

ROOT ROT OF ANNONA IN RELATION TO CERTAIN FERTILIZERS AND FUNGICIDES

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ABSTRACT

Isolation trials from rotted roots of annona plants collected from different localities revealed the presence of seven fungal genera including *Botryodiplodia theobromae*, *Curvularia* sp., *Fusarium oxysporum*, *F. semitectum*, *Macrophomina phaseolina*, *Pythium ultimum*, *Rhizoctonia solani* and *Nigrospora* sp. However, *F. oxysporum* was the most dominant fungi followed by *R. solani*, *B. theobromae* and *M. phaseolina*. Among the isolated fungi, *F. oxysporum* was the most pathogenic causing yellowing, wilting and mortality on the seedlings. Applying ammonium sulfate at the rate of 5 and 10 gm/plant, significantly increased the percentage of infection than the other fertilized treatments. The lowest percent of infection was detected in the treatment with potassium sulfate (2.5 and 5 gm/plant). All fertilizers tested significantly increased plant growth parameters compared with the control. Kristalon (NPK) at the rate of 2 and 4 gm/plant, showed the highest values of plant height and number of leaves per plant. Soaking seeds in suspensions of the tested fungicides reduced the percentage of seedlings infected with *F. oxysporum*. However, Vitavax/T and Homai were the most effective in controlling the disease. Soil drench with the tested fungicides revealed that Topsin M followed by Vitavax/T gave the highest percentages of healthy survival seedlings, 90 days after transplanting.

INTRODUCTION

The genus *Annona* belongs to family Annonaceae, which include 60 or more species. It is one of the evergreen fruit crops and occupies a reasonable position among fruits in Egypt, being attractive and delicious (Said, 1982). The fruits contain a large amount of protein, lipids and sugars, in addition to be considered as a good source of vitamins A, B and C. The leaves and roots are usually used in medicine products (Ahmed, 1936). The agricultural policy nowadays aimed to increase the area cultivated with annona trees in the new reclaimed lands, especially with the most promising cultivar "Abdel-Razik" (Abdalla *et al.*, 1996).

Several soil borne fungi have been reported to attack annonas causing root rot and wilt disease, *i.e.* *Diplodia natalensis*, *Fusarium solani*, *Pythium debaryanum* and *Rhizoctonia solani* (Cook, 1975; Pathak, 1980; Rangswami, 1984 and Mourad *et al.*, 1992). Host nutrition was an important factor in the development of root rot and wilt incited by *Fusarium* spp. (Chi and Hansen, 1964). Application of fertilizers as a measure of disease management has been known to be effective against some plant disease including root rot and wilt disease (El-Helaly *et al.*, 1971; Saied, 1986 and Hilal *et al.*, 2001). Treating soil and seeds of different trees with fungicides is recommended as an effective mean for successful control against damping-off and root rot diseases (Saied, 1986; Sharma and Mohanan, 1986; Mahdy, 1988; Radwan *et al.*, 1996; Abo-Rehab, 1997; Abdel-Aziz, 1999; Verma & Navtej Singh, 1999 and Abd El-Ghany, 2001).

The present work was planned to identify the causal pathogens of annona root rot and study their pathogenic capabilities. Also, the effect of mineral fertilizers and certain fungicides on disease incidence were investigated.

MATERIALS AND METHODS

Isolation, identification and frequency of the associating fungi to root rot symptoms: Naturally infected root samples were collected from the surveyed orchards and nurseries of Beheira, Ismailia and Menofia Governorates during two successive seasons (1999-2000). Yellowing and/or partial or complete wilt are, however, often appeared as foliar symptoms on rotted roots. Infected root tissues were plated on PDA medium for 7 days at 25°C. The developed fungal colonies were counted and frequency of each fungus was recorded. Pure cultures from the isolated fungi were prepared using single spore and hyphal tip technique (Dhingra and Sinclair, 1985) and identified according to Booth (1977) and Barnett and Hunter (1987).

Pathogenicity tests: Inocula of the tested fungi were prepared by separately growing on maize meal sand medium at 25°C for 2 weeks. Formalin-sterilized pots, 25 cm diam., packed with formalin-sterilized clay sand soil (1: 1 w/w) were infested with each of the tested fungi at the rate of 2 % (w/w). Annona seedlings (6-months old) were transplanted in the pots (one seedling per pot). Three pots were served as one replicate and five replicates were used for each particular treatment. Percentages of infected plants were recorded after 2, 4 and 6 months from transplanting.

Effect of fertilization on disease incidence and plant growth parameters: Annona seedlings, Abdel-Razik cv., 12 months old, were transplanted in pots (20 cm diam.), filled with soil infested with *F. oxysporum*. Five replicates were used for each treatment and four plants were served as one replicate. Nitrogenous (N) and potash (K) alone, NK and NPK were tested in this experiment. The treatments were carried out per each plant as follows:

- 1- 5.0 gm Ammonium sulfate (20.5 % N)
- 2- 10 gm Ammonium sulfate (20.5 % N)
- 3- 2.5 gm Potassium sulfate (48.0 % K)
- 4- 5.0 gm Potassium sulfate (48.0 % K)
- 5- 2.5 gm Ammonium sulfate (20.5 % N) + 5 gm Potassium sulfate (48.0 % K)
- 6- 2.0 gm Kristalon (NPK, 15-5-30)
- 7- 4.0 gm Kristalon (NPK, 15-5-30)
- 8- Without fertilization (control)

Addition of these treatments was achieved every 15 days intervals started from May and terminated at the end of September. Percentages of infection and two plant growth parameters (plant height & No. of leaves/plant) were recorded, 6 months after transplanting in all treatments.

Effect of soaking seeds in fungicide suspension on the percentage of infection: Four fungicides namely; Homai 80 (50 % thiophanate-methyl + 30 % thiram), Captan 83 (83 % orthoicide), Topsin M70 (70 % thiophanate-methyl) and Vitavax/Thiram (37.5 % carboxin + 37.5 % thiram) were used at the rate of 3 gm/L water. The seeds were soaked in each of these fungicidal suspensions for 24, 48 and 72 hrs. The fungicide-treated seeds and untreated ones (soaking in water only) were planted in soil infested with *F. oxysporum* (2 % w/w). Four pots with 8 seeds per pot were used for each treatment. Percentages of infected seedlings as well as healthy survivals were recorded, 90 days after planting.

Effect of soil drench with the four fungicides on disease incidence: The tested fungicides, i.e. Homai 80, captan 83, Topsin M 70 and Vitavax/Thiram at the rate of 3.0 gm./L water were used. Each fungicidal suspension (200 ml) was applied just before transplanting to drench infested pots (20 cm diam.) with *F. oxysporum*. One annona seedling, Abdel-Razik cv., 6 months old, was transplanted in each pot. Five replicates were used per each treatment. Treated pots with water only were served as control. Percentages of infection and healthy survivals were recorded, 90 days after transplanting. The statistical analysis of the present data was carried according to Snedecor and Cochran (1980). Significant differences among the means of various treatments were established by L.S.D. at 5 %.

RESULTS AND DISCUSSION

Several isolated fungi belonging to seven genera, were identified as: *Botryodiplodia theobromae*, *Fusarium semitectum*, *F. oxysporum*, *Nigrospora* sp., *Macrophomina phaseolina*, *Pythium ultimum*, *Rhizoctonia solani* and *Curvularia* sp. (Table, 1). However, *Fusarium oxysporum* (38.85 %) showed the highest mean percentage of occurrence, followed by *R. solani* (18.9 %), *B. theobromae* (16.99 %) and *M. phaseolina* (13.12 %). Whereas, the other isolated fungi were present in low frequencies (0.82-7.00 %). On the other hand, *F. oxysporum* was frequently isolated from rotted roots collected from Beheira and Ismailia Governorates (51.64 % and 41.32 %, respectively). While *R. solani* was the most dominant fungus isolated from samples collected from Menofya Governorate (32.15 %). Similar results have been reported by Kranz *et al.* (1978), Mourad *et al.* (1992), Mahrous (1994), Abo-Rehab (1997), Abdel-Aziz (1999), Singh (2000) and Abd El-Ghany (2001).

Data presented in Table (2) show that all fungi tested, except *Curvularia* sp., were able to infect annona plants, within 2 months after transplanting. However, percentages of infected seedlings were increased from 2 to 6 months with the pathogenic fungi, except *Pythium ultimum*. Also, it could be noticed that, the fungi tested were found to be vary on their pathogenic capabilities, *F. oxysporum* was the most virulent fungus resulted in 86.7% infection followed by *B. theobromae* (40.0 %), *M. phaseolina* (26.7%) and *R. solani* (20.0 %). The other tested fungi yielded less infection percentages ranged between 0.0%-13.3% at the end of the experiment.

Annona spp. are greatly affected by soil - borne fungi which cause considerable losses (Cook, 1975; Pathak, 1980; Rangswami, 1984 and Mourad *et al.*, 1992). On the other hand, *F. oxysporum* was found to be the most predominant and highly pathogenic among the isolated fungi in this study. However, it has been reported as root rot inciting pathogen affecting different species of fruit crops (Kranz *et al.*, 1978; Mahdy, 1988; Dwivedi, 1990; Mahrous, 1994; Abo-Rehab, 1997; Singh, 2000 and Abd El-Ghany, 2001). According to the available literature, *F. oxysporum* which was found to be the most virulent pathogen to annona plants, was not recorded before on *Annona* spp. in Egypt.

Infection with *F. oxysporum* showed wilt symptoms, whereas wilted plants were often stunted, withered, yellowed and/or defoliated foliage as well as rotted roots and basal stems (Fig. 1). The aforementioned symptoms, which occurred under artificial inoculation, were usually similar to those recorded as a result of naturally infection in nurseries and orchards (Fig. 2).

Table (1): Frequency (%) of fungi isolated from rotted roots of annona plants, collected from three Egyptian Governorates during seasons,1999 - 2000.

Fungi	% Frequency in Governorates			Mean (%)
	Beheira	Ismailia	Menofya	
<i>Botryodiplodia theobromae</i>	20.59	18.18	12.20	16.99
<i>Curvularia</i> sp.	1.10	0.00	2.43	1.18
<i>Fusarium semitectum</i>	0.00	12.13	8.88	7.00
<i>F. oxysporum</i>	51.64	41.32	23.58	38.85
<i>Macrophomina phaseolina</i>	7.37	11.24	20.76	13.12
<i>Pythium ultimum</i>	3.28	8.26	0.00	3.85
<i>Rhizoctonia solani</i>	15.20	7.22	32.15	18.19
<i>Nigrospora</i> sp.	0.82	1.65	0.00	0.82

Table (2) : Pathogenicity tests of the isolated fungi to annona seedlings (cv.Abdel-Razik) 2 , 4 and 6 months after transplanting under greenhouse conditions.

Fungi	% Infection after (months)		
	2	4	6
<i>Botryodiplodia theobromae</i>	6.7	13.3	40.0
<i>Fusarium semitectum</i>	0.0	0.0	16.7
<i>F. oxysporum</i>	73.3	80.0	86.7
<i>Pythium ultimum</i>	13.3	13.3	13.3
<i>Macrophomina phaseolina</i>	20.0	26.7	26.7
<i>Rhizoctonia solani</i>	13.3	13.3	20.0
<i>Curvularia</i> sp.	0.0	0.0	0.0
Control (without fungus)	0.0	0.0	0.0
L.S.D. at 5 %	3.71	8.15	8.34



Fig. (1): Symptoms of root and basal stem rot on annona seedlings (30-days-old) caused by *Fusarium oxysporum*, characterized with wilting and reduction in root system formation. Healthy seedling on the left.

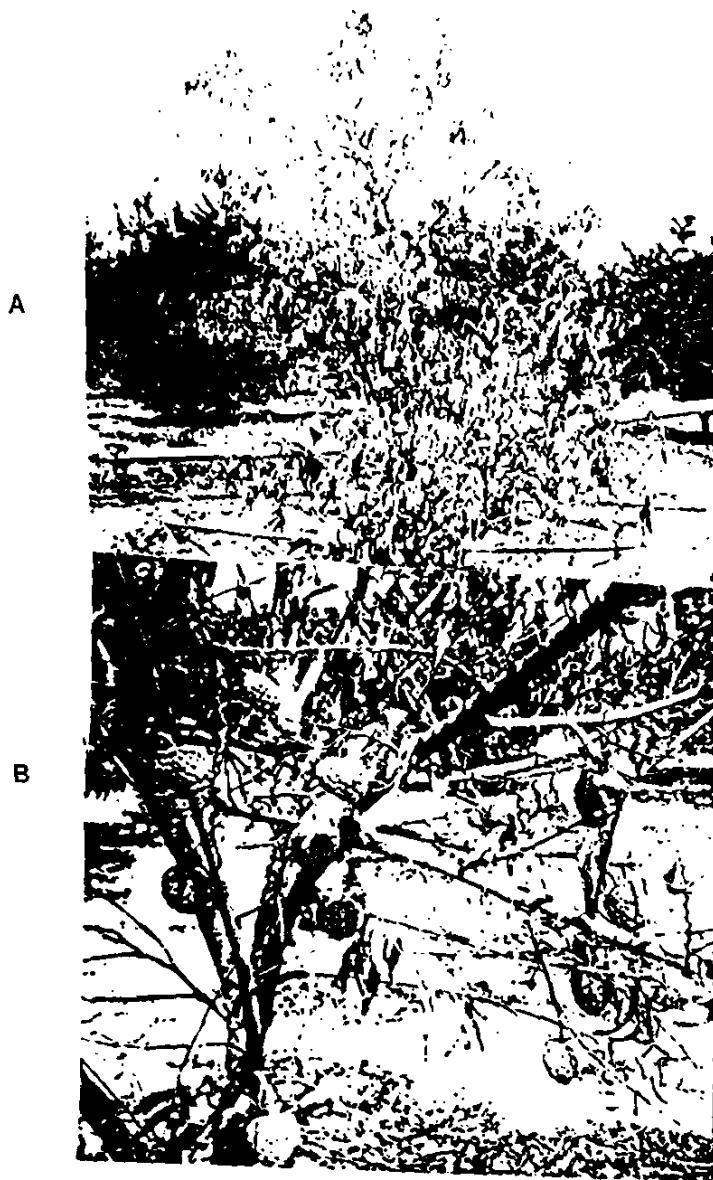


Fig. (2):

A- Annona tree (cv. Abdel-Razik, 5-years-old) showing symptoms of root rot disease. Yellowing and withering foliage are the main symptoms.

B- Premature annona fruits remain attached to the wilted branches throughout the growing season (in Nobarria locality).

Data presented in Table (3) show that potassium (K) alone, NK and NPK tested, significantly reduced the percentages of infected plants than the control. The highest percentage of infection were found when (N) alone was applied in fertilization at the rate of 5.0 gm or 10.0 gm ammonium sulfate per plant. Differences between these two treatments and the others were significant. On the contrary, (K) alone as potassium sulfate at the two rates tested (2.5 & 10 gm/plant) was the most effective in reducing the disease, which showed the lowest percentages of infection (30 % & 20 %, respectively). Also, it is noticed that no significant differences were observed in the two doses tested in case of ammonium sulfate or Kristalon. Similar results were reported by El-Helaly *et al.* (1971), Saied (1986) and Hilal *et al.* (2001), who stated that application of different nitrogen sources plus calcium superphosphate and potassium sulfate, resulted in significant reduction in *Fusarium* disease incidence and increased the survived gladiolus plants. In this respect, Chi and Hansen (1964) mentioned that, host nutrition was an important factor in the development of root rot and wilt incited by *Fusarium* spp. It affected both the rate of development and ultimate severity of these diseases. They also added that, application of potassium caused the greatest reduction of the disease.

As for plant growth parameters, it could be noticed that all fertilizers at doses tested, significantly increased plant height and number of leaves per plant, compared with the control treatment. Increases relative to control reached 22.00 % - 48.58 % and 3.89 % - 31.79 % in plant height and number of leaves per plant, respectively.

Table (3) : Effect of soil fertilization with N, P, NP and NPK on percentage of infection and plant growth parameters of annona in soil infested with *F. oxysporum* in the greenhouse, 2001 season.

Fertilizers and dose /plant (gm)	Infection (%)	Plant growth parameters			
		Plant Height (cm)	*Increase (%)	No. of leaves /plant	*Increase (%)
5.0 gm, Ammonium sulfate (N)	90	56.0	22.0	47.4	28.8
10 gm, Ammonium sulfate (N)	95	60.3	31.4	39.6	7.5
2.5 gm, Potassium sulfate (K)	30	60.9	32.7	38.6	3.9
5.0 gm, Potassium sulfate (K)	20	63.6	38.6	41.8	13.6
2.5 gm, Ammonium sulfate (N) +5.0 gm Potassium sulfate (K)	45	63.4	38.1	44.8	21.7
2.0 gm Kristalon (15-5-30, N:P:K)	50	65.2	42.1	47.9	30.2
4.0 gm Kristalon (15-5-30, N:P:K)	55	68.2	48.6	48.5	31.8
Control (without fertilization)	70	45.9	0.0	36.8	0.0
L.S.D. at 5 %	8.5	5.4	-	1.2	-

* Increase relative to the control.

The highest plant height (65.2 cm & 68.2 cm) and number of leaves/plant (47.9 & 48.5) were recorded with Kristalon treatment at the two doses tested. In contrast, ammonium sulfate (5.0 gm/plant) showed the lowest plant height values; whereas, the lowest number of leaves/plant was detected in case of the treatment with ammonium sulfate (10 gm/plant) or potassium sulfate (2.5 gm/plant).

Data in Table (4) indicate that all fungicides tested, in general, reduced the percentage of infected plants compared with untreated control. However, Vitavax/Thiram showed the highest reduction of infection (87.13 %) followed by Homai (76.92 %) after 72 hours soaking in the fungicide suspension. In contrary, Topsin M and Captan were less effective, whereas their reduction of infection were 34.62 % and 50.01 %, respectively. On the other hand, it was noticed that, increasing soaking period from 24 to 72 hours, clearly increased the percentage of healthy survivals. Treating seeds with fungicides was reported to be an effective mean in improving germination and emergence and controlling seedborne and soil borne diseases (Maude *et al.*, 1969 and Radwan *et al.*, 1996) who mentioned that soaking seeds in fungicidal suspension gave good results against root rot disease.

Table (4) : Effect of soaking seeds of annona in four fungicidal suspensions before planting on the percentage of infection, 90 days after planting in soil infested with *F. oxysporum*, under greenhouse conditions.

Fungicides	Soaking Periods (hrs.)	Infection (%)	* Reduction (%)	Healthy survivals (%)
Homai	24	34.37	65.63	65.63
	48	21.87	76.67	78.13
	72	18.75	76.92	81.25
Captan	24	68.75	31.25	31.25
	48	59.37	60.00	40.63
	72	53.12	50.01	46.88
Topsin M	24	71.87	28.13	28.13
	48	37.50	60.00	62.50
	72	40.62	50.01	59.38
Vitavax/Thiram	24	40.62	59.38	59.38
	48	12.50	87.13	87.50
	72	12.50	87.13	87.50
Control (untreated)	24	100.00	0.00	0.00
	48	93.75	0.00	6.25
	72	81.25	0.00	18.75

* Reduction in infection relative to control

L.S.D. at 5 % for:

Fungicides (F)	3.36
Periods (P)	3.38
F x P	7.60

Data presented in Table (5) show that soil treatment, soil drench with the tested fungicides caused a noticeable increase in the percentage of survived annona seedlings compared with untreated control. However, Topsin-M followed by Vitavax-Thiram were superior in controlling the disease which showed the highest percentages of survived plants (93.33 % & 80.00 %, respectively) after 90 days from transplanting in potted soil infested with *F. oxysporum*. In contrast, Homai and Captan gave the least percentages of survivals (33.33 % and 26.67 %). Similar results were reported by Abd El-Ghany (2001) who mentioned that drenching soil with Vitavax/Thiram was the best effective treatment against root rot pathogens of mango seedlings.

Applying fungicides as an effective procedure of soil borne diseases control for seedlings of different trees were recorded by several investigators (Cooly, 1983; Sharma and Mohanan, 1986; Mahdy, 1988; Abdel-Aziz, 1999 and Verma & Navtej Singh, 1999).

Table (5) : Effect of drenching soil with four fungicides on the percentage of healthy survival plants of annona, 90 days after transplanting in soil infested with *F. oxysporum* under greenhouse conditions.

Fungicides	% Infection	% Reduction*	% Survivals
Homai	66.67	9.08	33.33
Captan	73.33	0.00	26.67
Topsin M	6.67	90.90	93.3
Vitavax-Thiram	20.00	72.73	80.00
Control	73.33	-	26.67
L.S.D. at 5 %	-	-	5.80

* Reduction relative to the control.

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عفن جذور القشطة وعلاقته بالتسميد والمبيدات

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أسفرت عمليات العزل من جذور نباتات القشطة المصابة المأخوذة من مناطق مختلفة عن وجود سبعة أجناس فطرية وهي:

Botryodiplodia theobromae, *Curvularia sp.*, *Fusarium oxysporum*,
F. semitectum, *Macrophomina phaseolina*, *Pythium ultimum*,
Rhizoctonia solani and *Nigrospora sp.*

M. phaseolina أكثر الفطريات المعزولة شيوعاً يليه *F. oxysporum* وقد كان الفطر

B. theobromae, *R. solani*

وكان أكثر الفطريات في الشدة المرضية عند إجراء العدوى الصناعية بالصوبة الزجاجية هو الفطر *F. oxysporum* حيث سبب اصفراراً وذبولاً وموتاً للبادرات.

لوحظ أن إضافة سلفات الأمونيوم بمعدل ٢,٥ جرام/نبات قد أدت إلى زيادة فعالية فسي النسبة المئوية للإصابة عن باقي معاملات التسميد. كما أن أقل نسبة مئوية للإصابة قد لوحظت في المعاملة بسلفات اليوتاسيوم بمعدل ٢,٥ و ٥ جرام/نبات. وكانت المعاملة للأسمدة المختبرة قد أدت إلى زيادة معنوية في قياسات النمو بالمقارنة بمعاملة الكنترول وقد أعطت المعاملة بالكريستالون ٢ و ٤ جرام/نبات أعلى القياسات في أطوال النباتات وعدد الأوراق لكل نبات.

أدى نقع بذور القشطة قبل الزراعة في محلول المبيدات المختبرة إلى خفض النسبة المئوية للإصابة بالفطر *F. oxysporum* وقد كان المبيدان فيتافاكس ثيرام وهومي هما أكثر المبيدات كفاءة في مقاومة المرض، وعند معاملة التربة بالمبيدات المختبرة وجد أن التوبسين م يليه فيتافاكس ثيرام قد أظهر أعلى نسبة مئوية للنباتات السليمة بعد ٩٠ يوماً من العدوى.