Egypt. J. Phytopathol., Vol. 34, No. 2, pp. 17-27 (2006)

Pathological Studies on Heart rot Disease of Banana in Egypt

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Banana heart rot began to appear as a serious problem in banana orchards in Egypt causing a great economical loss. *Fusarium moniliforme* (Sheldon) found as the causal organism of Banana heart rot in Egypt. Different isolates were collected from different banana growing orchards from three localities.

Banana plants artificially inoculated with the pathogen succeed under greenhouse conditions to study the development and progress of the disease symptoms as well as the control procedures.

The resulted symptoms from the artificial inoculation on banana plants were typically identical as those appeared on naturally infected plants collected from different orchards. The severity of infection varied on different banana tested cultivars where, cv. Williams exhibited the highest disease severity followed by cvs. Maghrabi and Grand Nain.

Different fungicides had inhibitory effect on *Fusarium moniliforme* growth *in vitro*. Under greenhouse, soil drench treatment using Kema Z around the diseased corm found as the most effective fungicide against the heart rot disease only if applied after cutting the diseased pseudostem to level of healthy tissue appearance.

Key words: Banana, control, fungicides, heart rot and *Fusarium moniliforme*.

Banana (*Musa* spp.) is considered one of the most important favourable and popular fruits in Egypt and all over the world. In Egypt, the cultivated area reached 45.802 thousand feddans which produce about 760.505 thousand tons of banana fruits (Anonymous, 2000). Serious diseases attacked banana plants; some of which are fungal like panama, black and yellow sigatoka. Some others are viral and nematode diseases which are considered the most important problems of the cultivated areas in Egypt where they cause serious losses to banana production (Rezk, 2000 and Azmy, 2006).

Heart rot disease appeared in the last years as one of the serious diseases attacking banana plants, in this respect, the heart rot symptoms may occur by different abiotic stresses such as mineral deficiency or unfavourable environmental conditions (Ko *et al.*, 1997) and some by biotic stresses such plant diseases (Mali and Deshpande, 1977). Pseudostem heart rot of banana usually associated with plant injuries. However, it was considered to be serious on young plantations when

attacked by severe storms and floods (Temkin-Gorodeiski and Chorin, 1971 and Stover, 1972). In some cases, both saprophytic fungi and bacteria occur as a secondary infection in the decayed tissue which caused by abiotic factors (Wardlaw, 1972). Many plant pathologists reported that *Fusarium* species as the causal pathogen of disease symptoms on banana plants where all of Abdel-Sattar *et al.* (1977), El-Sheikh (1989), Mahdy *et al.* (1993), Abd-Alla (1994), Abdel-Hafiz (1997) and Krauss *et al.* (1998) reported that *F. moniliforme* is the causal pathogen of fruit, corm, root, crown, rhizome and pseudostem rots. It worthily to mention that Abdel-Sattar *et al.* (1977) named the symptoms of fruit rots as heart fruit rot. El-Nasr *et al.* (1990) isolated *F. poae* for the first time from banana plants showed heart rot symptoms in one of the Egyptian banana orchard. The main symptoms of the heart rot disease on banana plants were severe tip rot with pronounced brown to blackening of young rolled central leaves tissues. These may remain folded or become nearly fully unrolled as they emerge from the crown. This decay was sometimes present only in the upper portion of the pseudostem but it could be tending to take a downward direction in the core of the pseudostem. This work aimed to throw the light on banana heart rot disease in Egypt and identification of the causal agent as well as its control.

Materials and Methods

1. *Isolation and identification:*

Samples of banana plants showing heart rot symptoms were collected from three governorates, *i.e.* Beheira (West Nobaria), Menofia (El-Sadaat) and Qalubia (Toukh). The pseudostems of diseased banana plants were split longitudinally to small pieces of the internal leaf of heart then, surface sterilized with 2% sodium hypochlorite solution for two min., rinsed in sterilized water and then dried between folds of sterilized filter papers before placing onto ready PDA plates (9cm.) .The plates were incubated at 25°C up to 7 days with daily observation. The emerged fungi were picked up, purified and identified according to the description of Nelson *et al.* (1983). Pure cultures stocks of the isolated fungi were kept under 5°C for further studies.

2. *Pathogenicity test and cultivars reaction:*

a) *Inoculum preparation:*

Disks of pure culture of three isolates of *F. moniliforme* obtained from different locations mentioned before were sub cultured onto potato dextrose agar (PDA) plates and incubated at 25°C for 7 days. After which resulting colonies, some growing cultures were used as starter cultures for inoculating sorghum grain medium (Kung'u and Jeffries, 2001). Each sterilized polyethylene bag of grain was inoculated with 7 day-old starter culture and mixed by squeezing the bags. Inoculated grain bags were incubated at 25°C for two weeks. To prepare stock suspension, the rest culture of *F. moniliforme* was used. Spores were collected in sterile water and separated using a camel hair brush, passed through 3 layers of cheesecloth to remove mycelial fragments and then counted using a Spencer haemocytometer to give the concentration of 10^6 spores/ ml.

b) *Inoculation techniques:*

Three cultivars, *i.e.* Grand Nain, Maghrabi (seedling about 6 months) and Williams (tissue culture plants about 6 months) were used for pathogenicity screening and cultivars reaction against the main isolated fungus *Fusarium moniliforme* under greenhouse conditions. Banana plants of the three cultivars were grown in plastic pots No.40 filled with a mixture of sand and clay soil (1:1 v/v). The experiments was divided into four replicated trails as follows: In the first one trail, three plants were inoculated two months after planting with the prepared grains inocula which used as soil infestation at rate 5% (w/w) around the base of the pseudostem, deep enough to reach the root system and then buried with soil. Control plants were inoculated with sterile uninoculated grains treated in a similar manner. The second trail was confirmed by inoculating banana plants with the prepared spore suspension as stem injection technique where injection was made in banana pseudostem just above 5 cm height from corm using a syringe 5ml until the syringe needle reach the young central or heart leaf. The third trial, banana plants were injected using the same injection technique but above 10cm. height from the corm. Control plants of second and third trails were injected with sterile water as mentioned above. The fourth trail was done by spraying banana seedlings with an aqueous suspension of 10^6 spore/ml using hand atomizer. Control plants were sprayed with sterile water. All plants in the latter three trails were covered with plastic bags for two days after inoculation then the bags were removed. Plants were irrigated when needed to maintain optimum moisture in the soil. Reisolation and identification was done from the appeared typical symptoms of heart rot in case of injection technique 5cm. heights from corm (trial 2). In this respect, the infected banana tissues were surface sterilized before transferred to PDA plates and incubating at 25°C for 7 days as mentioned before. The emerged fungus was picked up, then purified and identified according to the description of Nelson *et al.* (1983).

3) *Disease assessment:*

Disease symptoms of heart rot were assessed periodically every two weeks up to 3 months where the final assessment on the remaining plants was carried out. The corm and pseudostems were cut open longitudinally and the various levels of disease symptoms were scored and assessed using the scale of 0 to 6 grades. Banana plants were rated as shown in Table (1).

Table 1. Heart rot of banana assessment for external and internal symptoms

Disease scale	Symptom categories and description
0	No symptoms
1	Death of heart leaf tip
2	Most of central rolled leaf is death (external symptoms)
3	5-25% downward rotting of the heart leaf
4	25-75% rotting of the heart leaf
5	100% rotting of heart leaf
6	The young bud at soil level is finally rotted (internal symptoms)

The disease severity (DS) was calculated according to the formula described by Moore *et al.* (1993) as follows:

$$\text{Disease severity (\%)} = (\Sigma (n \times v)/6N)\ 100$$

whereas, n= No. of seedlings at rate v (disease score), N= total No. of inspected plants and 6 = highest disease severity rate.

4. *Chemical control:*

a) *In vitro study:*

Seven different concentrations of each tested fungicides, *i.e.* Bellis (Pyraclostrobin; methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl] oxymethyl} phenyl)-N-methoxycarbamate+Boscalid; 3-pyridinecarboxamide, 2-chloro-N-(4' chloro [1,1'biphenyl]-2-yl); Cabriotop 38 % WG (Pyraclostrobin; methyl N-(2-{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl)-N-methoxy carbamate +Metiram; zinc ammoniate ethylenebis(dithiocarbamate)- poly[ethylenebis(thiuram disulfide)]); Kema Zed 50%WP (Carbendazim; Methyle-N-bezimidazol-2-yl carbamat) ; Kopral 50% WP (Cupper oxychloride); Nimrod 25% EC (Bupirirmat; 5-n-Butyl-2-ethylamino-6-methypyrmidin-4-yldimethysulfamat); Torando 24.5% WP (Metallic copper) were prepared in previously calculated volume of autoclaved PDA. The investigated concentrations were added to the medium directly before solidifying and poured into the plates to determine their inhibitory effect on growth of *F. moniliforme* (Beheira isolate). Each plate was inoculated with one equal disk, (5mm), of 7 days old culture of the desired fungus. Three replicates were used for each treatment. All plates were incubated at 25°C. The mycelial linear growth was measured when the full growth of the tested fungus observed in the check treatment.

b. *In vivo* study:

Fungicidal application, as a therapeutically treatment was carried out under greenhouse conditions. Banana plants (cv. Williams), which previously inoculated with the tested fungus and showed external disease symptoms, were used for evaluation both Kema Z and Kopral, the most effective fungicides *in vitro* test, on the disease recovery. The inoculated plants were divided into two groups; the first one was applied by spraying the desired fungicides with the recommended dose as a foliar treatment while the check treatment was sprayed with sterilized distilled water. The second trail was applied as soil drench around pseudostem cuttings. The fungicidal application was repeated twice with 15 days interval and other diseased plants were kept without fungicide treatment as a comparison check plants. Three plants were used for each treatment as replicates. Observations of disease development were recorded 45 days post application time.

When necessary, the results were statistically analyzed using factorial experiment design suggested by Snedcor and Cochran (1982).

Results and Discussion

1. Symptoms:

The external symptoms of heart rot disease in field infections (Fig. 1) appeared as follows: in early stages, the young rolled central leaves is subjected to severe tip rot with pronounced brown to blackening of tissue spots (Fig. 1b); the leaves may remain folded or become nearly fully unrolled as they emerge from the crown. Spots were sometimes not only present in the upper portion of the pseudostem but also it could be tending to take a downward direction in the core of the pseudostem. The surrounding sheath tissue remaining apparently unaffected (Fig. 1c). This rot may descend to the upper region of corm. If a bunch is present it may fall to ground and the internal tissues of the petiole and trunk undergo a uniform brown discoloration.

Fig. 1. Healthy plant (a). Natural symptoms of heart rot disease indicating blackened tip of young central heart leaf (b). Longitudinally section in pseudostem showing the results of downward rotting of heart leaf (c).

2. Pathogenicity and cultivar reaction trails:

Pathogenicity of the three isolates of F. *moniliforme* indicates that injection the corm just above the heart leaf within 5cm height (trail 2) only caused the infection of heart rot to all three inoculated cultivars meanwhile the other methods of inoculation did not reveal any symptoms (Table 2). Banana seedlings artificially inoculated with the fungus which developed typical symptoms of heart rot with tip rot of the central leaf and take a brown colour (Fig. 2a) were used to reisolate the main pathogen which identified as F. *moniliforme* again. The inoculated plants revealed rotting of the heart leaf and core of pseudostem. This rotting begins from the site of inoculation as a canker then, progress upward and downward in the pseudostem (Fig. 2c). These results are in agreement with the results obtained by

Table 2. Pathogenicity tests for three isolates of F. moniliforme on banana plants (cv. Williams) using different inoculation techniques

F. moniliforme infestation	Disease severity (%)		
	Isolate 1 (Beheira)	Isolate 2 (Menofia)	Isolate 3 (Qalubia)
Soil infestation	0.0	0.0	0.0
Injection above corm directly, 5cm.	83.33	38.89	50.00
Injection,10 cm above corm	0.0	0.0	0.0
Spraying the leaves	0.0	0.0	0.0
Control	0.0	0.0	0.0

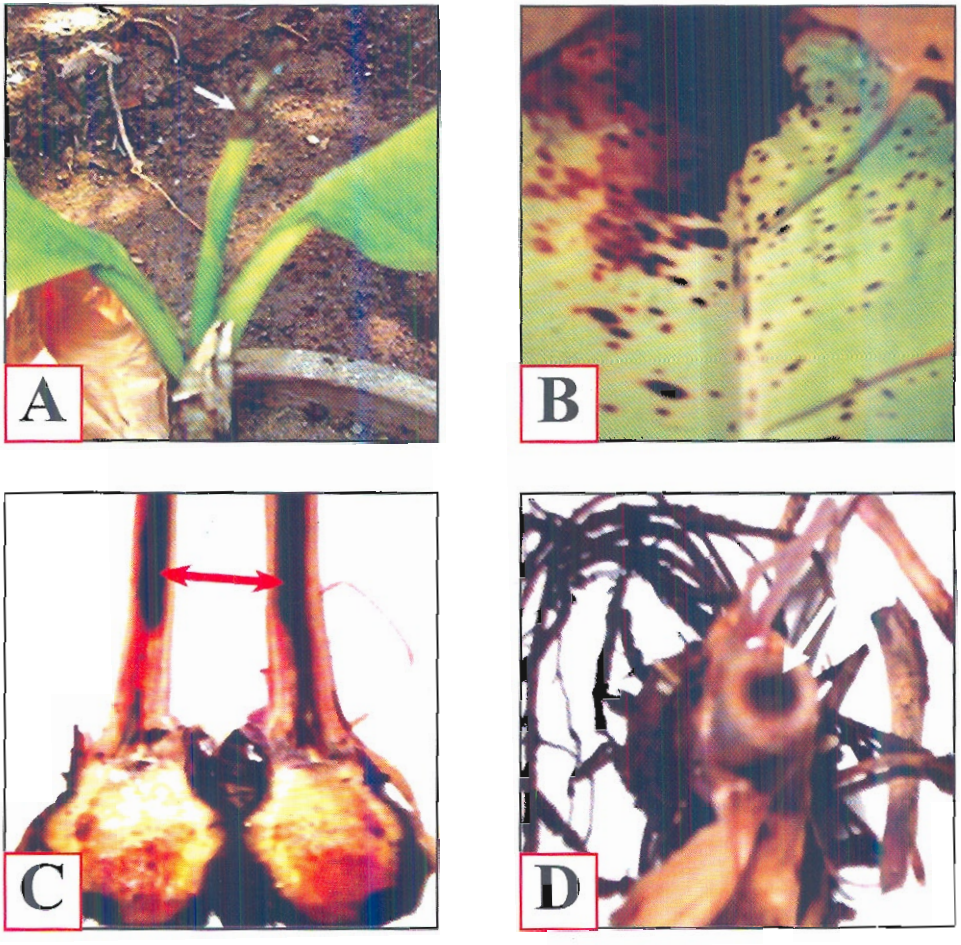

Fig. 2. Showing tip rot of young rolled central leaves through artificial inoculation of banana seedlings (a). This decay may be tending to take a downward direction in the core of the pseudostem (c and d). Note tip rot and spotting of unfolded leaf (b).

many workers (Wardlaw, 1972 and El-Nasr *et al.*, 1990). In this respect also, Ocfemia and Mendiola (1933) found that *F. moniliforme* var. *subglutinans* was the principle associated fungus with heart rot of banana when applied to wounded or unwounded, unrolled; heart leaves of plants maintained under moist conditions caused water- soaked, dark brown areas to appear surrounding leaf sheaths. They also added that the effects being most severe when the tissues had been previously wounded. The fungus did not produce any disease symptoms when applied to the corm. The same trend was obtained when the soil inoculated with conidial suspensions at the crown between the leaf- sheaths meanwhile, positive infections were obtained when the sheaths and furled leaves within the pseudostem were wounded and inoculated (Waite, 1956 and Wardlaw, 1972). Cheng *et al.* (1999) found that the highest rate of development of banana sheath blight caused by *Rhizoctonia solani* occurred in the leaf segments taken from banana plants at 8-25cm pseudostem height.

These results could be interpreting in light the finding of factors responsible for the development of heart rot disease. Also, the height (5cm) containing the meristemic tip of banana plant and this method of inoculation (trail 2) might help the pathogen to reach this region. These symptoms were more pronounced on cv. Williams which exhibited the highest percentage of disease incidence 83.33% (Table 3) followed by Maghrabi and Grand Nain cvs. (55.56 and 33.33%, respectively). These results are in agreement with those obtained by Abd-Alla (1994), Abdel-Hafiz (1997) and Korra (2005). Such differences between cultivars in their susceptibility might be due to differences in their genetic structure which affect the quality and quantity of excretions released from wounds of the inoculated cultivars (Blackeman and Fokkema, 1982).

Table 3. Heart rot severity based on symptoms caused by *F. moniliforme* on three cultivars of banana

Treatment	Disease severity (%)			Mean
	Cv. Williams	Cv. Maghrabi	Cv. Grand Nain	
F. moniliforme	83.33	55.56	33.33	57.41
Control	0.0	0.0	0.0	0.0

L.S.D. at 5% level for: Cultivars (C)= 16.63; Treatment (T)= 11.99 and C x T= 20.76

3. *Chemical control:*

a) *In vitro:*

Laboratory screening of the fungicidal effect against *F. moniliforme* prove good indicator for understanding the efficiency of chemical control on heart rot disease of banana. Data in Table (4) reveal that Kema zed completely inhibited the growth of the tested fungus at 10ppm, while Nimrod and Kopral gave the same effect at 200 and 500ppm respectively. Torando was the least effective in this respect. In general, *F. moniliforme* was less sensitive to the rest tested fungicides in spite of the growth was gradually decreased with the increase of fungicides concentrations. Similar results concerning the response of these fungicides at different concentrations were also reported by Korra (2005).

Table 4. Effect of different fungicides concentrations on the linear growth of *F. moniliforme*

Fungicide	Linear growth (mm) at different concentrations(ppm)								Mean
	0	10	50	100	200	300	400	500	
Bellis	90.0	66.3	34.7	30.7	28.0	24.3	20.0	17.0	31.6
Cabriotop	90.0	90.0	90.0	48.7	48.0	45.3	42.3	42.0	58.1
Nimrod	90.0	90.0	49.7	46.3	45.0	29.3	20.3	0.0	40.1
Kopral	90.0	90.0	42.3	19.7	0.0	0.0	0.0	0.0	21.7
Kema Zed	90.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Torando	90.0	90.0	90.0	90.0	90.0	90.0	67.0	58.7	82.2

L.S.D. at 5% level for: Fungicides (F)= 0.63; Concentrations (C)= 0.65 and F x C= 1.95

b) *In vivo*:

Studies on the fungicidal effect against *F. moniliforme* revealed that Kema Z and Kopral were the most effective fungicides caused the highest growth reduction of the tested fungus. Satisfactory results were obtained on inhibition of heart rot infection when used Kema Z fungicide under greenhouse conditions as soil drench of the diseased banana plants with removal of vegetative infected parts. Kema Z decreased the disease severity to level reached 73.34 as compared with the control (Table 5). On the other hand, the two fungicides were not effective to reduce disease incidence when used in spray programs without removal of diseased parts.

Table 5. Effect of fungicidal application on reduction (%) of diseased banana plants affected by heart rot disease

Fungicide	Dose/l	Disease severity (%)			Reduction (%) in disease severity	
		Before application	After application			
			Foliar treatment	Soil drench	Foliar treatment	Soil drench
Kema zed	3.0 gm	83.33	83.33	22.22	0.00	73.34
Kopral	1.5 gm	77.78	77.78	77.78	0.00	0.00
Control		72.22	72.22	72.22	0.00	0.00
L.S.D. at 5%		N.S.	N.S.	12.71		

The effect of Kopral was nil in both trails. These results are in agreement with the results of Abdel-Kader *et al.* (2004) who found that application of irrigation scheme with Rizolex-T and Topsin M alternatively could successfully be used as a control measure for root rot and wilt complex disease of banana plants. Reviews conducted on the mode of action of carbendazim (such, Kema zed) indicate that the fungicide acts in many ways. The major action has been noticed on the biosynthetic process of the fungi. In this regard, carbendazim inhibits the synthesis of DNA and other related processes due to its antimetabolite nature (Vyas, 1984).

Also, Temkin-Gorodeiski and Chorin (1971) reported *F. moniliforme* as the causal of banana black heart rot disease in Israel and obtained good control by removal of pistils not later than 14 days after bunch shooting, following immediately by spraying with 1% zineb.

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(Received 10/07/2006; in revised form 12/09/2006)

عفن قلب الموز المتسبب عن الفطر
فيوزاريم مونيليفورم فى مصر

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الأعراض الرئيسية للإصابة بعفن القلب فى الموز تتمثل فى عفن قمة الأوراق المركزية الصغيرة الملفوفة والتى قد تظل منطوية أو تبدأ فى الإنبساط عند خروجها من قمة منطقة التاج. التدهور قد يبقى فقط فى الجزءالعلوى من الساق الكاذبة ولكنة يمتد أحياناً لإسفل فى إتجاة مركز الساق الكاذبة. كان الفطر فيوزاريم مونيليفورم هو الفطرالأساسى المعزول من النباتات المصابة فى ثلاث مناطق بمحافظات البحيرة والمنوفية والقليوبية وكان قادراً على إحداث الإصابة عند إجراء عدوى صناعية به عن طريق الحقن فى مسافة ٥ سم أعلى كورمة النبات فى حين أن الطرق الأخرى للعدوى لم ينتج عنها أى أعراض للإصابة. كانت أعراض المرض أكثر وضوحا على الصنف وليامز والذى أظهر أعلى نسبة للإصابة يلية صنف المغربى ثم الصنف جراندنان. الإختبارات المعملية للمبيدات أظهرت أن مبيد الكيمازد ثبط تماماً نمو الفطر عند كل التركيزات المستخدمة كذلك مبيدات الكوبرال والنمرود عند تركيز ٢٠٠ و ٥٠٠ جزء فى المليون. المبيد كيمازد كان الأكثر فعالية فى مقاومة المرض تحت ظروف الصوبة عند إضافته لتربة النباتات المصابة مرتين بينهم ١٥ يوم مع إزالة الأوراق الظاهرية المصابة.