) were significantly delaying the

emergence in the 2nd, 3rd and 4th weeks (Fig. 10).

As a result of the effect of the same *Fusarium* spp., *Orobanche* dry weight was significantly reduced (Fig. 7a). The corresponding tomato height was increased (Fig. 8) but not significantly compared to the control (C-) (Fig. 7b).

Table 13: Influence of different fungal isolates on the emergence of *O. ramosa* and corresponding increase in tomato dry weight[a]

Tested isolates[b]	No. of emerged *O. ramosa*/pot	Reduction (%)	To ato dry weight (g/plant)	Increase (%)
F. oxysporum (isolate 1)	3.0 bc	79.3	7.4*	32.1
F. solani var. *solani* (isolate 2)	1.3 c	91.0	5.6	0
F. nygamai (isolate 4)	1.5 c	89.7	7.3*	30.3
F. proliferatum (isolate 5)	4.3 bc	70.3	6.7	19.4
F. gibbosum (isolate 7)	2.3 bc	84.1	7.3*	30.3
Fusorium sp. (isolate 8)	1.0 c	93.1	7.3*	30.3
Fusarium sp. (isolate 13)	0.5 c	6.6	5.5	0
F. oxysporum (isolate 16)	2.3 bc	84.1	6.7	19.4
F. oxysporum (isolate 19)	2.8 bc	80.7	6.9	23.3
Fusarium s.p (isolate 26)	7.0 b	51.7	5.6	0
Control (C-+N)	2.3 bc	84.1	5.7	1.8
Control (C-)	14.5 a	0	5.6	0
LSD (0.05)	5.3	-	1.7	-

[a]: Data are presented as the mean of one trial with 4 replicates. Means with the same letter are not significantly different.
[b]: Control (C-) include tomato and *Orobanche*, control (C-+N) include tomato + *Orobanche* + wheat grain.
*: Significantly different

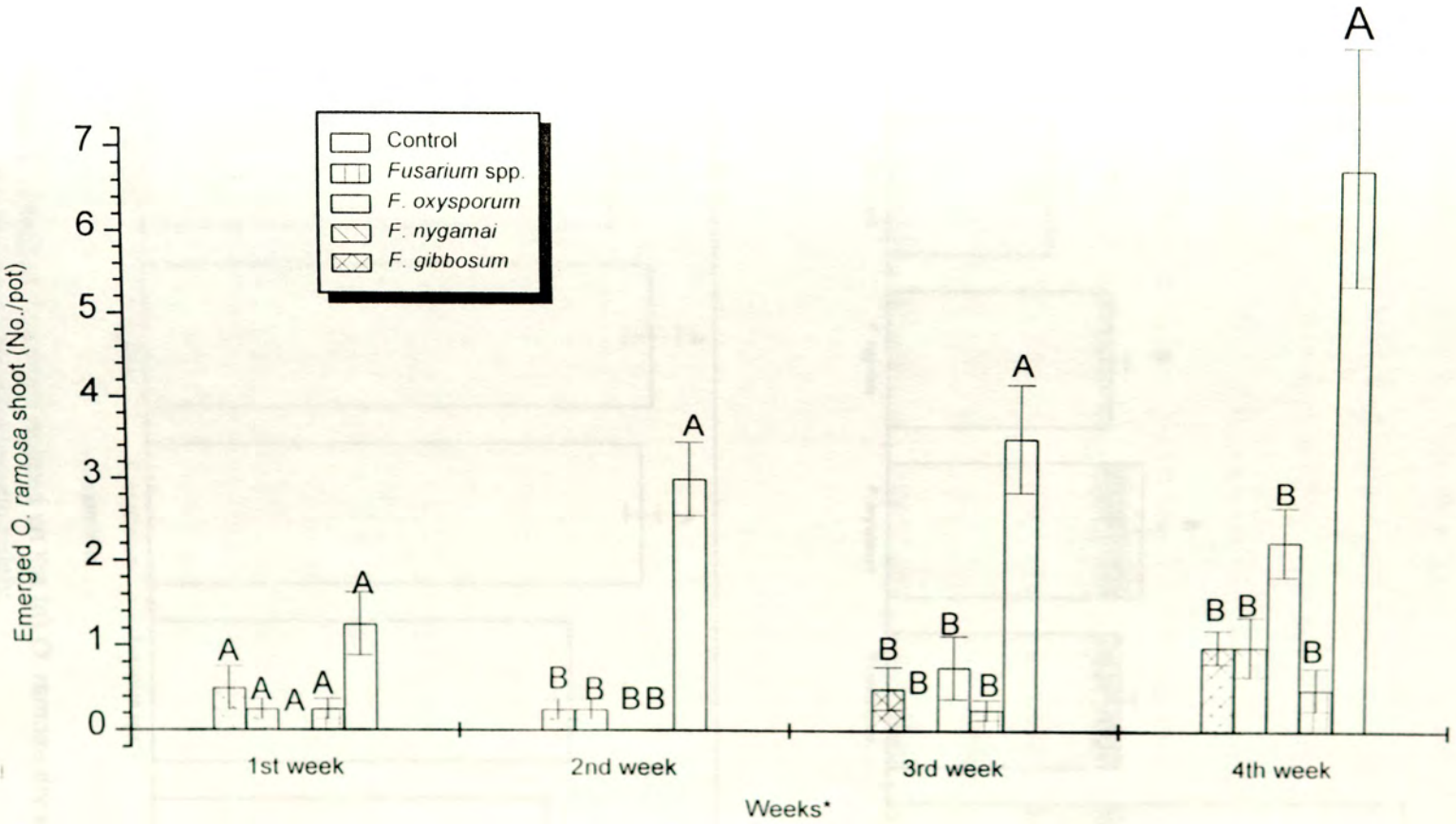


Figure 6: Effect of *Fusarium* isolates on the emergence of *O. ramosa*.

*: Weeks after emergence of the first *O. ramosa* shoot until harvesting of tomato plants.

Note: Data are presented as the mean of one trial with 4 replicates. Vertical lines represent the SE of the means. Means with the same letter per each week are significantly different from the control using LSD at $\alpha = 0.05$. Evaluation was done for all tested isolates, but only four of them which have shown significant increase in tomato dry weight were presented.

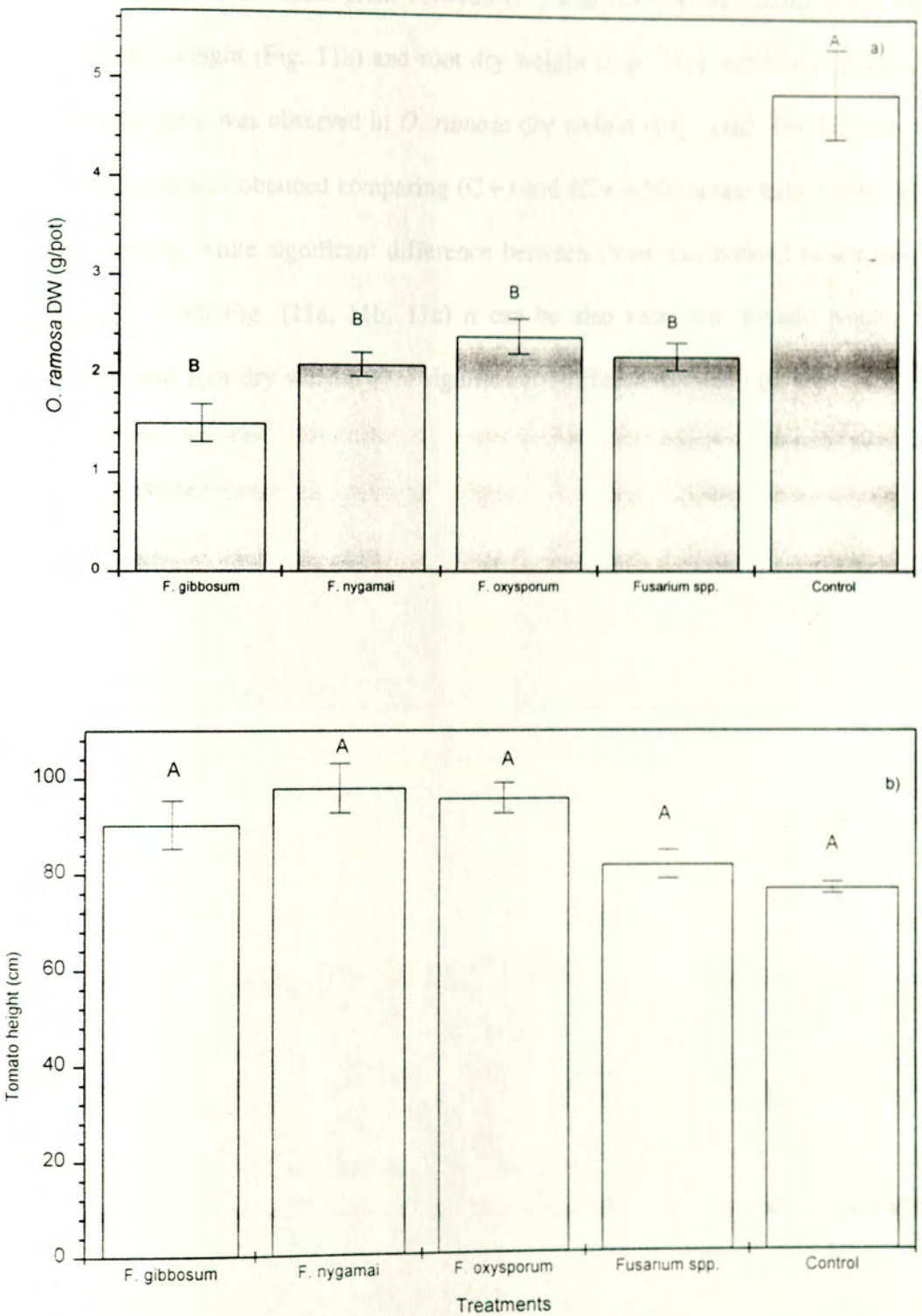
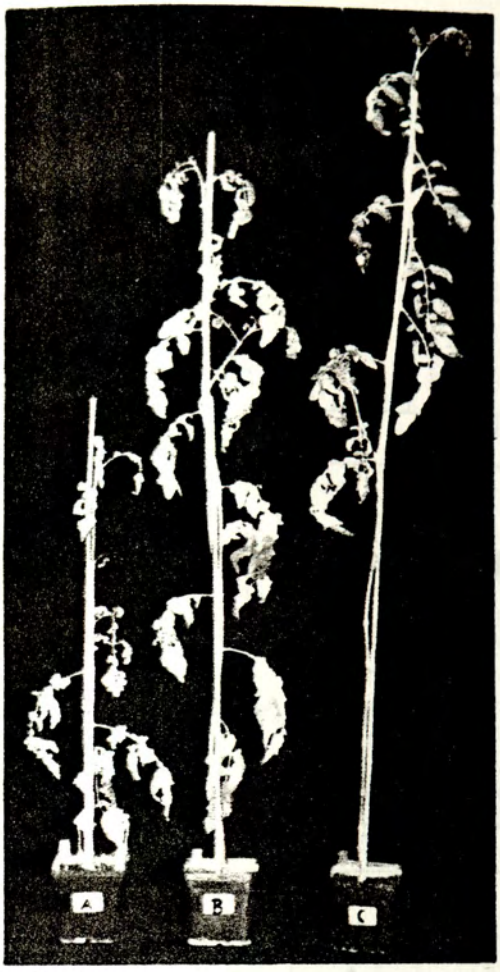


Figure 7: Effect of *Fusarium* isolates on the (a) *O. ramosa* dry weight, and (b) corresponding tomato height.

Note: Data are presented as the mean of one trial with 4 replicates. Means with the same letter are not significantly different at $\alpha = 0.05$. Vertical lines represent SE of the means. Evaluation was done for all tested isolates, but only four of them which have shown significant increase in tomato dry weight were presented.

The results of the control treatments comparison showed that no significant difference due to the presence of wheat grain between (C-) and (C-+N) in tomato height (Fig. 11a), shoot dry weight (Fig. 11b) and root dry weight (Fig. 11c), while significant difference between them was observed in *O. ramosa* dry weight (Fig. 11d). On the other hand, the same result was obtained comparing (C+) and (C++N) tomato height (Fig. 9) and root dry weight, while significant difference between them was noticed in tomato shoot dry weight. From Fig. (11a, 11b, 11c) it can be also seen that, tomato height, shoot dry weight and root dry weight were significantly different between (C++N) and (C-+N).

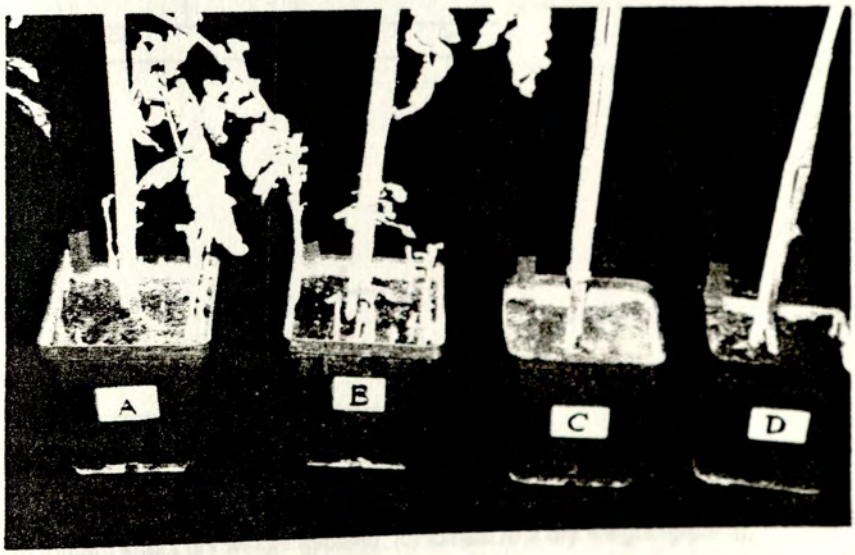


g 8 :Tomato height in the (A) control
th *O. ramosa* (B) *F. oxysporum* (C)
ntrol without *O. ramosa* and
eat grain



Fig.9 :tomato height in the control A
with *O.ramosa* (B) with *O. ramosa* and
wheat grain (C) without *O. ramosa* and
with wheat grain D without *O. ramosa*
and wheat grain

g. 10



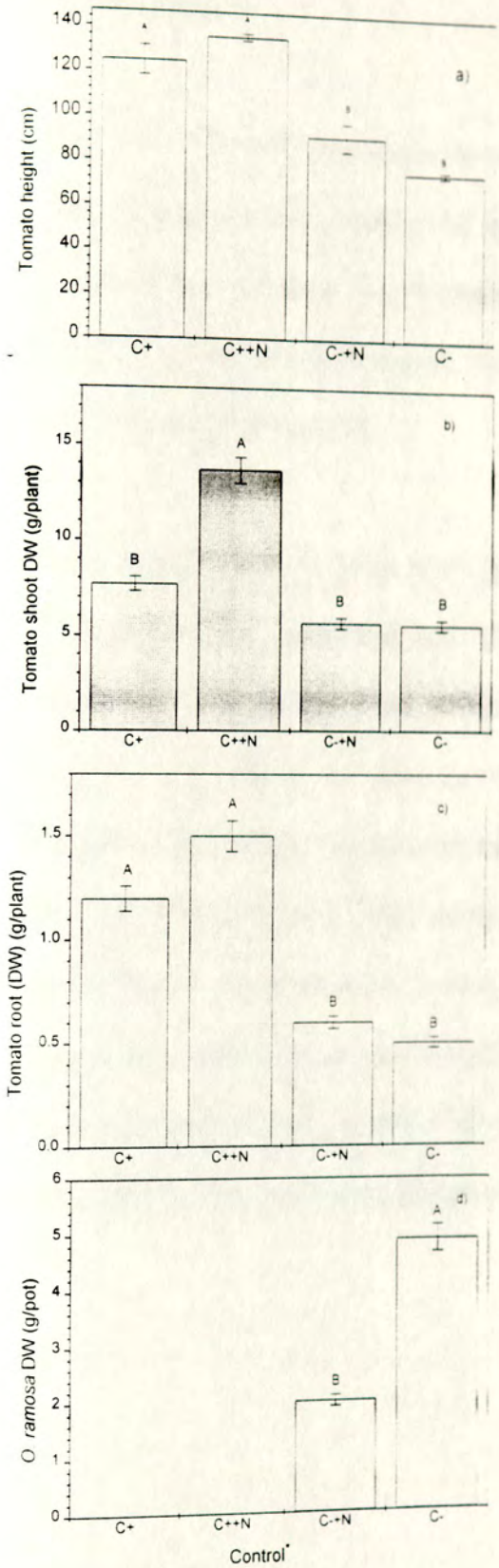


Figure 11: Effect of wheat grain in the control treatments (a) tomato height (cm), (b) tomato shoot dry weight (g/plant), (c) tomato root dry weight (g/plant), and (d) *O. ramosa* dry weight (g/pot)

Note: Data are presented as the mean of one trial with 4 replicates. Treatments with the same letter are not significantly different using Duncan's at $\alpha = 0.05$. Vertical lines represent SE of the means.

*: Control considered are (C+) tomato without *O. ramosa*, (C++N) tomato without *O. ramosa* but with wheat grain, (C-+N) tomato with *O. ramosa* and wheat grain, (C-) tomato with *O. ramosa* only.

5.5 Root chamber experiment

The results of the first root chamber experiment showed that only *F. oxysporum* (isolate 1) and *F. nygamai* (isolate 12) were found to be more pathogenic to *O. aegyptiaca* tubercles (Table 14). Disease symptoms like wet rotting, change in the tubercles colour to dark brown or black were observed. Therefore, these isolates were tested again in a separate trial to confirm their pathogenicity.

In the second root chamber trails, the effect of *F. oxysporum* and *F. nygamai* on the tubercles of *O. aegyptiaca* were observed 10 and 15 days after inoculum application. Both species significantly reduced the number of the healthy tubercles. However, *F. oxysporum* killed 81% and 82% of the tubercles 10 and 15 days after inoculation respectively compared to the controls. On the other hand, 41% and 48.6% of tubercles were killed by *F. nygamai* after 10 and 15 days respectively (Fig. 12a, 12b). As a result of the effect of both isolates on the tubercles, tomato plant performance was improved (Fig. 13). The colour of diseased tubercles was changed to dark brown or black compared to uninoculated tubercles (Fig. 14, 15). Symptoms like rotting were also observed. Later, the same symptoms were noticed on the underground *Orobanche* shoots (Fig. 16).

Table 14: Pathogenicity of the fungal isolates to *O. aegyptiaca* tubercles in the root chamber*

Isolate tested		pathogenicity to *O. aegyptiaca***
No.	species	
1	*F. oxysporum*	+++
2	*F. solani*	+
5	*F. proliferatum*	+
11	*F. oxysporum*	-
12	*F. nygami*	++
16	*F. oxysporum*	+
	control	-

*: Evaluation was done 2 weeks after inoculation with isolates

**: (-) no reaction, (+) 1-25% of the tubercles diseased/killed, (++) 26-50% of the tubercles diseased/killed, (+++) 51-75% of the tubercles diseased/killed, (++++) 76-100% of tubercles diseased/killed

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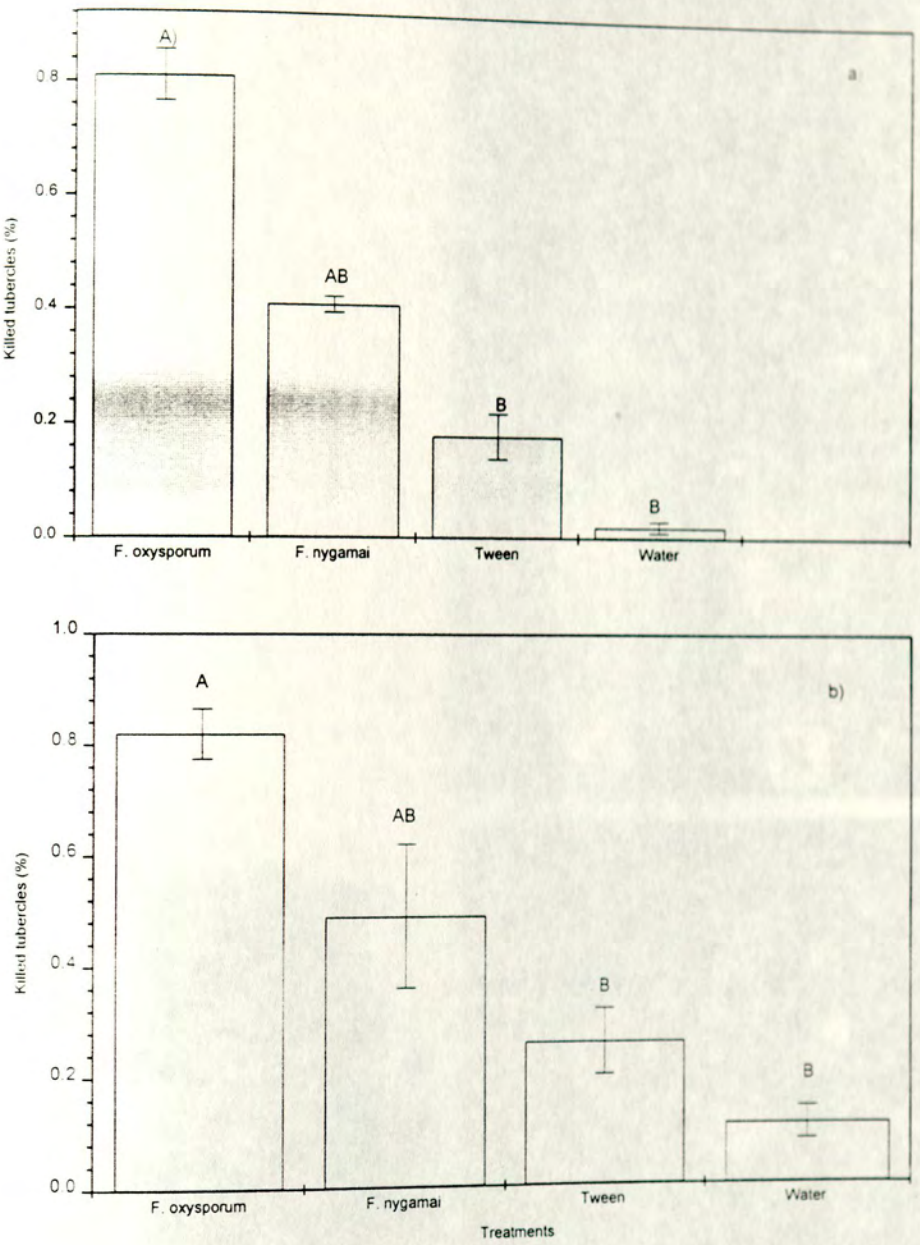


Figure 12: Effect of *F. nygamai* and *F. oxysporum* as compared to two controls (tween and water) on the underground stages of *O. aegyptiaca* at a) 10 and b) 15 days after inoculation.

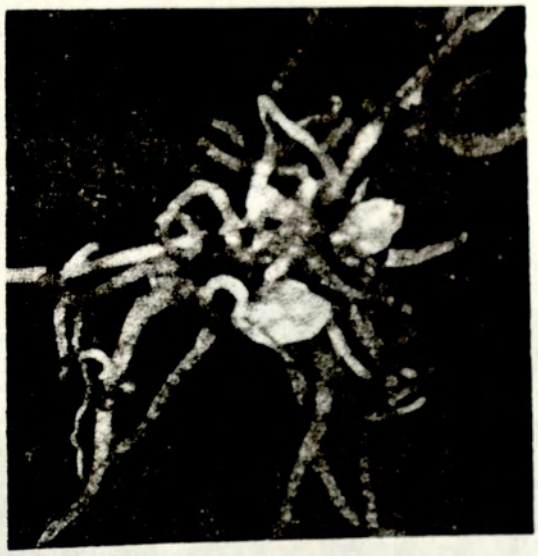
Note: Treatments with the same letter are not significantly different at $\alpha = 0.05$ using Duncan's test . Killed tubercles are % of total inoculated tubercles. Vertical lines represent SE of the means.

Fig. 13: Root chamber showing
A: Diseased, uninoculated
B: effect of *F. oxysporum*
on the under ground stages of
O. aegyptiaca and the
performance of tomato plant



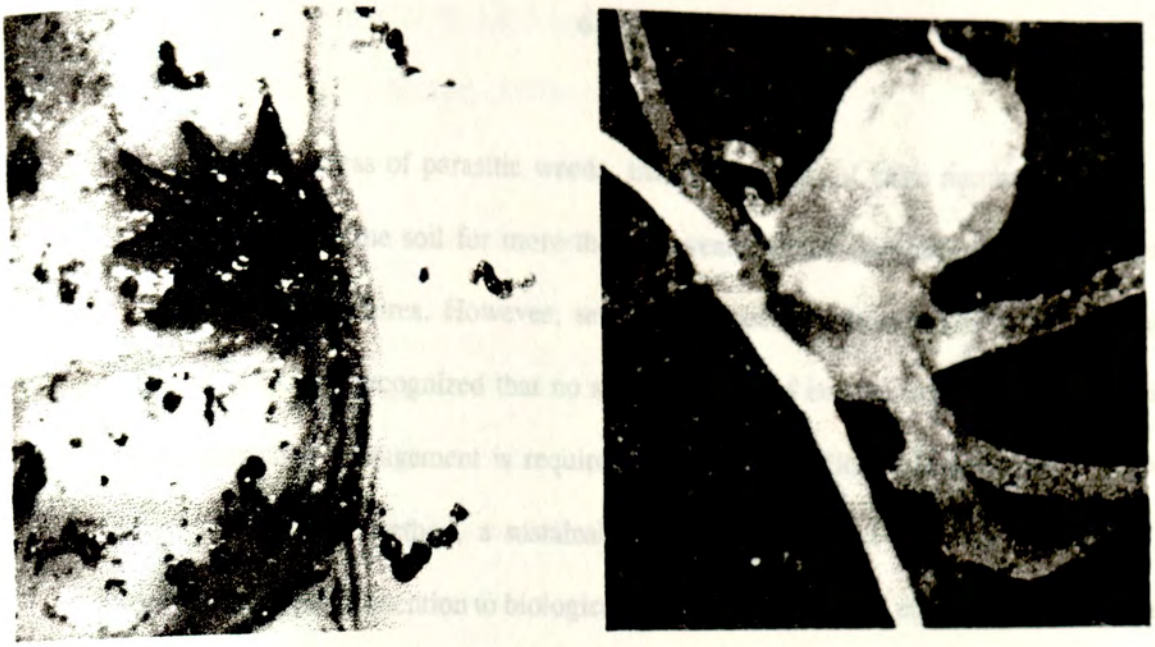


a)



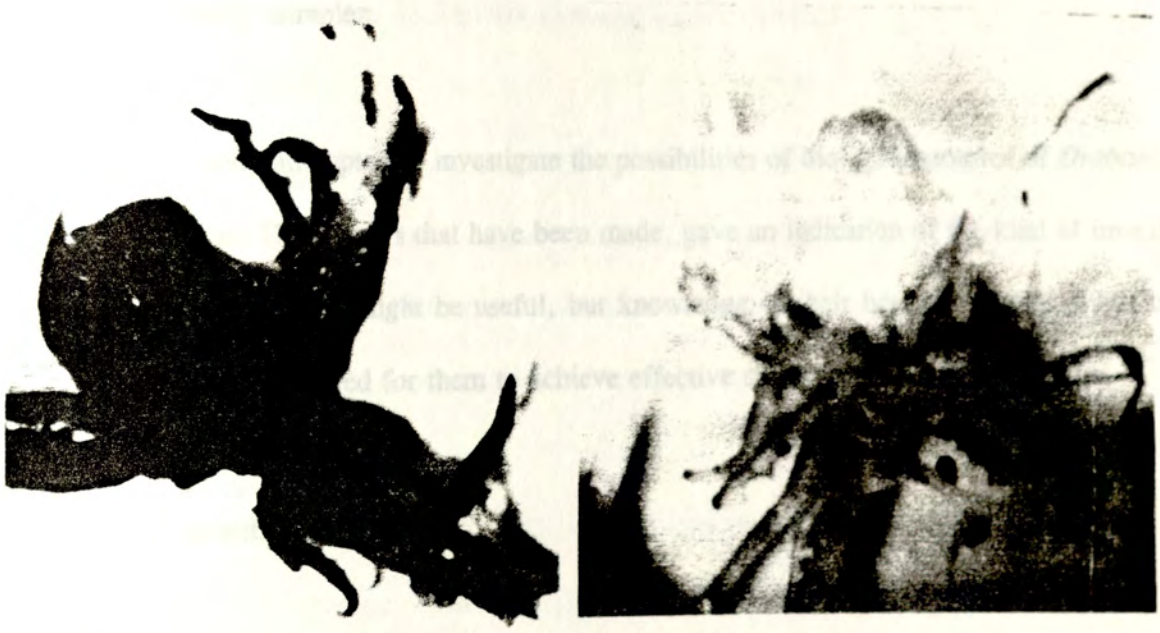
b)

Fig. 14: Effect of *F. oxysporum* on the tubercles of *O. aegyptiaca*
(a) diseased (X6.5) (b) healthy (uninoculated) (X6.5)



a) b)

Fig.15 : Effect of *F. nygamai* on the *O. aegyptiaca* tubercles (a) diseased (X10) (b) healthy (uninoculated) (X16)



a) b)

Fig.16 : Effect of *F. nygamai* on the bud stage of *O. aegyptiaya* (a) diseased (X10) (b) healthy (uninoculated (X10)

6. DISCUSSION

The aggressiveness of parasitic weeds, their production of large number of seeds which remain viable in the soil for more than ten years, limits the application of conventional weed control measures. However, several methods have been tested to control parasitic weeds, it is well recognized that no single method of control can solve the problem and that integrated management is required. Due to the difficulty in controlling *Orobanche* spp., by a single method, a sustainable long-term program of integrated control could benefit from more attention to biological control. Many natural enemies of broomrape have been reported, but a few systematic studies of individual antagonists and their influence on populations of the host plants have been conducted. A major reason for this is that the interactions between the natural enemies of the parasite and its hyperparasites are extremely complex.

This study attempted to investigate the possibilities of biological control of *Orobanche* in Ethiopia. The surveys that have been made, gave an indication of the kind of insects and pathogens which might be useful, but knowledge of their host plants ranges and of the conditions required for them to achieve effective suppression is still incomplete.

6.1 Insects

A number of polyphagous insect species were found associated with *Orobanche*. *Phytomyza orobanchia* is the only monophagous insect (i.e. the larvae live exclusively on *Orobanche*) reported that has great value in controlling *Orobanche*. It has been shown that this insect provided excellent control of *Orobanche* spp. in USSR (Klyueva and Pamuchki,

1983), in Turkey (Giray and Nemli, 1983), in Syria (Linke et al. 1990) and in India (Manjunath and Nagarkatti, 1977).

The foregoing results indicated clearly that the occurrence of *P. orobanchia* in Ethiopia seems to be limited. The absence of *P. orobanchia* in the fields of Nura Era and Merti Jeju state farms, might be associated with the application of insecticides at a high rate in these farms (Table 8). A wide range of insecticides are used in the state farms, this includes Thiodan, Sumicidin, Mitac, Cymbush and Deltanet (Ibraheim Assabi[1], 1994, personal communication). This agrees with that of Mihjlovic (1986), Parker and Riches (1993) and Schroeder (1994) how reported that, the use of insecticides against crop pests can largely eliminate *Phytomyza*. In addition, Sauerborn (1991) reported that, 74.5% of *Orobanche* was damaged by *Phytomyza* in crops not treated with insecticides. While on the other hand only 33.75 were damaged in treated crops.

In this study, *P. orobanchia* was found in *O. ramosa* and *O. cernua* in Malima farm. These results go in line with that of Lekic (1974) and Puzzilli (1983), the *P. orobanchia* live mainly on *O. ramosa* and *O. cernua* (*O. cumana*) plants. Recently, Klein et al. (1995) have confirmed its occurrence and effectiveness on *O. crenata* in Morocco.

Under the condition of this study, it was found that, *P. orobanchia* is highly efficient in reducing *O. ramosa* infestation by destroying 81.4% of the seed capsules (Table 9). Similar results have been reported in USSR by Girling et al. (1979). AL-Khesraji and Abdel-Wahid (1988) observed that, larvae of this insect led to 21% reduction of *O.*

1: Ibraheim Assabi, Head, Research section, Nura Era Horticulture Development Enterprise, Ethiopia.

aegyptiaca seeds in Iraq. On the other hand, the fly effectively destroyed *O. cernua* seed capsules (Table 9) (Fig. 5). This insect is efficient in reducing seed capsules of *O. cumana* (*O. cernua*) by 96% in Yugoslavia (Mihajlovic, 1986), and Horvath and Wittmann (1988) confirmed its effectiveness on *O. cumana* in Hungary.

It can be concluded from the present work that, *P. orobanchia* could be a promising biological control agent of *Orobanche* spp. in Ethiopia, especially if artificial mass rearing and field release techniques could be adopted. More detailed studies are however needed.

6.2 Fungi as pathogens of *Orobanche*

Since fungi are usually aggressive parasites and are frequently host specific (Charudattan, 1985), they could be used as a mycoherbicide for *Orobanche* control. Since *Orobanche* spp. has an adverse effect on the crop even before emergence (Sauerborn, 1991), soil-borne pathogens could be very effective as biological control agents, because they can infect the parasite pre- or post-emergence.

Results from the present investigations showed that, *Orobanche* spp. could be infected with different fungi. These are: *F. gibbosum, F. nygamai* Burgess & Timboli, *F. oxysporum, F. proliferatum* (Matsushima) Nirenberg, *Fusarium* sp and *F. solani*. Our results are in accordance with the previous findings that, *F. gibbosum* was isolated already from *O. mutellii* in USSR (Taslakh'Yan and Grigoryan, 1978). Within the present work *F. nygamai* and *F. proliferatum* were isolated for the first time from *Orobanche* (Table 12). The most common species which has been identified from *O. ramosa* and *O. cernua* is *F. oxysporum*. The isolation of *F. oxysporum* and *F. solani* from *Orobanche* spp. have

been reported by many authors from different countries. Both species were isolated from *Orobanche* spp. in Hungary (Hodosy, 1980) and from *O. crenata* in Egypt (Al-Menoufi, 1980) and in Syria (Linke et al. 1992). In addition, *F. oxysporum* f.sp. *orthoceras* was also isolated from *O. aegyptiaca* in USSR (Panchenko, 1974) and from *O. cumana* (Bedi, 1994). Moreover, *F. nygamai* and *F.oxysporum* were isolated from diseased *Striga hermonthica* plants from Sudan and Mali (Sauerborn, 1993; Abbasher, 1994; Ciotola et al. 1995). Many other fungal species have been isolated and identified from *Orobanche* spp. by Linke (1992) and Linke et al. (1993).

6.3 Pre-planting pathogenicity experiment

Fusarium spp., as soil-borne pathogens, could probably be of use as natural antagonists for *Orobanche* control because, they could reduce *Orobanche* population through the decay of the seeds, by attacking the soft tissue of the parasite before or after their attachment at the underground stages, and by causing the death of the plants shortly after emergence. The pathogenicity test revealed that, all tested fungal isolates markedly limited the number of emerged *O. ramosa* compared to the control (Table 13) (Fig. 6). A reduction and or a delay of *Orobanche* emergence was sufficient to increase tomato dry weight. Similarly, in the present work, significant improvement of tomato dry weight was obtained by *F. oxysporum, F. nygamai, F. gibbosum* and *Fusarium* sp. (isolate 8) (Table 13). The same *Fusarium* spp. significantly reduced *O. ramosa* dry weight, and corresponding increase in tomato height was noticed (Fig. 7, 8). The effectiveness of *F. oxysporum* in the control of *Orobanche* was reported from different countries. However, it has been shown that this fungus provided 70-80% control of *Orobanche* spp. in Ukraine (Kovalev and Lebedev, 1969). In Iran the same fungus decreased 75% of *O. cernua* Loefl. infestation, and

increased the yield of tobacco by 80.5% (Mazaheri, et al., 1991). *F. oxysporum* f.sp. *orthoceras* provided excellent *Orobanche* control, and has been recommended by many workers as the most promising mycoherbicides. Earlier results obtained by Kott (1969) showed 86-100% reduction of broomrape in tobacco field and yield increase was also observed. Recently, Bedi and Donchev (1991), and Bedi (1992; 1994) used the same fungus to achieve more than 90% control of *O. cumana* on sunflower in Bulgaria. Others have reported good control with *F. oxysporum* f.sp. *orthoceras* on *O. aegyptiaca* and *O. ramosa* (Timchenko and Dovgal, 1972; Panchenko, 1974), and on *O. cumana* (CAB International, 1987; Sauerborn et al., 1994).

F. nygamai was isolated from *Orobanche* species for the first time in this work. Thus, there are no previous reports about its pathogenicity to *Orobanche*. This fungus was isolated from diseased *Striga hermonthica* plants from Sudan by Abbasher (1994), and effectively controlled *Striga*. Significant increase in the sorghum height, shoot dry weight and panicle yield was achieved. Many other *Fusarium* spp. were tested for their pathogenicity to *Orobanche*. However, low pathogenicity of *F. gibbosum* was reported (Bozonkov and Konzmanova, 1994; Schroeder, 1994).

It would seem from the results that the pathogenicity of all tested *Fusarium* spp. is low considering the increase in tomato dry weight and the aerial symptoms on emerged *Orobanche* plants. The reasons for this could be attributed to the long storage period of the *Fusarium* spp. on a complete medium (Malt Extract Agar) for more than 8 months which can decrease the virulence of the concerned species. In this connection, Vanderplank (1968) and Bilai, 1977 (cited in Bedi, 1994) suggests that in the absence of a host, the virulence of *F. oxysporum* f.sp. *orthoceras* and some other fungi decreases. Bedi (1994)

confirmed the decrease of the virulence of this species during storage, but also found that after passage for one generation through *O. cumana* this fungus had regained its virulence like original isolates for 6 months. Vanderplank (1968) hypothesized that the decrease of the virulence may be due to the enzyme-producing mechanism of the fungus remaining non-functional after such long period of storage. This behavior was observed with *F. oxysporum* f. sp. *orthoceras* by Bedi (1994).

Fusarium species had many advantages as soil-borne pathogens in the control of parasitic weeds. However, the main advantage is their greater independence from environmental conditions (moisture and temperature), which limit the use of foliar pathogens (Abbasher, 1994). The second advantage of these species is that, they can remain viable for more than 2 years, and Bedi (1994) found that, *F. oxysporum* f. sp. *orthoceras* survived for at least 25 months on artificial culture medium as well as in the field. Thus, the persistence of this fungus in the soil can lead to residual weed control (Boyette et al., 1984). Additional advantage of this species is that, lost virulence could be regained in very short time (5-7 days) (Bedi, 1994). These advantages of *Fusarium* spp. are of great value for broomrape control and so they are potential candidate for a mycoherbicides. Thus, further tests to prove and to confirm their effectiveness against *Orobanche* spp.in Ethiopia are needed.

6.4 Inoculum substrate

Generally the use of cereal seed, flour and straw (barely, maize, sorghum and wheat) as inoculum substrates for pathogenic fungi was recommended by many authors (Hildebrand and Mcain, 1978; Boyette and Walker, 1985; Bedi and Donchev, 1991; Abbasher, 1994; Ciotola et al., 1995). Because it was found to be (a) simple and economical system

(inexpensive) for inoculum production; (b) available with minimum price in local market; (c) easy to quantify, to disperse and to incorporate pre-planting (Boyette et al., 1991); (d) can serve as a food base for the fungus, leading to a longer period persistence (Abbasher, 1994); (e) less to be washed a way from the treated areas than the liquid spores. Based on these advantages our isolates were propagated on wheat grain. To evaluate the effect of wheat grain, a comparison between the controls was made. The results showed that significant increase in tomato height, shoot dry weight and root dry weight was obtained by control (C++N) compared to the control (C-+N) (Fig. 11). Meanwhile, significant difference in tomato shoot dry weight between control (C++N) and control (C+) was also observed (Fig.11b). This may be due to the effect of wheat grain. In this regard, Abbasher (1994) reported that, *F. nygamai* gave better control of parasitic weeds and improved the performance of the host plant (sorghum) when it was propagated on sorghum grain. The reasons for this could be attributed to the carbon, nitrogen, and mineral ratio according to the same author. The significant difference in *O. ramosa* dry weight and in the number of emerged *O. ramosa* shoots between control (C-) and (C-+N) (Fig. 11d; Table 13)., may be due to the effect of wheat grain, but there are no previous studies on the ways by which the wheat grain can effect the seeds or underground stages of *O. ramosa*. More research are needed in this area.

6.5 Root chamber experiments

The effect of *F. oxysporum* and *F. nygamai* on the underground stages of *O. aegyptiaca* was evaluated using root chambers. Disease symptoms like rotting and changing of the tubercles colour to dark brown or black were observed by both species (Fig. 13, 14, 15, 16). Similarly, Linke et al. (1992) observed wet rot symptoms on the black tubercles of

O. crenata inoculated with aggressive pathogenic isolates. These results along with initial findings of Bedi and Donchev (1991) that *O. cumana* is susceptible to *F. oxysporum* f. sp. *orthoceras* at all stages of the life cycle from seed germination up to the flowering and seed formation. In addition, they have reported that, the same fungus killed the germinated *O. cumana* seeds before their attachment to the host root by infecting the soft tissue of the radicle. This might be the reason why the number of emerged *Orobanche* were significantly reduced compared to the control treatment in pot experiment (Table 13). Previous studies on the infection of *Orobanche* seeds by pathogenic fungi before germination was not observed. However, Krenner (1958) reported that, the black pigment present in the seed coat of broomrape protects the seed from fungal attack in the soil. In contrast, recently Abbasher (1994) found that in the root chamber *F. nygamai* and *F. semitectum* var. *majus* are able to infect *Striga* seeds and reduced their germination up to 93% and 84% compared to the control respectively. Using electronic microscope, Sauerborn et al. (1996) proved that, the infected *Striga* seed had poor inner cellular contents and were dead.

Infection of broomrape tubercles with *F. oxysporum* f. sp. *orthoceras* was reported by Bedi and Donchev (1991) and Linke et, al.(1992). Similarly, from our root chamber experiment, the susceptibility of *O. aegyptiaca* tubercles to *F. nygamai* and *F. oxysporum* were noticed. However, 81% and 41% of the tubercles were killed by *F. oxysporum* and *F. nygamai* after 10 days of inoculation (Fig. 12a). The respective figures for killing after 15 days were 82% and 48.6% compared to the control (Fig. 12b). The effectiveness of *F. nygamai* on *Striga hermonthica* control was confirmed by Sauerborn et al. (1994).

In pot experiment as well as in root chamber trails, no disease symptoms were observed on tomato plants due to the presence of the investigated isolates. These observations are in agreement with the previous findings of Timchenko and Dovgal (1972) that *F. oxsysporum* had no pathogenic effect on tomato. However, the non-pathogenicity of this fungus to tomato was confirmed by other authors (AL-Menoufi, 1986; Bedi, 1994; Hallmann and Sikora, 1994).

7. CONCLUSION

In this study the possibilities of biological control of *Orobanche* spp. in Ethiopia have been investigated for the first time. The surveys showed that, the fly *Phytomyza orobanchia* occurs only in Malima farms. The fly effectively destroyed the seed capsules of *O. cernua* and *O. ramosa* under natural condition of Malima.

Following surveys for microorganisms pathogenic to *Orobanche* spp. 20 fungal isolates were isolated and identified from diseased *Orobanche* plants. These are: *F. gibbosum*; *F. nygamai* (two isolates); *F. oxysporum* (eleven isolates); *F. proliferatum* (two isolates); *F. solani* var. *solani* and *Fusarium* sp (three isolates). All of these fungal isolates are reported for the first time from Ethiopia, and two of them are new records for *Orobanche* spp.

The screening for effective mycoherbicides for *O. ramosa* in pot experiment resulted in four isolates of *Fusarium* seems to be promising. *F. gibbosum, F. nygamai, F. oxysporum* and *Fusarium* sp. (isolate 8) significantly reduced the number of emerged *O. ramosa* shoots. The same isolates significantly decreased the dry weight of *O. ramosa* and significantly improved the dry weight of tomato. More tests are needed to confirm their pathogenicity to *Orobanche* spp.

In root chamber trials, *F. oxysporum* and *F. nygamai* proved to be pathogenic to the early stages of *O. aegyptiaca*. Both isolates were effectively killed the tubercles of *O. aegyptiaca*. *F. oxysporum* is more effective compared to *F. nygamai* as indicated by the percent of killed tubercles.

7.1 Prospects for biological control of *Orobanche* in Ethiopia

In conclusion, many future activities could be proposed from the foregoing investigations for development of biological control of *Orobanche* spp. in Ethiopia. These are:

1 Systematic field surveys for insects and pathogens associated with broomrapes throughout their main distribution areas. Special attention should be paid to the places outside cultivated areas (e.g. field peripheries) because a large complex of organisms may be encountered there.

2 Since *Phytomyza orobanchia* showed effective destruction of *Orobanche* seed capsules, more evaluation of this fly as potential bioagents should be carried out under field conditions. However, it is necessary to increase the activity of this fly by making its population as dense as possible when broomrape begin to germinate. This would mean that all factors affecting its population density should be avoided. In addition, artificial mass rearing and field release techniques should be adopted.

3 Lepidoptera (*Noctuidae*) insect should be identified first, and then its life history and biology, habitat requirements and host specificity should be well studied.

4 Since *F. oxysporum* and *F. nygamai* showed to be effective against *Orobanche* under controlled conditions, more tests for their pathogenicity and host specificity need to be conducted under natural and glasshouse conditions. Crops and vegetables of economic importance in the areas where *Orobanche* occurs should be included in the host specificity tests.

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5 More efforts would be required to study the conditions affecting the survival and effectiveness of *F. oxysporum* and *F. nygamai* such as soil temperature, humidity and inoculum substrates. Since both species were found to control the underground stages of *Orobanche*, the inoculum should be incorporated pre-planting into the soil.

6 Laboratory and fields studies should be conducted to integrate the insects and pathogens of *Orobanche* in to the overall crop production system, with special attention to the widespread use of fertilizer and pesticides.

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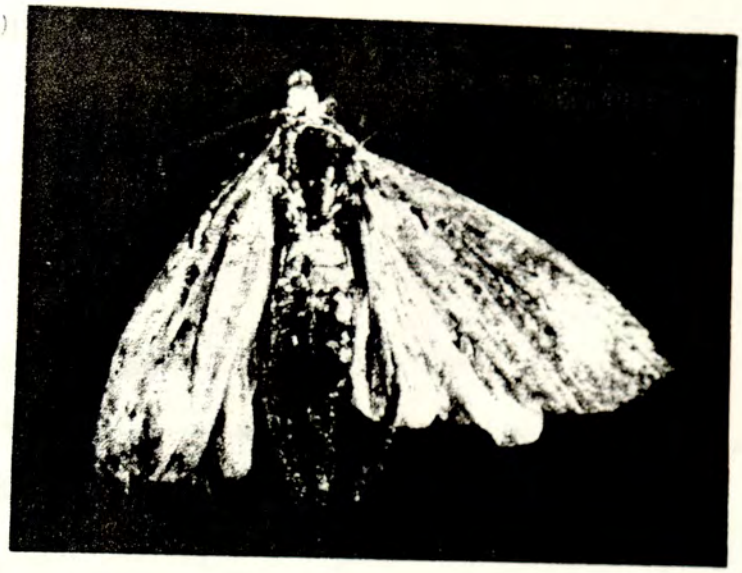
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Appendix 1: Unidentified insect (Lepidoptera, Noctuidae) a) adult b) larvae c) pupae

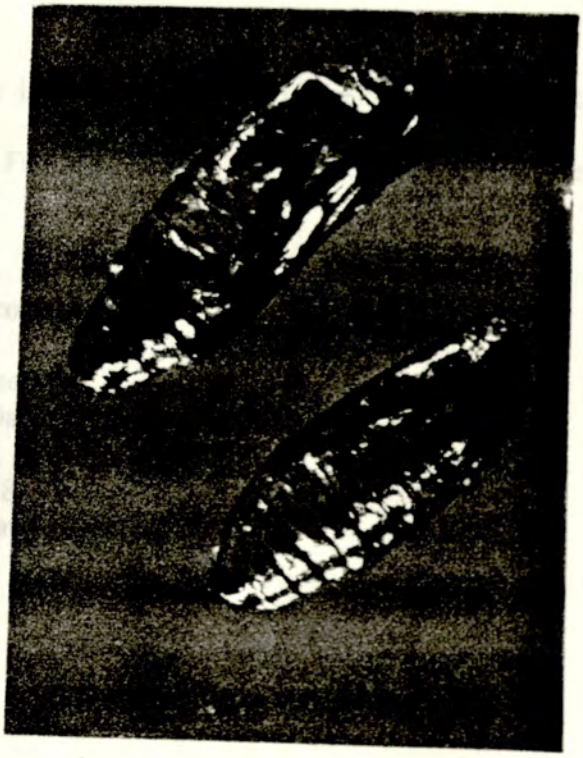
a)



b)



c)



Appendix 2: Storage code numbers of the identified fungal isolates from Ethiopia.

Fungus	Number[a]	Date of identification
F. proliferatum (isolate 5)	BBA 69673	Nov., 1995
F. oxysporum (isolates 1, 9, 16)	BBA 69678	Nov., 1995
F. oxysporum (isolates 11, 19)	BBA 69674	Nov., 1995)
F. oxysporum (isolate 18)	BBA 69767	April, 1996

[a]: Numbers under which the isolates were stored in Federal Biological Research Centre for Agriculture and Forestry, Institute for microbiology, Berlin, Germany.

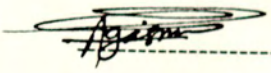
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DECLARATION

I, the undersigned, declare that this thesis is my original work and it has not been presented for a degree to any other university.

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Signature: 

Place and date of submission:

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Addis Ababa University

June, 1996