A STUDY ON FUSARSIUM WILT DISEASE OF MUSKMELON (Cucumis L.)
In Khartoum North

By

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Nov. 2003
DEDICATION

To the soul of my father and mother. To my brothers, sister, dearest ones and to all my dear friends.

I dedicate this work.
Acknowledgement

I gratefully acknowledge the help and encouragement of my supervisor Dr. Ahmed Hashim Ahmed for his suggestions and good guidance. My thanks are also extended to Dr. Mohammed Osman Idris for his help and assistance.

I am also deeply indebted to Mr. Husam Mohammed Omer Ereibi or his keenness and help in typing this work. My thanks are also extended to all staff members and my colleagues at the department of Crop Protection in the Faculty of Agriculture, University of Khartoum.
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بسم الله الرحمن الرحيم

خلاصة الاظروحة

o آثار الإصابة كهفية nز عازل KADZ

.2002/2001 لتي تؤدي إلى تكوين تحت سيئة

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ورفعة أثاثية وقفلي السوئي فرخم أراز ابكا

f. oxysporum f. sp. melonis لـ آثلة أثلة أثلة

يأتي، أو آثارا، آثارا جميا، ترجع إلى اكما

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Abstract

The present study was conducted to investigate the infection of muskmelon growing at Elsilate Scheme with the *Fusarium Wilt* during 2001/2002 growing season. The symptoms of the disease were describe as brown necrotic strikes beginning at or below the ground and extending for several inches along one side of the stem and wilting was accompanied by progressive yellowing of leaves.

The causal organism was identified as *Fusarium oxysporum f. sp. Melonis*.

The results of the disease survey conducted in Elsilate Scheme revealed a significant difference in susceptibility among the melon varieties tested.

The varieties evaluation revealed that the variety Primal was resistant while the varieties Aminal and Standard Galia were very susceptible.

The present study also confirmed the positive relationship between *Fusarium oxysporum f. sp. melonis* (the causal organism) and the presence of the nematodes (Pretyleichus sp.).
CHAPTER ONE

INTRODUCTION

Muskmelon (Curcumas melon I.), as a member of the family *Cucurbitaceous*, is an important vegetable crop worldwide. The cultivated area grown with watermelon and muskmelon as well as the total production area is not stable, as seen in Table (1). Muskmelon is also an important vegetable crop in the Sudan as it is grown for local consumption as well as for export.

The main production areas in the Sudan are Khartoum, El damer, Atbara, Kosti, El dewaim and Sennar. The melon and varieties grown in the Sudan are Annanas, Hales Best Jumbo and Honey Dew for local marketing (Heinz *et al.*, 1984).

Most of the muskmelon varieties are grown in the Sudan for export. The fruits of these varieties are heavily netted, medium sized, nearly round with green-flesh, with excellent flavor, sweetness aroma, small seed cavity and good shelf life. Such fruits are of great demand in the European markets.

The most important handicaps for the production of muskmelon in Sudan are the pests and diseases. The major disease is the Fusarium wilt, incited by *Fusarium oxysporum f. sp melonis*, it is wide spread in melon fields as the Fungus is soil- borne and could survive in organic matters for six years or more. Hence, it is very difficult to be eradicated from the soil.
Table (1) : Area of Melons production in the Sudan

<table>
<thead>
<tr>
<th>Years</th>
<th>Area (1000 ha)</th>
<th>Production (1000 MT)</th>
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<tbody>
<tr>
<td>86-1990</td>
<td>4.98</td>
<td>137.20</td>
</tr>
<tr>
<td>1991</td>
<td>5.05</td>
<td>144.00</td>
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<tr>
<td>1992</td>
<td>5.00</td>
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</tr>
<tr>
<td>1993</td>
<td>5.39</td>
<td>142.73</td>
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Source : (Arab Agricultural Statistics Year Book (14), 1994)
In recent years there has been expansion in melon production such that it became an economical crop in the national income. According to this expansion a lot of disease problems appeared, thus the present study was initiated to study the following aspects of the wilt disease:

1- To describe the symptoms of the disease.
2- To identify the causal agent(s).
3- To survey the occurrence of the disease in Seilate Scheme.
4- To present evidence of resistance to the wilt disease in the commonly grown Melon varieties.
CHAPTER TWO

LITERATURE REVIEW

2-1 Botanical Description and Taxonomy

Muskmelon (Cucumis melo l.) is a member of the family Cucurbitaceae. The general botanical characteristic of the cultivated melon describe that the roots are well developed and superficial, stems are short, with fine hairs, the leaves are alternate and orbicular. The flowers are monoecious or undermonoecious (Male and Female flowers occurring on the same plant). The fruits are very variable in size and shape, globular, smooth or furrowed, rind glabrous and smooth to rough and reticulate, pale to deep yellow, yellow-brown or green, flesh yellow pink or green with many seeds.

The seeds are flattened, black, red-brown, white or cream smooth, 5-15mm in length, the edible seed kernel containing approximately 46% oil and 36% protein and 30 seeds/gram (Tindal, 1983). The national system of classification classified muskmelon as:

- Division : Phanerogram (Flowering plants)
- Sub Division : Angiosperms (Closed seeded plants)
- Class : Dicotyledons
- Sub Class : Polypetals
- Order : Cucurbitales
- Family : Cucurbitaceae
- Genus : Cucumis

2.2 The Center of Origin and Distribution
Center of origin is possibly Africa, then it was introduced into Europe and Asia during the last 2000 years. Now it is widely distributed throughout the tropics (Tindal, 1983).

2.2.1 Area of Cultivation

South east Asia (India, Indonesia, Philippines, Malaysia); China and east Africa, the Caribbean and throughout tropical and sub tropical regions (Tindal, 1983; Ib Libner, 1989).

2.3 The important Diseases of Melons

2.3.1 Fusarium Fruit rot

Fusarium fruit rot caused by Fusarium roseum, a soil borne fungus normally attacks only ripe fruits, causing spots 13 to 25mm on the melon surface, it is favored by wet conditions in the field or storage (Lincoln, 1987).

2.3.2 Powdery mildews

Powdery mildews have been serious problems for many years. Control in melon has been achieved by breeding resistant cultivars, first by introducing cultivars sulphur resistant. Enabling growers to apply an effective chemical control. And later by breeding for resistance to control the causal organism (Lincoln, 1987). Most of the research work in the control of powdery mildew diseases in the Sudan was done at Gezira Research Station on cucurbits (Omer, 1972).

2.3.3 Muskmelon mosaic
This disease is caused by a virus, the leaves show dark-green banding around the larger veins and later develop yellow and green mottling. Flowers of infected plants are often deformed but fail to set fruits. The virus is readily transmitted by aphids (Thompson and Kelly, 1957).

2.3.4 Fusarium wilt

Melon and cucumber are widely grown in several parts of the world although they are highly vulnerable to Fusarium wilt in many parts of the world, the infection could reach 70% (Marshal et al. 1981).

Wilt of Melon is caused by *Fusarium oxysporum* f. sp. *melonis* (John, 1952; Alan and Arden, 1986). Zungia and Zitter (1993) stated that forty-six isolates of *F. oxysporum* f. sp. *melonis* were isolated from soil. Samples throughout melon fields in New York State (USA) were collected and the fungus was identified on the basis of morphological and pathogenic test. The pathogen infected *Cucumis melo*, which included muskmelon and Honey dew melon (Alan and Arden. 1986).

2.4 The Fungus

Pandey (1986) reported that, the fungus was classified according to Ainsworth report (1971), as belonging to the

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Fungi</th>
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<td>Division</td>
<td>Eumycota</td>
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</table>
Sub Division : Deuteromycotina
Class             : Hyphomycetes
Order            : Moniliales
Family           : Tuberculariaceae
Genus            : Fusarium

The hyphomycetes genus Fusarium is characterized by being fast growing, with pale or bright-colored colonies, felty arial mycelium and diffused or spordochial sporulation. There are three types of spores, namely microconidia, macronidia chlamydospors. The microconidia develop in the vessels host plant. They are small, oval or somewhat curved, unicellular or 1-2 time septate, 5-12x 3.3-3.5 µm. The macroconidia are longer, developing on the stoma of the mycelium found in the park of the stem. They are long, curved backward, pointed at their ends and septate. Each conidium is 15-20 µm 3-5 µm broad. Sometimes small conidiophores develope.

The chlamydospores are terminal or intercalary in hyphae, often in conidia, hayline, smooth walled or roughened 5-15 µm diam. Sclerotial pustules present in some isolates, pale to green or deep violet(Gerlach and Nirenbeng, 1982; Booth1971).

Fusarium oxysporum is a fast growing fungus, has an optimal temperature for growth between 25° and 30 C
(Zachariah et al. 1956 and Olutiala 1978) and maximum at or below 37°C, and minimum above 5°C (Buxton, 1955).

The thermal death point is between 57°C and 60°C for 30 min in soil. A wide range of pH between 2.2-9.0 is tolerated (Korobeinikova, 1960) with an optimum at pH 7.7 (Olutiala, 1978)

2.5 History and Distribution of Wilt disease in Melon

Chupp (1930) reported that the Fusarium wilt of muskmelon caused a trace to one percent loss in several fields in New York. Leach (1933) reported that the experiment of several melon growers indicated that the disease was present in the United States for at least three years. Records of the Department of Plant Pathology also indicated that the disease was observed in 1931 in USA.

Bouhot and Louret (1971) reported that the disease was first observed in 1965 through the introduction of the muskmelon cultivar “Doublon” and it was shown by soil analysis to be present all over France in 1965. Fusarium wilt of muskmelon, caused by *F. oxysporum f. sp. melon* has been major problem in France and in northern and central part of the United State for years. The disease was also reported from Washington state and from Riverside country in southern California (Gubler and Grogan, 1976).
Alan and Arden (1986) reported that, Fusarium wilt of muskmelon was recorded in 1930 in New York and Minnesota. It is now present throughout a wide area of North America including Washington, California, Northern Ontario as well as throughout Europe and Asia. The losses of individual field amount to 50% of seedlings during the first few weeks of growth, and from 90% to 100% cessation of melon production in one area of central Minnesota

2.6 Disease Symptoms

Wilted plants in later stages of maturity are characterized by brown necrotic streaks beginning at or below the ground and extending for several inches along one side of stem (Leach, 1933).

Mas et al. (1980) that, the Fusarium wilt fungus can attack melon at any age, even before the plants sprout, but especially when the fruits are maturing.

The diseased plants, either develop slow wilting accompanied by progressive yellowing, or sudden wilting without prior yellowing.

The first case is the most common one where by the veins of some leaves turn yellow on one side, then later these leaves become completely yellow, thickened and brittle. At this stage, the leaves have a slight color of violets. A longitudinal brown necrotic streaks then appear on the stems from which gum
exudes. In the final stage, the fungus sporulates in the necrotic zone and forms pinkish – colored.

In the second case the plants show sudden wilting without yellowing or odor. The tips of the stems are generally attacked first and shrivel; wilting then progresses towards the base of the plant.

In both cases a transverse section of the stem shows clear brown vascular discoloration corresponding to the vessels that have been attacked vascular discoloration is more predominant in those plants with yellowing symptoms (Nelson et al., 1981).

Alan and Arden (1986) recorded that the melon plants may be affected at any stage of development. In heavily infested soil and at low temperature, the seedlings may die before they emerge. Symptoms on very young plants could be confused with those of damping-off caused by other fungi. Root – rots on older seedlings especially in cool soils are obvious.

2.7 Etiology of the disease
2.7.1 Survival in the field

Banihashemi and Dezeeuw (1973) reported that, the survival units of *F. oxysporum f. sp. melonis* were the chlamydospores associated with previously invaded organic particles. Alan and Arden (1986) stated “the fungus can survive long, periods in soil, likely, as chlamydospores and in
association with melon plant residue. The fungus can colonize and survive on both host and non-host tissue and can compact successfully with other soil fungi”

2.7.2 The Parasitism of the Pathogen

Banihashemi and Dezeeuw (1975) reported that, the population of *F. oxysporum f. sp. melonis* in the soil increase in the presence of a living host and decrease in its absence, and was greater at the site of wilted plants and on the soil surface than between rows and at lower soil levels.

Pandey (1986) stated that "this fungus is a facultative parasite and can survive in the soil in the absence of host plants”.

2.7.3 Factors that favor the infection

Leach (1936) reported that, melon wilt does not depend upon high soil temperatures for best development. Melon seeds did not germinate well in soil below 20° C, and seedlings grow best at 35° C, within this range the wilt was most severe at the lower soil temperature, that the infected plant is killed by wilt late in the season.

Miller (1945) reported that, external factors can play and important role in the development and severity of Fusarium
wilt of melon but it is difficult to assess the influence of each factor separately. The most severe symptoms were observed at 18°-22°C. At high temperature (30°C) the plants were infected without showing symptoms. Wilt of melon is a disease that occurs in cool soils and early in the season, since the most severe symptoms are found at temperature between 18° and 20° C. Insufficient illumination and short day length increase the severity of vascular wilt. Also, Miller observed that the frequency of the disease is higher under dry conditions, and symptoms occur more quickly and more severe when the relative humidity of the air is between 50 and 65%, and rarely occur when it goes beyond 80 and 90%.

Wensley and Mckeen (1965) reported that, mineral nutrition also influence Fusarium wilt of melon. The addition of nitrogen increases the severity of disease either by increasing susceptibility of the host or by increasing virulence of the fungus. The addition of potassium or calcium decreases disease severity.

Banihashemi and Dezeeuw (1975) stated that “low winter temperature does not affect the population of the pathogen, but it declines it sharply in spring, This drop in population appears to be due to progressive decomposition of infected melon residue, which perhaps is an important source of inoculum in the field”. Alan and Arden (1986) observed that disease development was favored by high nitrogen, low calcium and potassium levels.
High nitrogen levels increase the susceptibility of melon plants, and high potassium levels increase the activity of beneficial competitive fungi around melon root.

Sezgin and Onano (1987) reported that, growth, virulence and incidence of the disease on melon were reduced by urea application.

2.8 Pathogenicity

Gubler and Grogan (1976) tested the pathogenicity of *F. oxysporum* isolates by inoculation of muskmelon cultivars PMR-US and topmark. He found that the cotyledons developed 7-8 days after inoculation and the plants were killed within 15 days. Non-inoculated controls (root dipped in distilled water) healthy, isolation from wilted seedlings yielded *F. oxysporum f. sp. melonis* in pure culture.

Sneh et al. (1987) found that inoculated plants with Fusarium displayed symptoms within 21 days in a glasshouse experiment.

2.9 The effect of Nematodes on the incidence of wilt
Athinson (1982) reported, the association between Fusarium wilt of cotton and root rot nematodes, similar results of these findings were observed in melon by Leach and Currence (1939) and Ahmed (1997).

It has been suggested by Minton (1963) and Smith (1953) that penetration by Fusarium occurs through those portions of vessels exposed after the cortex has been damaged by the action of nematodes or other soil organisms.

Jenkins and Coursen (1957) stated that the genus *Meloidogyne* is concerned in so many recorded association with melon wilt together with its wide host range and distribution. Wilt was more severe when nematode inoculation (Pouter and Powel, 1967) confirmed by Ahmed (1997).

Control of nematodes with soil fumigants and crop rotation often results in considerable decreases in melon wilt (Gubler et al 1976) and in large yield increases. However, usually only a part of this benefit of fumigation can be ascribed to the control of nematodes or decrease in wilt. Kesayan and Prasal (1975) pointed out that some of the benefits are derived from greater mineralization of soil nutrients following fumigation.
CHAPTER THREE

MATERIALS AND METHODS

3.1 The field experiment

The field experiment was conducted at the Faculty of Agriculture Research Experimental Farm (latitude 1° 40 N and longitude 32° 32 E) during the winter of 2001-2002, to study the susceptibility of six varieties of muskmelon to wilt disease. The recommended agricultural practices for the cultivation of muskmelon were adopted in the experiment. The field was divided into four blocks. Each block was divided into six mastabas each 150 cm in width and 10m in length and 30cm between the plants. Mastabas were running north south. The seeds were sown on the mastabas at the rate of two seeds per hole.

The climate is semi-arid, tropical with an average annual rain fall of 160 mm, mostly during July – September. The mean maximum and mean minimum temperature are as high 48°C in summer and as low as 18° C in winter, respectively.

The soil of the experiment is clay loamy type, slightly alkaline with pH 7.8.
The varieties tested in the experiment were:

1- Aminal
2- Ideal
3- Royal
4- Rodin
5- Green star
6- Annanas

Each treatment was replicated four times. The plants were irrigated every 7 to 9 days. A complete Randomized Block Design (CRBD) was adopted. The first count of the diseased plants was performed five weeks after sowing. The layout of the experiment is shown in fig 1.
Fig 1. plan of field experiment

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<tr>
<td>Rodin</td>
<td>Annanas</td>
<td>Aminal</td>
<td>Royal</td>
</tr>
<tr>
<td>Ideal</td>
<td>Rodin</td>
<td>Ideal</td>
<td>Ideal</td>
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<tr>
<td>Royal</td>
<td>Royal</td>
<td>Rodin</td>
<td>Aminal</td>
</tr>
<tr>
<td>Annanas</td>
<td>Green star</td>
<td>Green star</td>
<td>Rodin</td>
</tr>
<tr>
<td>Green star</td>
<td>Aminal</td>
<td>Royal</td>
<td>Annanas</td>
</tr>
<tr>
<td>Aminal</td>
<td>Ideal</td>
<td>Annanas</td>
<td>Green star</td>
</tr>
</tbody>
</table>

3.2 Disease Survey

Regular surveys were carried out in muskmelon fields at Elsilate Scheme during 2001/2002. The disease incidence was recorded as a percentage of muskmelon plant showing the typical wilt symptoms.

The observations were taken every two weeks and the inspected plots were chosen at random from three fields, every field was divided into four blocks. Each block was divided into ten mastabas.

The Muskmelon varieties surveyed were:

1. **Aminal**
2. **Standard Galia**
3. **Primal**
The sampling was conducted as follows; the first block was left as boarder and the sampling started from the second block at mastaba number four. The second sample was taken at the third block at mastaba number six. The third sample was taken from the fourth block at mastaba number seven. The fourth sample was taken from the tenth block at mastaba number three. The counts were made 35 days after muskmelon plantin.

3.3 Isolation of Fungus

3.3.1 Isolation from Soil

Soil samples were taken randomly from the infected muskmelon fields in plastic bags and brought to the laboratory for isolation of the fungus. The soil samples were mixed thoroughly one gram of soil was taken and added to 100 ml sterilized distilled water (S D W), 5 ml of this suspension were taken by the micropipette and added to 95 ml of S D W in a flask. Three samples were taken for study. To each of the four Petri dish all through containing Potato Dextrose Agar (PDA), one ml of the suspension from the last dilution was added. The Petri dish were incubated at 25° C for one week and observed under the microscope for fungus growth and identification.
3.3.2 Isolation from Roots

Infected roots of muskmelon plants showing the characteristic symptoms of wilt disease were obtained from Elsilate Scheme during February 2001. The infected roots were washed in running water and then cut into one inch segments and then surface sterilized for two minutes in 0.1% solution of mercuric chloride. The segments were then washed in five changes of SDW on sterile filter papers and placed on PDA (4 segments/petri dish). The Petri dish was then incubated at 23 - 25°C for one week. The dishes were then examined under the microscope for the fungal growth subcultures were made from the periphery of the growth of different colonies to obtain pure cultures. Fungal hyphal tips were taken from the growing colonies and transferred to agar slants and then incubated at 23-25°C for one week. The identification of the isolated fungus was based on shape of the fungus under the microscope.

3.4 Isolation of the Nematodes

Soil samples and infected plants materials were collected from the surrounding of the roots of muskmelon crop in the field and kept in plastic bags for nematode extraction by using Baermann Funnel Techniques (Warwick, 1975). The soil samples containing nematodes were placed in a square of butter...
muslin which is flooded to enclose the soil, and then gently submerged in the water in the funnel. Nematodes emerge out and sink to the bottom of the funnel stem, after one day, part of the water was run-off and examined the presence of the nematodes.
CHAPTER FOUR

RESULTS

4.1 Field Experiment

The field experiment was conducted in the Faculty of Agriculture Experimental Farm to screen six muskmelon varieties for their resistance to Fusarium wilt. The experiment was executed as described in chapter three (Materials and Methods). Unfortunately the experimental exposed to heavy rain soon after irrigation and resulted in flooding of the muskmelon plants which were much affected and no reliable data was obtained.

4.2 Disease Symptoms

Wilt symptoms were observed on muskmelon plants during the survey conducted at Elsilate Scheme during 2001/2002 cropping season. The Fusarium wilt symptoms were observed in muskmelon before the plants are mature, but severe symptoms were observed when the plants are maturing (Fig. 2). Various degrees of yellowing, stunting, vascular discoloration, wilting and stem streaking were produced on the
muskmelon crop, usually resulting in premature death. Yellowing and wilting generally advanced upward from the stem base. The symptoms were first pronounced in young leaves (Fig. 3). The pathogen advanced from the xylem into the cortex causing the death of muskmelon plants (Fig. 4). Transverse sections of the steins showed clear brown vascular discoloration corresponding to the vessels that have been attacked. Vascular discoloration was more predominant in plants with yellowing symptoms (Fig. 5).

4.3 Disease survey

The data obtained from the survey conducted at Elsilate Scheme is shown in (Fig. 6). It shows different degrees of susceptibility of muskmelon varieties to Fusarium wilt. The variety Primal was resistant while the Aminal and Standard Galia were highly susceptible (Fig. 6).

4.4 The Isolation of Fungus from the Roots and Soil

Isolation of the causal agent can easily be made in pure culture from soil and roots of infected melon plants showing symptoms. The causal agent was identified as *Fusarium oxysporum f* sp. melonis collected from infected plant roots and soil. The fungus was characterized by fast growing pale or bright colored
colonies with arial mycelium and diffuse sporulation (Fig. 7), and the shape of macroconidia, microconidia and chlamedospores.

4.5 Isolation of Nematodes

The soil samples obtained from soil surrounding the roots of the infected muskmelon plant (from Silate Scheme) were tested and the results of these tests showed the presence of nematodes (Pretylelchus sp.). Plants growing in farm field or in other place are constantly exposed to attack by the nematodes and multiple infection of root system is common rather than exceptional (Ahmed, 1997).
Figure 6: Disease Incidence of the three melon varieties obtained during 2001/2002 season at Elsilate Scheme.
CHAPTER FIVE

DISCUSSION

In the present study the muskmelon varieties grown in Silate Agricultural Scheme in 2001/2002 were evaluated for the resistance of the Fusarium wilt disease.

The wilt disease of melon caused by *Fusarium oxysporum f. sp. melonis* is a major disease of muskmelon and can attack muskmelon at any age of the plant growth, but more serious when the fruits are maturing.

The symptoms of the disease on muskmelon plants were recorded from the result of the Elsilate Scheme in season 2001/2002 (The description of the symptom was given in the previous chapter), and was compatible with Mas and Risser (1966) descriptive monograph.

The results showed that disease was very common in the muskmelon grown in Silate Scheme: The results also showed that the variety Primal was highly resistant while the varieties Aminal and Standard Galia were highly susceptible to the wilt disease, such findings agreed with the previous reports that described the Standard Galia as susceptible to the Fusarium wilt (Anon, 1997).

Similar to the results reported by Marshal et al. (1981) The present study revealed a positive relation between the Fusarium
wilt of muskmelon and the presence of the plant parasitic nematodes in the soil, more over, Ahmed (1997) showed that the combination of the Fusarium and the nematodes in the soil resulted damage.

Further Future Research should include the following:

- More varieties should be evaluated.
- The effect of the sowing date on the resistance of Muskmelon to the wilt disease need to be evaluated.
- Field experiments need to be conducted in sick plots (contaminated with both the Fusarium and the nematodes) to confirm the resistance of the varieties.
REFERENCES


APPENDICES

Appendic 1: Mean Incidence of wilt on three melon varieties obtained during 2001/2002 at Elsilate Scheme.

<table>
<thead>
<tr>
<th>Varieties</th>
<th>% of Mean disease Incidence</th>
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<tbody>
<tr>
<td>Aminal</td>
<td>14.81</td>
</tr>
<tr>
<td>Standard Galia</td>
<td>11.93</td>
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<tr>
<td>Primal</td>
<td>0.51</td>
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Appendic 2 : Anova table of Wilt Incidence in of melon

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<th>Sources</th>
<th>D.F</th>
<th>S.S</th>
<th>M.S</th>
<th>F.cal</th>
<th>F.tab</th>
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</thead>
<tbody>
<tr>
<td>Varieties</td>
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<td>11517.04</td>
<td>5758.52</td>
<td>45.77</td>
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<tr>
<td>Error</td>
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<td>29819.48</td>
<td>125.82</td>
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<tr>
<td>Total</td>
<td>239</td>
<td>41336.52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD 5%</td>
<td></td>
<td>3.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD 1%</td>
<td></td>
<td>4.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*, ** : significant at 0.05 and 0.01 levels