J. Agric. Sci. Mansoura Univ., 32 (2): 903 - 911, 2007

OCCURRENCE AND IMPORTANCE OF WATERMELON WILT DISEASEE IN DAKAHLIA

El-Hadad, S. A.
Plant Pathol. Res. Inst., Agric. Res. Center, Giza.

ABSTRACT

Wilt of water melon, caused by *Fusarium oxysporum f.* sp *niveum*, is one of the most important economic diseases, which attacks watermelon in any stages of plants development causing great losses under open field.

Fusarium spp. was the most prevalent fungi isolated from watermelon seeds samples followed by *Aspergillus* spp., *Penicellium* spp. and other field fungi. Highest percentage of infection and disease severity were recorded in new reclaimed land in El-Gamalia and Sherbeen district in Sandy soil.

Different isolates of *F. oxysporum f.* sp. *niveum* caused percentage of wilt from 10 % to90 %. Total count microorganisms in watermelon rhizosphere at maturity stage very high compared with control. All watermelon cultivars were infected with *F. oxysporum* f. sp. *niveum* Charleston gray; Giza 1 and Dixie Queen were more susceptible cultivars, while Bicok 60 and Giza 2 were more resistant.

INTRODUCTION

Watermelon (*Citrulius lanatus* L.) is one of the most important cucurbit crops in Egypt and many countries in the world. Wilt disease caused by *Fusarium oxysporum* f. sp. *niveum* was considered the most vital pathogen and limiting factor of watermelon production in Dakahlia governorate (Gimeon, 1990; El-Zayat *et al.*, 1993 and El-Kahky, 2005).

Watermelon seeds were reported to bear important phytopathogenic mycoflora. *Fusarium oxysporum* f.sp. *niveum* is a seed borne pathogen of watermelon, and the seeds play an important role in spreading wilt disease (Tymchenko, 1967, Sun and Huang, 1979; Martyn, 1985 and Michail *et al.*, 1989). *F. oxysporum* f. sp. *niveum* the watermelon wilt Pathogen might induce seed decay and damping off to its host (Walker, 1952; Hine, 1962; Naik *et al.*, 1993; Larkin *et al.*, 1996; Michail and *et al.*, 2002).

Filiz and Turahn (1992) isolated *F. oxysporum* f. sp. *niveum* from wilted water melon plants were sampled in 123 fields in 3 provinces of Turkey over 2 years the isolates of 103 *F. oxysporum* f. sp. *niveum* obtained form the diseased plants were tested for pathogenicity towards the susceptible cultivar sugar baby.

Wei *et al.* (1991) demonstrated that, the main diseases in the regions of China were mosaic, fusarium wilt, anthracnose and fruit rot in watermelon plants.

Biles and Marteyn (1989) stated that watermelon cultivars differentially resistant to fusarium wilt were pre inoculated with *F. oxyasporum* f. sb. *cucumerinum* or avirulent races of *F. oxysporum* f.sp. niveum 24 or 72 hr prior to challenge with virulent race of *F. oxysporum* f. sp. *niveum*.

Thus, this work was designed to solve this problem by studying the following points.

1- Study seed healthy testing of watermelon.

2- Survey watermelon wilts disease.
3- Isolation, purification and identification of the causal pathogen.
4- Pathogenicity test.
5- Study rhizospheric microflora.
6- Study varietal reaction.

MATERIALS AND METHODS

1- Seed health testing of watermelon:

Seeds were tested be the two standard methods, agar and the blotter method (IST A, 1966) two hundred seeds of each cultivar were first soaked in 1 % sodium hypochlorite solution (chlorax) for three min., then transferred to PDA medium supplemented with 0.2% difico yeast extract (PDY A) (Booth, 1971) in pyrex glass plates at the rate of 10 seeds/dish for agar test. In case of bolter method another 200 seeds were directly plated on three moistened bolters at the rate of 10 seeds/ dish. All plates of both tests were incubated at 30°C under alternating cycles of 12 hours of neon ultraviolet light (NUV) then complete darkness for 12 hr for seven consecutive days. The NUV light was emitted for two Phillips Black lighter tubes TI 40w/80 BLB. (Chnaiprasit *et al.*, 1974).

2- Survey studies:

During the growing season 2005 a quantitative survey on the distribution of wilt disease of watermelon was carried out to determine the percentage of infection and disease severity on different locations of new reclaimed land as well as old land. The disease severity of wilt disease in mature stage was determined according to the scale was suggested by Martyn (1965) as follows:

0 = no symptoms.....(resistant)
1 = hypocotyls browning, no wilt, no stunting.....(intermediate)
3 = cotyledon lesion, no wilt, no stunting.....(intermediate)
5 = slight wilt, stunted.....(susceptible)
7 = severe wilt, stunted.....(susceptible)
9 = dead.

3- Isolation, Purification and Identification of the causal pathogen:

Samples of diseased watermelon plants were collected from different locations of Belkas district especially new reclaimed land in Dakahlia governorate. The infected shoots and roots washed carefully with tap water then dried between two filter papers and were cut into small pieces. The pieces were surface sterilized by immersing them in 0.1 % Sodium hypochlorite solution for two min., then rinsed for several times in sterilized distilled water. Sterilized samples were dried between two sterilizes filter paper then were plated on plain agar medium in Petri dishes and were incubated at 25 -28°C for several days.

The isolated fungi were purified by using the single spore technique and/or hyphal tip technique. Detected fungi were transferred to slants of Potato Dextrose Agar (PDA) medium and were kept at 5 °C for further studies.

4- Pathogenicity test:

Pathogenicity tests of isolated fungi were carried out under greenhouse conditions. Sterilized pots (50 cm. in diameter) filled with five kg. autoclaved sandy loom soil (50 % sand and 50% loam w/w) soil inoculum was prepared in barley kernels medium. Conical flasks (500 ml.) containing 100 gm. of clean barley kernel and 100 ml. of water were autoclaved at 121°C for 15 min.

Autoclaved barley medium were incubated at 25°C for 10-15 days. The previously prepared fungal inoculum was used to infest autoclaved soil at the rate of 4 % soil weight (w/w). The above mentioned infested soils were mixed and rubbed together to release mycelium spores from barley; the mixture was sieved thoroughly at 2 mm mesh screen to separate barley kernels. The pots were watered and mixed thoroughly with inoculum and left for one week to ensure distribution of the inoculated fungi. At the same time three pots containing autoclaved soil was prepared to serve as a control. Seeds of watermelon (Giza 1) were surface sterilized by immersing them in 0.1 % Sodium hypochlorite solution for 2 min then washed several times with sterilized water. Dried watermelon seeds were sown at rate of ten seeds per pot. A set of three replicates were used in each particular treatment. Disease incidence was recorded as percentage of wilted plants and healthy survival after 60 days of sowing date.

The inoculated wilt fungi were tentatively re-isolated from infected plants and checked microscopically.

5- Rhizosphereic microflora:

Some samples of infected soil with pathogen were collected from different locations which were sown for several times with watermelon.

One gram of soil sample was transferred to bottles containing 99 ml. sterile distilled water. The bottles were shaken thoroughly on a mechanical shaker for 15 min. This gave on approximated 1/100 dilution was diluted to obtained serial dilation till $1/10^6$ by using sterile distilled water as dilution to study the microflora. The rest solution (99 cm^3) in the bottle containing the rhizosphere soil was dried at 105°C for 24 hours to obtain actual weigh of rhizosphere soil, the count of microbial floral gm/ dry weight were obtained by the decimal plate count technique .

A- Actinomycetes count:

Jensen's medium (Johanson *et al.*, 1960) was used for counting this group of microorganisms. One ml from $1/10^4$ dilution platted on the medium and incubated at 28°C for 6 days. Results were recorded and corrected to the dilution of 10^6.

B- Total fungal count:

Martin's medium (Martin, 1950) was used to obtain the total fungal count by adding 1.0 ml of $1/10^5$ dilution to Martini's medium before solidification and incubated at 25°C for 5 days. The results were recorded and corrected to 10^4 dilution.

C- Total bacterial count:

The total bacterial count, 1.0 ml from 10^6 dilution plated on Topping's medium (Allen, 1959) and incubated at 30°C for 3 days. The results were recorded.

6- Varietal reaction

Some watermelon cultivars namely Giza 1, Giza 21, Chilean black, Charleston gray, Bicok 60, Dixie Queen and Eswan hyprid were tested in this experiment. The autoclaved soil was infested with inoculums of pathogenic fungus which was prepared as mentioned before in pathogenicity test experiment at the rate of 4% of soil weight (w/w). The sterilized plastic pots (25 cm in diameter) were filled with preparing soil. Ten seeds from each cultivar were sown in each pot. Three pots were sown for every cultivar. Disease incidence was recorded as percentage of pre and post emergence damping of after 30 and 60 days respectively, while healthy survival plant was recorded also after 90 days from planting.

RESULTS AND DISCUSSION

Data in table (1) identification trials showed that the isolated fungi belong to 14 genera and 23 species. Among the so called (field fungi) *Fusarium oxysporum*, *F. solani*, *F. semitectem*, *F. moniliforme* and *F. equisti* which occurring higher percentage followed by *Aspergillus niger*, *A. flavus*, *Rhizopus nigricans*, *Mucor* sp. *Phoma* sp. and *Cladosporium* sp. The other isolated fungi had low percent of occurrence. Variation in the percentage of detected fungi might be due to increase frequently sowing watermelon in the same soil. These results were in agreement with those obtained by Jiang *et al* (1998).

Data in table (2) show that the highest percentage of infection and disease severity were recorded in El-Gamalia and Sherbeen districts which gave the highest percentage of infection and disease severity followed by Belkas and El Manzala districts. On the other hand, Kalabsho and Ziane gave the lowest percentage of infection. Percentage of infection and disease severity were high in sandy soil compared with clay soil. The wilt watermelon disease was surveyed in all locations.

Variation in the percentage of disease incidence and severity in different locations might be attributed to one or all of the following factors sowing watermelon in the same soil for several once causing increase frequency spores of pathogenic fungi and gave aggressive pathogenic isolate, temperature, soil moisture, relative humidity, differences in planting date, agricultural practices used, pathogenic fungus strain and interaction with host and environmental. These results were in agreement with those obtained by Mclaughlin and Martyn (1982) and Filiz and Turhan (1992).

Data in Table (3) indicate that the tested isolates from 1 to 16 of *F. oxysporum* f. sp. *niveum* were pathogenic to watermelon plants and caused wilt disease with virulent percent. Isolates No. 11, 2, 13, 14, 8 and 6 gave the highest percentage of wilt plants, while the isolates No. 3, 5, and 16 gave moderately percent of wilt plants. On the other hand isolate No. 4, 15, 10, 12 and 7 gave the lowest percent of wilt infection. Differences in pathogenicity might be due to differences among isolates in genetic structure and differences in producing enzymes and toxin essential for their pathogenicity. Such results are in line with the results obtained by Michail *et al.* (1989) and Wei *et al.* (1991).

Table (1): Incidence of seed borne fungi in sample of watermelon by using agar and blotter methods:

Fungi	Agar method		Blotter method	
	No. of sample infection	% of detected fungi	No. of sample infection	% of detected fungi
Fusarium oxysporum	46	90	49	95
Fusarium solani	9	18	11	25
Fusarium semitectem	18	35	14	34
Fusarium moniliforme	14	40	13	45
Fusarium equisti	6	25	8	39
Aspergillus niger	30	45	26	38
Aspergillus flavus	24	56	17	66
Penicillium digitatum	7	27	6	37
Penicillium chrysogenum	8	16	7	27
Rhizopus nigricans	21	34	17	46
Phoma sp.	14	38	10	41
Mucor sp.	19	25	14	45
Cladosporium sp.	15	36	10	32
Nigrospora sp.	5	25	5	27
Trichoderma viride	8	34	3	42
Trichoderma sp.	10	18	11	28
Trichoderma hamatum	7	26	8	32
Trichoderma herzianum	8	32	5	18
Epicoccum sp.	6	34	3	17
Curvularia sp.	4	18	8	22
Drechslera sp.	1	32	5	24
Chaetomium sp.	4	14	10	36
Verticillium sp.	3	18	7	27
L.S.D. at 0.05	10.43	15.45	16.34	9.97

Table (2): Survey of wilt disease of watermelon in Dakahlia governorate during 2005 growing season.

Location	Soil texture	% of infection	Disease severity
Belkas	Clay	50	3
	Clay	30	1
	Clay	40	5
	Sandy	80	7
Sherbeen	Sandy	70	7
	Clay	20	5
	Sandy	80	9
El-Gamalia	Sandy	80	3
	Clay	50	3
	Sandy	80	9
El-Manzala	Sandy	60	5
	Sandy	30	3
	Clay	50	3
Kalabsho	Sandy	60	5
	Clay	30	3
Ziane	Sandy	60	7
	Clay	20	3
L.S.D. at 5 %		9.48	1.21

Table (3): Percentage of infected and healthy survival plants of watermelon (Giza 21) cultivar infected with *Fusarium oxysporum* f. sp. *niveum*.

Fungal isolates	% of infected plants	% of healthy survival plants
1	70	30
2	80	20
3	50	50
4	40	60
5	50	50
6	70	30
7	30	70
8	70	30
9	30	70
10	40	60
11	90	10
12	30	70
13	80	20
14	50	50
15	40	60
16	60	40
L.S.D at 5%	15.36	20.42

Data in table (4) indicate that the total count of all tested microorganisms in rhizosphere of watermelon plants at maturity stage very high compared with the total count microorganisms in control soil. Total count of bacteria gave highest count, followed by fungi count while the count of actinomycetes gave the lowest count.

Data show that decrease microbial count in unsowing soil compared with watermelon rhizosphere. The differences on count of microflora might be due to the activity of root exudates of watermelon which contain sugar, amino acids, growth regulates and vitamins encourage increasing microflora count.

Table (4): Count of microflora on the rhizosphere of watermelon at different locations of cultivated area.

Microorganisms		Locations				
		1	2	3	4	5
Control	Bacterial count	74	63	68	85	70
	Fungal count	17	15	14	20	8
	Actinomycetes count	1	2	3	1	1
Watermelon	Bacterial count	285	280	239	245	120
	Fungal count	40	38	25	46	20
	Actinomycetes count	1	3	5	6	8

Location : L, Microorganisms : M, Treatment : T
L.S.D. at 0.05, L = 8.9, M = 10.2, T = 10.9
L x M = 17.3
M x T = 16.8
L x T = 18.6
L x M x T = 21.3

Data in Table (5) indicate that, the all cultivars were infected with *F. oxysporum* f. sp. *niveum*. Giza 1, Charleston Gray and Dixie queen were more susceptible which gave high percentage of pre and post emergence damping off and low percentage of healthy survival plant. On contrast Bicok 60, Giza 21 and Eswan hybride which gave high percentage of healthy survival plants and low percentage of pre and post-emergence damping off. Differences among cultivars in their resistance and susceptibility might be due to the differences in their genetic make up and characters correlated to this genetic structure such as morphological characters of plants, root exudates and chemical components of seed or plants. In addition, to genetic make up, factors in relation to both host and pathogen might play a role in cultivar susceptibility. These results were in agreement with those obtained by Michail *et al* (2002) and Attia (1995)

Table (5): Varietals reaction of watermelon plants to wilt infection caused by *F. oxysporum* f. sp. *niveum*.

Cultivars	% of		
	Pre-emergence damping off	Post-emergence damping off	Healthy survival
Giza1	30	50	20
Giza2	2.	20	60
Chelian black	30	30	40
Charleston Gray	30	60	10
Bicok 60	10	30	60
Dixie Queen	30	40	30
Eswan hypride	20	30	50
L.S.D at 5%	6.3	7.4	8.6

REFERENCES

Allen, O. N. (1959). Experiments in soil microbiology 2nd. ed. Burgen Pul. Co. Minnesota.

Attia, (1995): Studies on root rot disease of some cucurbits in reclaimed land M.Sc. Plant Path., Fac. Agric., Zagazig Univ., Egypt.

Biles, C.L and Marteyn, R.D. (1989): Local and systemic resistance induced in watermelon by forme specials of *Fusarium oxysporum* Phytopathology, 79 (8) : 856- 860

Booth, C. (Editor) (1971). Methods in microbiology. Vol. 4: Academic Press London and New York. 795 pp.

Chnaiprasit, C.; Mathurand, S.B. and Neergaard, P. (1974). The light factors in seed healthy testing. Seed Sci.& Technol. 2: 457- 475.

El-Kahky, M. T A. (2005). Study on wilt diseases infesting some important cucurbit crops and its control. M.S.C. Thesis, Fac. Agric., Mansoura Univ., Mansoura, Egypt.

El-Zayat, M. M; Mahrous, M. M.; Sallam, A. A. and El-Korachy, M. M. (1993). Cucumber diseases at Ismailia under protected and open field cultivation in relation to environmental comditions. Zagazig J. Agric. Res. 20(3)1051-1062

Filiz, N. and Turhan, G. (1992): Investigations on the determination of *Fusarium oxysporum* f.sp. *niveum* races in the Aegean region of Turkey. Zeitsehrift fur Pflanzkranheiten and Pflonzschuz. 99(1): 5 6-61.

Gimeon (1990). Collapse of melon on the valancia coast centero de invetigaciony Technologia Agraria (1988), 87 ISBN, 84-86840-00-7 (C.F. Review of Plant Pathology, 79: 2, (1990).

Hine, R. B. (1962). Saprophytic growth of *Fusarium oxysporum* f. sp. *nievum* in soil. Phytopathology. 52. 840-843.

I. S. T. A. (1966). Proceeding of international testing. International rules of seed testing. Wageningen, Netherlands, 152 pp.

Jiang, X.; Zhu, C.; Xie, D.; Ni, C, Jian, X.L; Zhu, C.X., Xie, D.I. and Ni, C.F. (1998): The mode of action of agricultural antibiotic 120 on Fusarium oxysporum f. sp. *niveum*. Acta Phytophylacinca.25 (4) 351- 354 .

Johanson, L. F.; Curl, E.A.; Bond, J.H. and Friboury, H.A. (1960). Methods for study soil microflora plant diseases relationships. Second printing, Burgess publishing company, pp.77

Larkin, R.P.; Hopkins, D.L. and Martin, F. N. (1996). Suppression of fusarium wilt of watermelon by nonpathogenic *Fusarium oxysporum* and other microorganisms recovered from a disease suppressive soil. Phytopathology 86, 812-819.

Martin J. P. (1950). Used of acid, rose Bengal and streptomycin in the plate methods for estimating soil fungi. Soil Sci., 69: 215- 233.

Martyn, R. D. (1985). An aggressive race of *Fusarium oxysporum* f. sp. *niveum* new to United States. Plants Diseases 69, 1007.

Mclaughlin, R.J and Martyn, R.D. (1982): Identification and pathogenicity of fusarium specie isolated from surface disinfested watermelon seed. Journal of Seed Technology. 7 (1): 97-107.

Michail, S. H.; Abdel Rehim, A. M.; Tarabeih, A. M. and Aly, M. A.. (2002). Effect of fusarium seed-borne infection levels on watermelon wilt incidence. Acta Phytopathologica et Entomological Hungerica 37C4), PP. 374-351.

Michail, S. H.; Sheir, H. M. and Rasmy, M. R. (1989). Cross protection of watermelon and cucumber against wilt by prior inoculation with an irrespective forma speciales of *Fusarium oxysporum*. Acta Phytopathologica et Entomologica Hungarica 24, 301-309.

Naik, M. K., Sen, B, and Nagarjan, S. (1993). Relationship of adaphic factors with fusarium wilt of watermelon. Indian Journal of Plant Protection 21, 95- 100.

Sun, S.K. and Huang, J. W. (1979) Variation in the watermelon wilt pathogen, *Fusarium oxysporum* f. sp. *niveum*. Plant Protection Bullectin, Taiwan, 21, 313-322.

Tymchenko, V.1. (1967). Character of pathological changes in fusarium of watermelon and the role of seed in spread of infection. In: Sokol, P.F. (ed.) pp. 141. 164. Izdat, Urozhai Kiv, Ukr.

Walker, J.C. (1952). Fusarium wilt of watermelon diseases of vegetable crops. Mcgrow Hilt Book. Company New York, PP. 190-191.

Wei, S. Q; Zhong, Y.; Ma, Z.T. and Jian, H. (1991). A survey on watermelon diseases in the northern China .China Fruits. 1: 37- 40.

أهمية وحدوث مرض ذبول البطيخ بالدقهلية

صفوت عبد الحميد الحداد
معهد بحوث أمراض النباتات – مركز البحوث الزراعية – الجيزة

يعتبر مرض ذبول البطيخ – المتسبب عن فطر فيوزاريوم أكسيسبوريوم نيفم – واحد من أخطر الأمراض النباتية الاقتصادية التي تصيب نباتات البطيخ وتسبب خسائر كبيرة في أعداد النباتات والمحصول تحت ظروف الحقل. وجد أن جنس الفيوزاريوم كان أكثر الأجناس تواجداً على بذور البطيخ المصابة، أعقبه جنس الأسبرجيلس، ثم جنس البنسيليوم، ثم بعض الفطريات الحقلية، وكانت أعلى نسبة إصابة بالذبول وأشدها في مركزي الجمالية وشربين في الأراضي حديثة الاستصلاح، حيث يتم زراعة البطيخ لمدة طويلة، وكانت أعلى نسبة وشدة إصابة بالأراضي الرملية، وعند إجراء اختبار القدرة المرضية للفطر فيوزريوم أكسيسبوريم نيفم ترواحت نسبة الإصابة بالذبول من ١٠% إلى ٩٠ % ، ووجد أن التعداد الكلي للميكروبات (بكتيريا – فطريات – أكتينوميسستات) في منطقة ريزوسفير البطيخ وهو في مرحلة النضج مرتفعة جداً مقارنة بالكنترول، وكانت جميع أصناف البطيخ المختبرة قد أظهرت قابلية للإصابة بالفطر الممرض، وكانت أعلى الأصناف قابلية للإصابة هي أصناف شركستون جراي وجيزة ١ ودوكس كوين، بينما كانت أكثر الأصناف مقاومة بيكوك ٦٠ وجيزة ٢١.