

**SEED-BRNE OF FABA BEAN IN THE
SUDAN WITH SPECIAL EMPHASIS ON**

Fusarium oxysporum f. sp. fabae

BY

Nsrein Ibrahim Ahmed Abu Elhassen

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Supervisor: Prof. Ahmed Mohammed Baghdadi

From the Department of Crop Protection

Faculty of Agriculture

University of Khartoum

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Dedication

*To my mother Fatma and
memory of my Father
with love.*

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ABSTRACT

The samples of faba bean collected from Gezira, Shandi, Aldamr, Shambat and Elhudebia Research Station Farm (northern Sudan) areas were tested by the standard international (ISTA methods 1966a), for seed-borne fungi.

Ten seed-borne fungi were encountered in all samples. *Fusarium oxysporum. f. sp. fabae* was found in a high percentage ranging between 16.66-37.5% while other fungi have shown rather low incidence.

Germination of faba bean naturally infected with these fungi was significantly reduced.

Chlorine pre-treatment of infected faba bean seeds showed that *Fusarium oxysporum. f. sp. fabae* was deeply seated in the tissues.

Czapek's dox agar media revealed better growth among other media tested. The fungicides Tilt 250EC and Nimrod25EC inhibited the fungal growth.

CHAPTER ONE

INTRODUCTION

Vicia faba L. is one of the oldest cultivated plants in the world. It has a number of common and English names; the common names are as faba beans and broad beans. It is known locally in the Sudan as Egyptian beans (Kambal, 1968). The English names are such as broad beans, field beans, faba beans and horse beans (Champman and Carter, 1976 ; Bond, 1979).

Vicia faba L. is the fourth most important pulse crop in the world. It is grown mainly for its dry seeds or as a green vegetable (immature seeds) both of which are important for human and animal consumption. It has presumably originated in western Asia and northern Africa (Hawtin and Stewart, 1979). The crop is widely produced in subtropical regions of the world and it can be found at high elevations within tropics such as Ethiopia and Northern Indian region of Latin America (Hawtin and Hebblethwaite, 1983).

The main producing countries in the world are; Ethiopia, Algeria, Morocco, Tunisia, Egypt, Sudan, Iraq, Afghanistan, China, France, Italy, USA, Mexico, Brazil and Argentina (Chapman and Carter 1976; Hawtin and Stewart, 1979; Hwatin and Hebblethwaite, 1983; and Bascur, 1993)

In the Sudan faba bean is grown for a long time as an irrigated winter-crop, mainly in the Northern State, where more than 70% of the crop is produced.

The Nile State ranks next and produces about 20% .Less yields is produced in Khartoum state, Central Sudan and the Jebal Marra in western Sudan. Area under broad beans production ranges from 47619-83333 feddans and the average yield is estimated to be 4.29 ton/fed as reported by Solh (1996) Mohamed (1996) and Ahmed (1996).

The importance of faba beans production has been rising in the last years due to the increase in population, and hence consumption.

Several pathogens are considered as seed-borne fungi of faba bean: *Fusarium oxysporum*, *Ascochyta fabae*, *Botrytis fabae*, *Uromyces fabae* (Vijendra and James, 1987).

In the present work, seed samples of faba bean, collected from different parts of the country were tested by standard seed health methods for seed-borne fungi.

As a result, *Fusarium oxysporum* was encountered in high percentage, while the incidence of other fungi was low.

Therefore, it was decided to investigate the role of fungus as seed-borne pathogen of faba beans seeds.

Accordingly, the following aspects pertaining to *Fusarium oxysporum* were investigated:

- I. The effect of seed-borne inoculum on germination of faba bean.
- II. Some information on the nature of the pathogen and its control.

CHAPTER TWO

LITERATURE REVIEW

2.1 Seed-borne fungi of faba beans:

According to Vijendra and James (1987), *Ascochyta fabae*, *Fusarium oxysporum*, *Botrytis fabae*, *B. ricini*, *Alternaria alternata* and *Uromyces fabae* are considered as seed-borne fungi of faba beans (*Vicia faba*. L) all over the world.

Moreover, Lenti (1993) reported that *Cladosporium herbarum* was seed-borne in faba beans in Hungary.

Gärber, et al (1993) suggested the possibility of systemic infection with *Ascochyta fabae* in seed of faba beans. Simay (1998) reported that *Alternaria spp*, *Fusarium spp*, *Trichothecium roseum* *Ascochyta fabae*, *A. pinodes*, *Botrytis cinerea* and *B. fabae* were observed on several seed samples of faba beans and some pathogenic fungi were also observed.

Kaiser, (1998) reported that *Ascochyta* blights of faba beans is caused by *Ascochyta fabae* which is an important seed-borne pathogen.

Recently, Dubey and Patel (2001) has shown the mode of perpetuation and spread of *Alternaria* blight of board beans.

Simay (1996) found that the germination of faba beans seeds was affected less by *Fusarium pallidoroseum* and most *F. oxysporum*.

2.2 *Fusarium oxysporum*:

This fungus is a serious seed-borne pathogen of world-wide distribution encountered in the survey of seed-borne fungi of faba bean. It has been reported in China (Yu and Fang, 1948), Japan (Ikata, 1951; Yamamoto *et al*, 1955), Canada (Coulombe, 1957), Russia (Dunin, 1962). In Egypt, the causal pathogen of broad bean wilt was indentified as *F. oxysporum f.sp fabae* (Ibrahim and Abdel Rahim 1965; Sahab 1970). In the

Sudan, the causal organism of faba bean wilt was reported as *F. oxysporum* (Ibrahim, 1978; Ibrahim and Owen, 1997).

Fusarium. oxysporum has been reported as causal of cortical rots, head blights, leaf spots, root rots, fruit rots, cankers, dieback and vascular wilt disease (Mace *et al*; 1981).

Fusarium wilt is much more common and destructive in warm temperature regions and in the tropical and sub-tropical zones, becoming less damaging in colder climates (Agrios, 1988).

The symptoms produced by *F. oxysporum* were described by (Hussein, 1982) and usually appear two to three weeks after sowing.

The symptoms of leaves yellowing and characteristic discoloration of xylem vessels of the root were observed. This is accompanied by a marked reduction in plant vigor and in severe cases, by death of the growing tip.

With regard to seed-borne *Fusarium oxysporum*, little has been published in the literature. However, Elliot and Crowford (1922) reported that the seed transmission of *F. oxysporum* frequently occurs when the fungus propagules such as conidia or chlamydosproes, are carried as surface contaminants on or in seed that remains after harvest.

Naturally infected seed may carry viable pathogen for at least seven months, serving to carry the organism over from one season to the next.

Randey (1986). Reported that, the fungus was classified according to Ainsworth report (1971), as belonging to the;

Kingdom: fungi.

Division: Eumycota.

Sub-division: Deuteromycotina.

Class : Hyphomycetes.

Order : Moniliales.

Family : Tuberculariaceae.

Genus : *Fusarium*.

The mycelium is inter- and intracellular hyaline, branched geniculate and septate. The reproduction takes place by asexual methods only. There

are three types of spores; microconidia, macroconidia and chlamydoconidia.

2.3 Factors affecting growth of the fungus:

2.3.1 Carbon Sources:

The importance of the source of carbon has long been emphasized. Nearly all fungi, which can be cultured, are able to utilize glucose.

Brown (1922-26) cited by Guma (1984) demonstrated the effect of carbon dioxide in suppressing spore production and the carbon-nitrogen ratio on the spores. In general, a low C/N ratio produces short spores with low septation; conversely, high C/N induces spores with increased septation. Also, a high glucose level produces excessive mycelial development and starch increase sporulation (Booth, 1971).

2.3.2 Hydrogen-ion concentration:

Most fungi tolerate a wide range of Hydrogen-ion concentration of the media (Hawker, 1950). In this context, Agarwal and Sarbhoy (1978) found that *F. oxysporum* and *F. soloni* grew best at pH 4.5 and 6.0 whereas *F. graminearum* and *F. equiseti* at pH 3.5 and 6.5 respectively.

Moreover, they concluded that all species of *Fusarium* drifted their pH towards acidic.

2.4 Seed treatment and chemical control of *F. oxysporum*:

Seed treatment with formaldehyde, Captan, and water at 70°C could check many seed-borne fungal root diseases and improve germination rate in faba bean (Castro, 1998).

Dubey and Patel (2001) reported that treating seeds of faba beans with Thiram (0.2%), Thiram (0.1%) + Topsin M (0.025%) and Captan (0.2%) gave maximum germination and elimination of seed-borne infection, respectively. In addition the three sprays by Topsin M (0.1%) gave best control of Alternaria blight of broad bean.

Seed treatment with Derosal (carbendazim) and Benlate (benomyl) at 10 p.p.m in a synthetic nutrient agar medium, completely inhibited growth of *F. oxysporum*, *F. soloni* and *F. moniliforme* also Topsin-M

(thiophanate-methyle) and vitavax (carboxin) gave 100% inhibition at 50 p.p.m Wahid,et al.(1996).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Faba bean seed samples supply:

Ten seed samples of faba beans (*Vicia Faba* L.) of mixed local varieties collected from Shandi, Gezera, Aldamer, Shambat and Elhudeiba research station farm (northern Sudan) were used in this investigation (Table 1).

The working samples were drawn according to the International Rules for Seed Testing (ISTA, 1966, 1976). They were collected in paper bags and taken to the laboratory for analysis.

3.2 Detection of seed-borne fungi in Faba bean seeds

3.2.1 Dry inspection:

The ten seed samples were examined under the stereoscopic binocular (16-40 x) for impurities, such as plant debris, sclerotia, galls....etc., and also for symptoms such as discoloration and malformation.

3.2.2 The Blotter method:

The above seed samples were tested by the standard blotter method (ISTA ,1966). Two hundred non-pretreated seeds and pretreated of each sample were plated in sterilized plastic Petri dishes (9 cm diameter) on moistened three layers of blotter at the rate of 10 seeds per plate. The seeds were placed equidistantly from each other. The plates were then incubated in a controlled environment chamber (Sohieb, 1983) at 25 - 30°C under alternating cycles of 12 hours near ultraviolet (NUV) light (at a distance of 40 cm) and 12 hours darkness for 7 days. Sterile water (about 3 ml) was added to each dish every 2 days so as to keep the blotter moistened throughout the test. The NUV tubes were of Philips black light lamps TL 40 w/80 Rs 40 BIB.

Table (1): faba beans seed samples collected for experimentation:

	1	2	3	4	5	6	7	8	9	10
Harvest year	1993	2000	2000	2002	2002	2002	2002	2002	2002	2003
Locality	Aldamer	Shambat	Shandi	Aldamer	Aldamer	Shandi	Shambat	Aldamer	Gezira	Gezira

On the 8th day the seeds were examined for presence of fungi under the stereoscopic binocular (16-40X) and whenever necessary, the compound microscope was used to confirm the identification.

3.2.3 The Agar plate method:

Two hundred seeds from each sample were first treated with 1 % sodium hypochlorite for 5 minutes and washed repeatedly in several changes of sterile water. The seeds were plated in the same way as in the blotter method (5 seeds / plate).

After the incubation period (7 days), they were examined and the incidence of seed-borne fungi was recorded.

The identification of fungi was done according to Barnett (1955-1960); Kulwantgingh et al (1991) and with the help of Baghdadi, Faculty of Agriculture, University of Khartoum.

3.3 Effect of natural infection by *Fusarium oxysporum* on germination of Faba beans:

The effect of *F. oxysporum* was studied by plating 200 untreated seeds from samples that have shown different levels of infection. Sample No. 10 I was used as control after chlorine pre-treatment. The above samples were tested by the standard Blotter method (ISTA , 1966)

3.4 Monosporic culture of *Fusarium oxysporum*:

Spore suspension was obtained by pouring a small amount of sterile water onto 10 days old culture of *F. oxysporum* on slant of (PDA) medium. The culture slant was rotated between the hands for release of spores of the fungus.

A loop of this diluted spore suspension was streaked on the surface of PDA medium. The plates were then incubated at 25⁰C for 24 hours for further investigations.

3.5 Effect of different media on linear growth of *Fusarium oxysporum*:

12 sterile Petri dishes (9 cm. Diameter) were prepared with three different types of media (four plates for each). These were potato sucrose – agar medium PSA, potato dextrose – agar medium (PDA) and Czapek’s dox agar medium.

Each plate was inoculated with 5 mm-disc from the advancing edge of a colony of *Fusarium oxysporum* cultured on PDA and were incubated for 6 days at 25°C. The colony diameter was measured daily for one week.

3.6 Effect of two fungicides on growth of the fungus in vitro:

This experiment was designed to study the efficacy of two fungicides against *F. oxysporum*. The fungicides used were Tilt (250 Ec) and Nimrod (25% Ec)*.

Three ml of each of the two chemicals was dissolved in a liter of sterilized distilled water to give 3000 ppm. From this solution 20,10,5 and 3 ml were completed to 100 ml by adding the required amount of sterile potato dextrose-agar in 100 ml Erlenmyer flasks, to give a final concentration of 200,100,50 and 30 ppm of each chemical, respectively. Each flask,content was poured into four sterilized Petri-dishes (9cm diameter).Ten plates were poured with PDA medium only serving as control. Two diameters were drawn on the back of the plate to aid in centering the inoculum which was 5 mm diameter disc cut from the edge of 5-days-old culture of *F. oxysporum*. The plates were then incubated at 25°C and the growth of the fungus was measured every day for one week. Further dilutions of concentration of certain chemical were used in the same manner, which will be mentioned in its appropriate place.

3.7 Pathogenicity test:

Two samples of faba bean were used to study the pathogenicity of *F.oxysporum* in seed inoculation technique.

* Ec = emulcifiable concentrate

3.8 Seed inoculation:

Healthy chlorine pre-treated faba bean seeds after washing several were divided into two samples. Each sample was soaked in the fungal suspension (*F.oxysporum*) using the 80 % for 30 minutes. Both samples were plated on top of wet blotters and incubated at 25⁰C under alternating cycle of light and darkness. Then the growth of the fungus was measured every day for one week.

3.9 Re isolation of fungi:

This was associated with faba bean rotted seeds, rotted seeds sodium were sodium soaked in sodium hypochlorite (Naocl₃) for 2 minutes , then washed for five times in sterilized distilled water to get rid of the chlorine. Seeds were plated in sterile Petri-dish containing PDA medium. Then the plates were incubated for 7 days at 25⁰C, after that they were examined for the presence of *F. oxysporum*.

CHAPTER FOUR

RESULTS

4.1 Detection of Seed-borne fungi:

4.1.1 In Dry inspection:

Dry inspection of the seed samples of faba beans, collected from the main faba bean growing areas, showed that 70% of these samples contained tiny black sclerotia scattered on surface of the seeds. Also brown to black seed discoloration was observed (plate 1). Inert matter was found mixed with all seed samples and identified as soil particles and pieces of plant residue.

4.1.2 Blotter test of untreated seeds:

On the 8th day of plating, the non-pre-treated seeds were examined and incidence of seed-borne, fungi was recorded. The following fungi were encountered in the blotter test: *Fusarium oxysporum*, *Botrytis fabae*, *B. ricini*, *Ascochyta fabae*, *Uromyces fabae*, *Alternaria alternata*, *Curvularia lunata*, *Aspergillus flavus*, *A.niger*, and *Penicillium pinophilum*. Their rate of occurrence was tabulated in (Table 2) and (Appendix 1). The result indicated that *F. oxysporum* was encountered in higher percentage of the tested seed samples, and their percentage of incidence ranged between 16.66 – 37.5 % with an average rate 20.84% (Table 2). Comparatively other fungi have shown rather low incidence.

During the blotter test *F. oxysporum* was found to produce the following effects on the seeds.

- I. Ungerminated faba beans seeds infected with *F. oxyporum* were covered by mycelia and conidia (plate 2).
- II. Consequently, sprouting seeds were attacked and further development was arrested (plate 4).
- III. The fungi severely attacked young seedlings and resulted in their death (plate 5).

Table (2): Percentage incidence of various fungi recorded in the samples of faba beans seeds tested by the blotter method. (200 seeds were tasted).

Fungi	Incidence %		
	Samples No.	Range	Average
<i>Fusarium oxysporum</i>	3	16.66-37.5	27.83
<i>Ascochyta fabae</i>	5	7.14- 20.00	14.03
<i>Curvularia lunata</i>	7	0.00-16.66	8.43
<i>Alternaria alternata</i>	2	0.00-18.51	7-49
<i>Botrytis fabae</i>	6	0.00-5.36	6.28
<i>Aspergillus flavus</i>	8	0.00-20.0	6.2
<i>Aspergillus niger</i>	1	0.00-17.95	5.19
<i>Penicillium pinophilum</i>	4	0.00-16.66	3.51
<i>Uromyces fabae</i>	1	0.00-12.85	2.38
<i>Botrytis ricini</i>	1	0.00-5.36	0.49

4.2 The Agar test of chlorine pre-treated seeds:

After pre-treatment of the 10 seed samples the following fungi were recorded. *F. oxysporum* was detected in sample 3 with range between 0.00 – 50.0 and an average percentage incidence 23.87 %. Other fungi were *Botrytis fabae*, *Alternaria alternata*, *Ascochyta fabae*, and *Curvularia lunata* have shown small percentage incidence in few samples (Table 3 and Appendix 2). From the result of the blotter and the agar plate method (Table 2 and 3), it becomes clear that *F. oxysporum* looked more important in faba beans seeds than other fungi. Therefore, further studies should be carried out on the pathogenic propensities of the fungus

Table (3): Percentage incidence of various fungi recorded in the samples of faba beans seeds from 4 localities tested by the Agar plate method. (200 seeds were tasted).

Fungi	Samples No.	Incidence %	
		Range	Average
<i>Fusarium oxysporum</i>	3	0.00-50.0	23.87
<i>Alternaria alternata</i>	2	0.00-25.00	11.5
<i>Ascochyta fabae</i>	5	0.00-28.57	10.30
<i>Curvularia lunata</i>	7	0.00-25.00	7.12
<i>Botrytis fabae</i>	6	0.00-14.29	5.19

4.3 Effect of *F. oxysporum f.sp. fabae* on germination of faba beans seeds:

The germination of faba bean seed was found to be affected according to the degree of infection by *F.oxysporum* Table 4 and Fig 1 shows that the samples 1, 9, 4, 5, and 3 with 9.99, 20, 29.5, 40, and 49.5 level of infection affected the germination level by 90%, 81.5% ,70.5% ,60% and 52.5% respectively.

4.4 Identification of *F.oxysporum*:

The aerial mycelium of *F.oxysporum* is delicate and white in color. Microconidia are formed from short phiallides. The macroconidia are mainly 3-5 separate and measuring about 26.4-38.4 X 3.6-4.8 M chlamydospores are intercalary or terminal on short branches.



**Plate (1): A) Faba beans showing symptoms of brown to black
Discolouration.
B) Normal seeds.**



Plate (2): Un germinated seed of faba covered by the growth of *F. oxysporum* .f. sp. *fabae*.



Plate (3): Normal germination of non infected faba beans.



Plate (4): Germinating seed of faba bean showing typical symptoms of *F.oxysporum* .f. *sp. fabae*



**Plate (5): Young seedling of faba bean attacked by
F. oxysporum f. sp. *fabae***

Table (4): Germination of faba beans seeds naturally infected with *Fusarium oxysporum f. sp. fabae*.

Samples No	% of infection	Germination %
10	0	100
1	9.99	90%
9	20.0	81.5
4	29.5	70.5
5	40	60.00
3	49.5	52.00

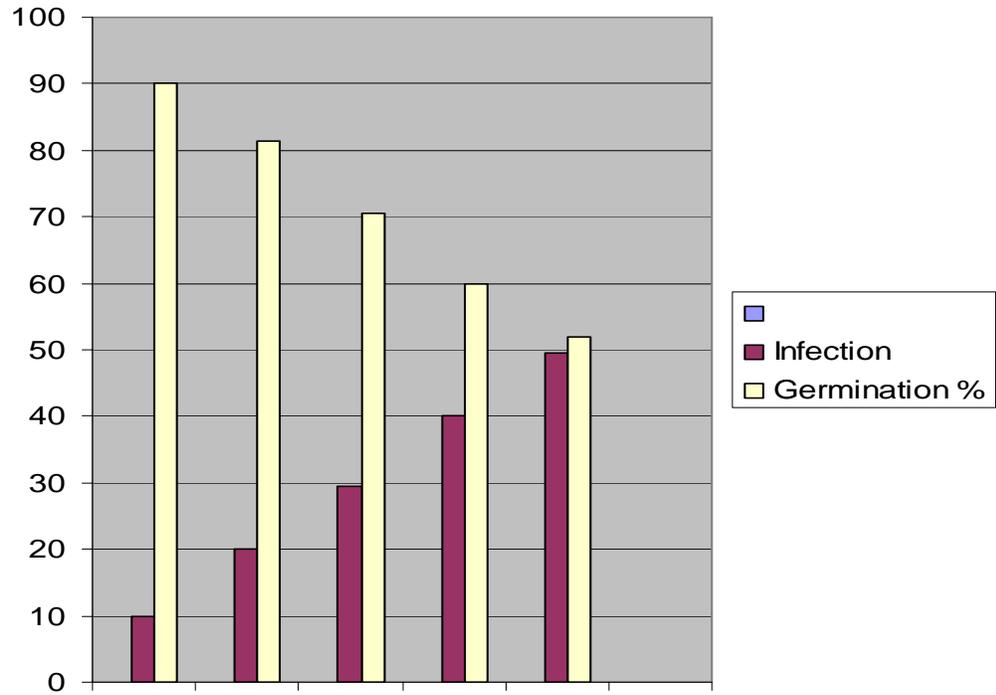


Fig (1): Germination of faba beans infected with *Fusarium oxysporum. f. sp. fabe*

4.5 Physiological studies:

4.5.1 Effect of different agar media on linear growth of *Fusarium oxysporum f. sp. fabae*:

Three agar media were chosen to study their effects on linear growth of the fungus *F. oxysporum*. The result showed that Potato Sucrose Agar (PSA) was the most suitable media for growth of the fungus followed by Czapek's dox agar and Potato Dextrose Agar PDA.

Differences were clearly observed between the three media in the density of the mycelial growth. PDA medium showed vigorous, fluffy and dense mycelial growth of the fungus, followed by Czapek's dox Agar and (PSA). Hence Czapek's dox agar was found to be favourable for the fungus as shown in (Table 5, Plate 6.7 and Fig 3).

4.5.2 Effect of different fungicides on the growth of *Fusarium oxysporum f. sp. fabae* in vitro:

Two chemicals were used in this study to observe effect of fungicides on the growth of *F. oxysporum* in vitro; these were Tilt (250 Ec) and Nimrod (25 % Ec). The growth was measured daily along the two diameters drawn on the plates. Mean diameter was calculated after four days from inoculation. Results showed that Tilt has completely inhibited the fungal growth at the lowest concentration (30 ppm). In case of Nimrod 25 % the growth was not inhibited even at the higher concentration (200ppm). (Tables 6, 7 and Fig 4).

Table (5): Effect of different agar media on linear growth of *Fusarium oxysporum f. sp. fabae*.

Media	Average linear growth (cm)			
	2 day	3 days	4 days	6 days
PDA	1.500	1.5	2.3	4.7
PSA	1.531	1.53	3.4	9.0
Czapek's dox Agar	1.525	1.53	2.5	8.0

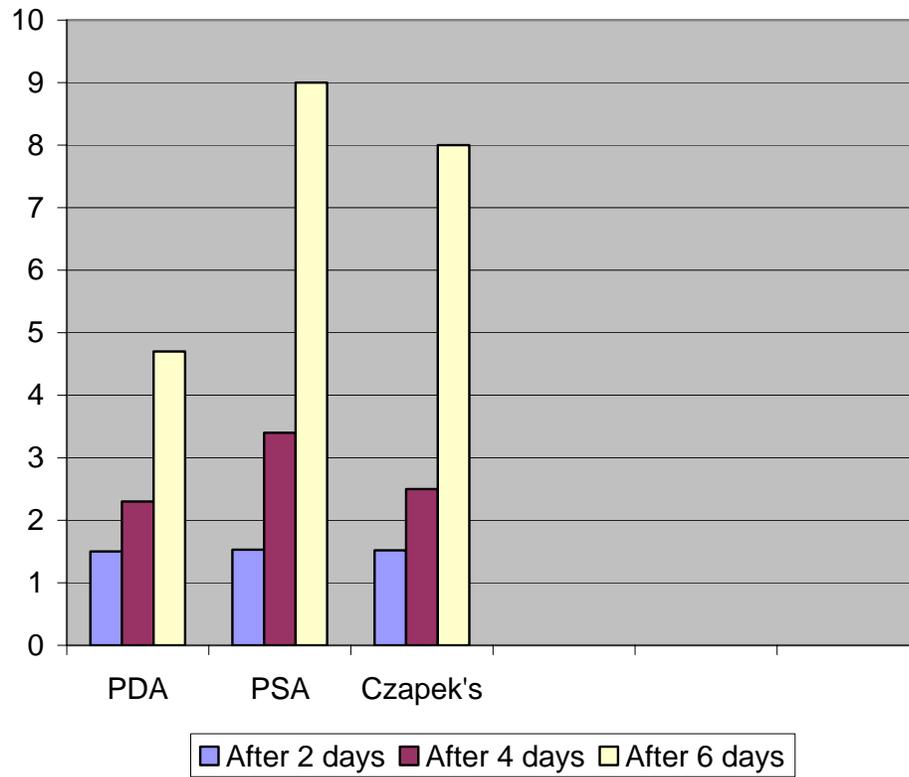
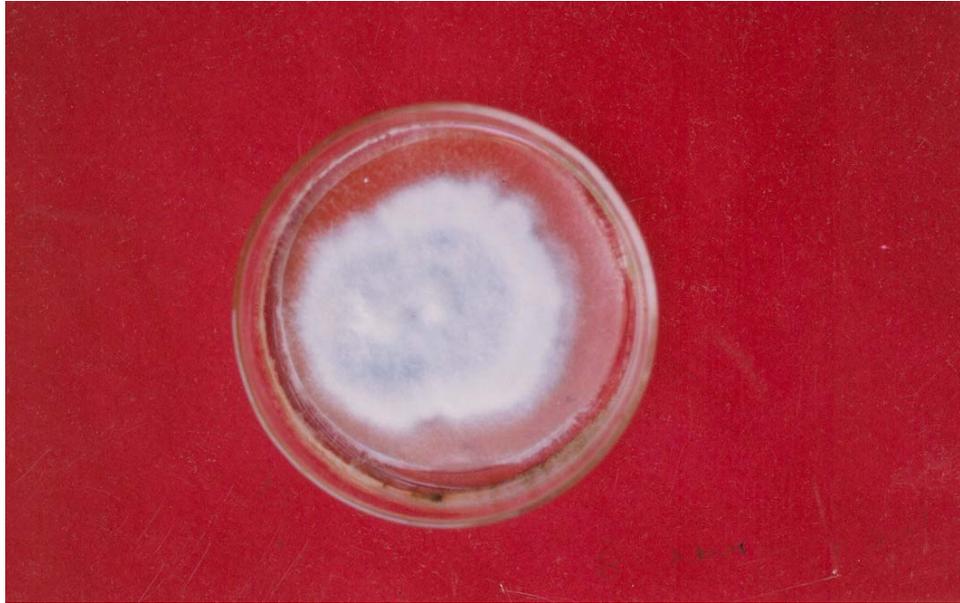


Fig (2):Effect of different agar media on linear growth of *Fusarium oxysporum f. sp. fabae*.



**Plat (6): Pure Culture of *F. oxysporum* on Potato Dextrose Agar
Medium (10 days old)**



Plat (7): Pure culture of *F. oxysporum. f .sp .fabae* on Potato Sucrose agar medium (10 days old).

Table (6): Effect of Tilt (250 Ec) On the linear growth of *Fusarium oxysporum f. sp. fabae*.

Treatments in ppm (Tilf)

Time in days	Mean colony diameter (cm)					inhibition of growth%	
	Zero	200	100	50	30	High (200)	Low (30)
1	0.275	0	0	0	0	100	100
2	0.770	0	0	0	0.043	100	93.86
3	1.200	0	0	0.1	0.1375	100	86.2
4	1.300	0	0	0.125	0.175	100	68.8

$$\% \text{ inhibition of growth} = \frac{\text{Control} - \text{mean colony diameter (cm)}}{\text{control}}$$

Table (7): Effect of Nimrod (25 % Ec) on the linear growth of *Fusarium oxysporum* .

Treatments in ppm (Nimrod)

Time in days	Mean colony diameter (cm)					% inhibition of growth	
	Cont	200	100	50	30	High (200)	Low (30)
1	0.275	0.006	0.0093	0.118	0.200	97.81	57.27
2	0.770	0.05	0.45	0.50	0.61	93.50	26.98
3	1.200	0.46	0.53	0.62	0.88	54	22.22
4	1.300	0.505	0.55	0.68	0.99	46.66	18.34

4.6 Pathogenicity Test

4.6.1 Faba beans rotted seeds:

Non-germinated rotted seeds inoculated in the pathogenicity test, were tested to confirm that the fungus *F.oxysporum* was the causal agent of seeds germination failure. All ungerminated seeds revealed occurrence of *F. oxysporum* when plated on PDA medium at 25°C for 7 days. Microscopic examination reflected the characteristic features of the fungus in all aspects as described by Booth (1971). Hence the fungus retarded seeds germination. (Plate 8 and Table 8).

Table 8: Effect of *Fusarium oxysporum* on germination using seed inoculation method on two samples.

	1 day	2 day	3 day	4 day	5 day	6 day
X	0	0	0.35	0.65	0.1	1.55
Y	0	0.2	0.8	1.4	1.9	2.15
S² poold	0	0.135	0.65	1.19	1.84	2.62
T cal	0	1.72 N.S	1.77 N.S	2.17 **	2.10**	1.17 x 1.5
T tab	At 0.05 2.025	At 0.01 2.716				

\bar{X} mean of sample (1).

\bar{Y} mean of sample (2).

S²pool = mean variance from **X** and **Y** variances.

N.S No significant difference.

** significant difference at $p \leq 0.05$.

T cal mean T calculated

Ttab =Ttabulated



Plate (8): Non-germinating seed inoculated by *F. oxysporum*. *f . sp . fabae*.

CHAPTER FIVE

DISCUSSION

In the present investigation, 70 % of the faba beans seed samples collected from different parts of the country have shown dark black tiny sclerotia on the surface of the seeds. Also brown to gray discoloration of seeds was observed and a number of fungi were detected in the blotter and the agar methods.

F. oxysporum was encountered in high percentage but other fungi have shown low incidence and some of them are new for faba bean. All seed samples infected by this fungus showed very poor germination and caused seedling mortality. The morphological and microscopic characters of *Fusarium spp*-described by Booth (1971) were coinciding with the isolated fungus associated with diseased seeds of faba beans.

The fungus is also similar to that reported by Yu and Fang, (1948) as *F. oxysporum Schl. f. Sp. fabae* and also according to Kulwant and Mathr (1991).

Result of dry inspection showed the presence of tiny sclerotia on the surface of the seeds and discoloration. In this context Neergaard (1977) reported that dry seeds may show symptoms in varying degrees due to necrosis or discoloration from stain produced by microorganisms. In the blotter and agar methods, *F.oxysporum* was detected in the tested seed samples. The fungus was found to attack germinating seeds, resulting in retarding the germination or complete prevention of further development.

When sown in blotter the fungus causes significant reduction in seed germination. The fact that *F. oxysporum* carried on or in seeds of faba beans and reduced their germination is well established, particularly from the report of Simay (1998). Other fungi have shown low incidence;

these were *Ascochyta fabae*, *Aletrnaria alternata*, *Botrytis fabae*, *B. ricini*, *Uromyces fabae*, *Aspergillus flavus*, *A.niger*, *Penicillium pinophlum* and *Curvularia lunata*. This is in agreement with Richardson (1979), Gärber; et al(1993); Simay (1998); most of these fungi are common seed-borne pathogens.

The occurrence of *C. lunata* in the agar method is a new record as seed-borne fungus in faba beans. Physiological studies of *F. oxysporum* indicated that, the suitable medium for *F. oxysporum* growth and sporulation was conducted according to the standard recommended techniques, Czapek's dox agar was recommended to be suitable for *F. oxysporum* as it gave vigorous, fluffy and dense mycelial growth than PSA. That may be justified by presence of both carbon and nitrogen sources (sucrose and sodium nitrate) while PSA contains carbon source only (sucrose). Among the four carbon sources, cornflour promoted better linear growth of *F. oxysporum*, while dextrose exhibited dense mycelial growth. This is in agreement with Booth (1971).

The pathogenicity of *F. oxysporum* was also demonstrated in the laboratory by testing the effect of fungus on germination of two samples of faba beans seeds. All inoculated seeds manifested visible symptoms. Susceptible sample revealed high significant difference than the resistant sample. This may indicate that the germination of faba bean seed is affected by *F. oxysporum* as reported by Simay (1996).

Chemical control of *F. oxysporum* in vitro has been attempted. It was found that Tilt(250 EC) was significantly effective against the fungus. While Nimrod (25 EC) was not effective. This finding was supported by the findings of Rauf; et al (1998) who found that when Tilt (0.3 %) was used in vitro it has prevented the occurrence of the disease in chick-pea infected by the same fungus.

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Appendix

Culture Media

a) PDA (patato Dextrose Agar) was prepared from:

- 1- 200 gm Patato (Pcefed & sliced).
- 2- 15 gm Dextrose.
- 3- 20 gm Agar.
- 4- 1 liter water.

b) PSA (Potato Sucrose Agar)

- 1- 500 ml. Potato extract.
- 2- 500 ml. Sucrose.
- 3- 20 gm. Agar.
- 4- 500 ml. Distilled water.

c) Czapek's Dox Agar

- 1- 1000 ml. Distilled Water.
- 2- 1.g Dipotasium (K_2HPO_4).
- 3- 2.g Sodium nitrate ($NaNO_3$).
- 4- 0.5 g Magnesium Sulphate ($FeCl_2$) $Mg(SO_4)_2$.
- 5- 0.5 g Potasium Chloride (KCl).
- 6- 15 g Agar.
- 7- 30 g Sucrose..

The water and potato extract are mixed together. Agar is added while the mixture is boiled, after that the Sucrose is added. Autoclaved at 15 psi for 20 minutes.

Chemicals used for controlling the fungus *Fusarium oxysporum*:

A / Nimrod

Trade name: Nimrod

Chemical name: 5-Butyl-ethyl amino-6 methylpyrimidin –4-yldim
ethysulphamate.

Application: systemic fungicide use for controlling powdery mildew
in fruit trees low doses.

B / Tilt

Trade name: Tilt

Chemical name: 1-2-3 Dichlorophenyl-4 propye 3-1 Dioclson-2-
xdmethyl-1-2-4 Trizol.

Application: Systemic fungicide related to that uses for inhibiting the
first stage of the fungal growth.

-:

(dry inspection)

-1

%70

-2

10

(Standard blotter method

-:

Fusarium oxysporum ,*Ascochyta fabae* ,*Botrytis fabae* , *B. ricini*
Alternaria alternata ,*Uromyces fabae* ,*Curvularia lunata* ,*Aspergillus*
niger ,*A. flavus* and *Penicillium pinophilum* .

F. oxysporum

-3

% 37.5 16.66

Media

-4

.Czapek`s Dox Agar

(25 Ec)

(250Ec)

-5

Fusarium oxysporum

()

2001

()

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2005

