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Pathogenic Fungi and Soil Conditions Causing Root Rot and Wilt Disease Complex during Acclimatization of Tissue Culture-Derived Banana Plantlets

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Rhizoctonia solani and *Fusarium oxysporum* were isolated from banana plantlets produced *via* tissue culture technique showing root rot-wilt complex disease. Isolation from culture soil mixture under banana plantlets resulted in fungal isolates identical to those isolated from diseased plantlets. These isolates proved to be pathogenic to banana plants causing complex disease symptoms. *In vitro* test, Rizolex-T and Topsin-M at 200 ppm have completely inhibited the growth of *R. solani* and *F. oxysporum*, respectively. Recorded results proved that the main source of *R. solani* and *F. oxysporum*, the causal pathogens of root rot and wilt complex disease, was the used soil culture medium in some surveyed nurseries. In addition, measures of soil pH and water holding capacity (WHC) of cultivated soil under banana plants revealed the records of pH 3.4 and 99.3% WHC. These unfavourable growth conditions affected negatively the plant which became more susceptible to attack by the soilborne pathogens. It could be suggested that application of irrigation scheme with Rizolex-T and Topsin-M alternatively for five days each at the rate of 2 g/l irrigation water and minimizing the irrigation time to 2 hr/day at the emergence of disease symptoms could successfully be used as a control measure for root rot and wilt complex disease of banana plants in production nurseries.

Key words: Banana, disease complex, *F. oxysporum*, *R. solani*, root rot, tissue culture and wilt.

Banana had a great attention in many countries for its consumption and various important purposes. In Egypt and most developing countries, the access to high quality planting material, improved varieties and control of pathogens is an essential demand. Plant tissue culture technique has an important role in the production of many plants. It is a multi-dimensional science that offers exciting prospects to future improvements in crop and land productivity (Punja, 2001). The main advantage of this technique is that a small piece of plant can produce hundreds of new *in vitro* plantlets which can be identical to the mother plant. Strategy of banana plants production *via* tissue culture technique passes through various stages. The first is growing separated cells of plants in containers with a nutrient medium under controlled *in vitro* conditions of temperature and light, until stimulation tissue differentiation, rooting and shooting development, which can then grow into a whole new plantlet. Plantlets, obtained *in vitro* should be transferred to a growth medium such as sand, vermiculite and peat-moss or their mixture and maintained for acclimatization in a greenhouse with special care of controlled temperature,

humidity, soil moisture and nutritional requirements. Plants produced *via* tissue culture tend to be disease free (Mink, 1991). While this does not eliminate disease once the plants are in the outdoor environment, it does give the plants a healthier start and a better chance of defense (Hwang and Ko, 1992).

Some problems were observed in many greenhouses and nurseries which affected production of tissue culture-derived banana plants. These problems increased their susceptibility to unfavorable environmental conditions as well as plant pathogens which is thus reflected on the infection with root rot and wilt disease complex during acclimatization and breeding periods.

In this regard, Dominguez, *et al.* (1996) reported that several soil characteristics have been studied in relation to Fusarium wilt in fields of banana in the Canary Island. The results indicated that there is a relationship between the development of disease incidence and the lower levels of soil pH which significantly affect the disease incidence and severity. Naseby and Lynch (1999) stated that the shoot and root weights and root length were reduced in pea plants at soil pH 4.4. They add that the decrease in pH increased total population of fungal and yeast and plant susceptibility to disease infection with indigenous pathogens. Furthermore, Peng, *et al.* (1999) recorded that chlamydospores of *Fusarium oxysporum* f.sp. *cubense* and the severity of Fusarium wilt in banana plantlets were correlated to water content and soil pH. They found that high water content (80% of field capacity) and low level of soil pH (3.6-4.2) enhanced disease incidence. On the other hand, Li and Nan (2000) reported that soil moisture content was one of the main environmental factors affecting the growth and development of root rot and wilt pathogens. They added that soil maintaining more than 50% water holding capacity was more favorable for root rot and wilt development in faba bean.

The present study concerned with investigating the role of unfavourable environmental conditions during acclimatization of tissue culture-derived banana and their relation to infection by root rot and wilt disease complex. Moreover, a rapid overview of some precautions, which can have a deep impact in banana nurseries, was also considered.

Materials and Methods

Isolation, identification and pathogenicity of the causal pathogens:

Samples of banana transplants, showing abnormal growth symptoms indicating disease incidence, were collected from some private nurseries of banana production *via* tissue culture technique. The percentage of disease incidence was recorded in acclimatization and breeding greenhouses (growth stages before transferring to the field) where disease symptoms were observed. Isolation of the pathogens was carried out from both diseased banana plants and the used soil medium. Infected banana roots were carefully washed with tap water in order to remove all the adhering soil particles. The roots were then surface sterilized with 2% sodium hypochlorite solution, washed in several changes of sterilized water and air dried between folds of sterilized filter papers. Small pieces of the internal cortex and the discoloured xylem tissues were cut and aseptically placed into ready PDA plates and incubated at 25±1°C for up to seven days.

Isolation from growth soil medium was carried out using the plate count technique as described by Allen (1961). Hyphal tips of the grown fungi were taken from the developing colonies appearing from plant or soil screening and transferred to PDA slants, then maintained for further studies. The fungi were identified according to cultural and microscopical characters described by Gilman, (1957), Barnett and Hunter (1972) and Nelson (1983).

Pathogenic ability of isolated fungi on banana plantlets was tested under greenhouse conditions. Banana plantlets at transplanting age for acclimatization stage (produced *via* tissue culture forming shoot and root systems) were used in this test. Plantlets were transplanted in pots (10-cm-diam.) containing autoclaved soil mixture (peat-moss, vermiculite and sand as 3:1:3, w/w/w) which previously infested individually or as mixture at the rate of 5% of soil weight with the inoculum of tested pathogens grown for two weeks on sand barley medium (1:1, w/w and 40% water). The acidity of soil mixture was adjusted to be pH 4 and constant of 80% water holding capacity throughout the experimental period. Twenty five pots, each containing one transplant, were used as replicates for each particular treatment as well as control (check). Plantlets showed root rot and/or wilt symptoms were recorded and the percentage of disease incidence was calculated 50 days after transplanting.

Determination of some culture soil characteristics:

Soil texture, pH and water holding capacity of the mixture culture soil (peat-moss, vermiculite and sand as 3:1:3, w/w/w) which commercially used in nurseries for growing banana plantlets, were determined. Soil samples, taken for analyses from cultivated pots with growing banana plantlets during acclimatization stage, were thoroughly mixed then divided to three replicated parts.

Sensitivity test against fungicides:

The inhibitory effect of fungicides on the linear growth of *Fusarium oxysporum* and *Rhizoctonia solani*, the banana disease incitants, was *in vitro* evaluated. Different concentrations, *i.e.* 0, 25, 50, 100 and 200 ppm, based on the active ingredient of each of Rizolex-T (*a.i.* 50%) and Topsin-M (*a.i.* 70%), were added to autoclaved PDA medium before pouring into Petri dishes. Check treatment was a fungicide-free medium. Plates were inoculated individually with 5-mm-disk of 10-day-old fungal cultures. Three replicates were used for each particular treatment as well as check. All plates were incubated at 25±1°C and examined after 7 days when the full growth of tested fungi was observed in the check treatment. Percentage of reduction in fungal growth was calculated relative to the check treatment.

Disease control:

Fungicidal application, as a therapeutically treatment, was carried out under natural conditions at one of the private nurseries, works in tissue culture-derived production. Banana plants which showed disease complex symptoms were used for evaluation of the applied treatment on the percentage of disease recovery. Treated banana plantlets were grown in plastic pots (10-cm-diam.) in acclimatization greenhouse, while those in breeding greenhouse were grown in plastic bags (Fig.1). The applied treatments were daily irrigated, alternatively each five days, with either

Rizolex-T or Topsin-M at the rate of 2 g/l water and one day-off between the two fungicides. The fungicidal application was repeated twice with interval of five days. The irrigation time was also minimized to 2 hr/day in order to reduce the undesirable water holding capacity in the growth soil mixture.

Three hundred diseased plantlets, as well as one hundred as replicates, were used for the evaluation of applied treatment in each of the adaptation and breeding greenhouses. Another set of one hundred diseased plantlets were kept as a comparison check treatment. Observations for diseased plantlets recovery were recorded after five weeks from the beginning of application time.

Statistical analysis:

Tukey test for multiple comparisons among means was utilized (Neler *et al.*, 1985).

Results and discussion

Disease symptoms:

Infected banana plantlets during *in vivo* adaptation and breeding periods, (Figs.1&2) exhibit root rot and wilt symptoms. Wilted leaves of diseased seedlings showed yellowish edges colour, which turned to light then deep brown and the whole leaves became dry and fell down. Young plantlets died speedily during the acclimatization stage (Fig.1-A) while the survival plantlets showed closed rolling symptom of the apical leaf at the following breeding stage (Fig.1-B), then slowly died. Upon splitting of the infected plantlets longitudinally, brown to dark colour observed in the vascular regions from the tip of the root to the top of stem (Fig.2-C). Furthermore, the primary roots became decayed and dark black in colour and the secondary roots and root hairs were rarely detected, (Fig.2-A&B).

The occurrence of root rot and wilt disease complex on banana plantlets were surveyed at different private nurseries, working with banana production *via* tissue culture technique. It was found that among different examined locations, the disease incidence was detected in few nurseries. The calculated percentage of diseased banana plantlets exceeded 50% and 30% at both adaptation and breeding greenhouses, respectively. In this regard, the observations of disease incidence in those nurseries might be attributed to the conditions of soil mixture, *e.g.* adjusting soil pH within the range of 7-8, ratio between soil mixture components and the addition of suitable fungicides. Furthermore, the peat-moss might be contaminated with various soilborne plant pathogens (personal communication).

Isolation, identification and pathogenicity of the causal pathogens:

Isolation trials from collected banana transplant samples, showing root rot and wilt disease complex symptoms, resulted in two fungal isolates identified as *Rhizoctonia solani* and *Fusarium oxysporum*. Furthermore, isolation from culture soil under banana cultivation revealed the occurrence of some soilborne fungi. Data presented in Table (1) indicate that *Aspergillus* spp. showed the highest frequency of the total isolated fungi followed by *R. solani* and *F. oxysporum* (being 26.7, 24.6 and 21.4%, respectively). Meanwhile, *Penicillium* spp. and unidentified fungal isolates represented 18.8 and 8.3% of the isolated fungal genera, respectively.

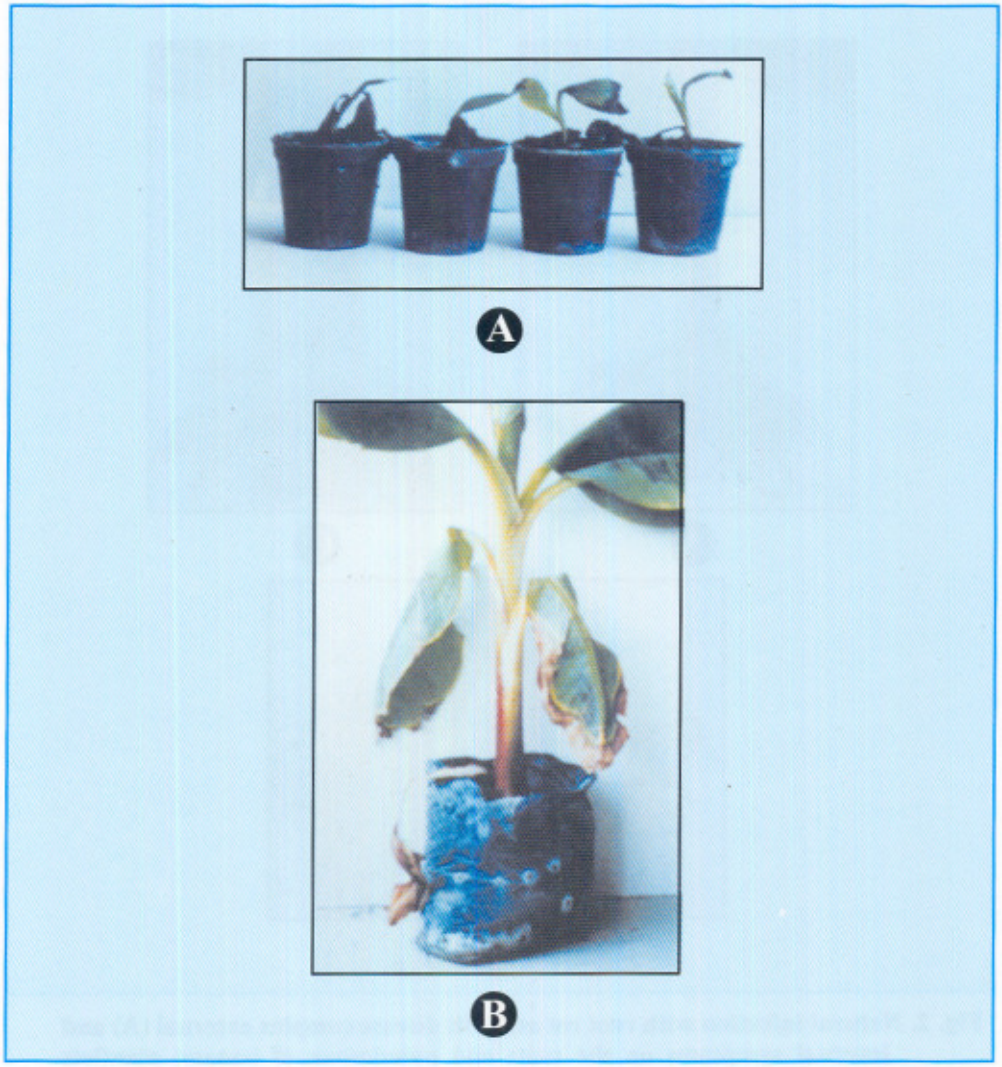

Fig. 1. Natural root rot and wilt disease complex symptoms on banana plantlets cultivated in acclimatization (A) and breeding (B) greenhouses.

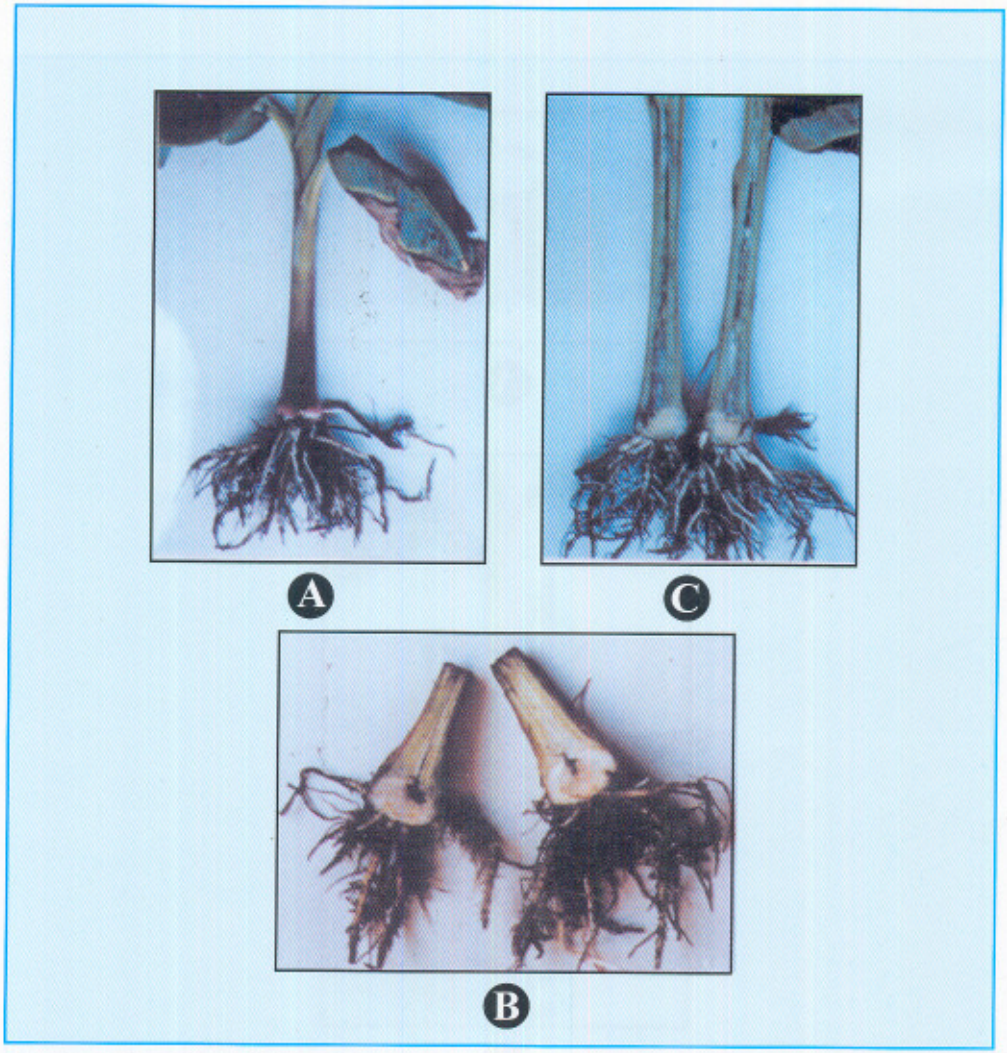

Fig. 2. Natural infection with root rot and wilt disease complex external (A) and internal symptoms on the roots and pseudostem of banana plantlets (A&B) and vascular longitudinal discolouration (C).

Table 1. Frequency occurrence of some soilborne fungi isolated from culture soil under banana plantlets cultivation

Isolated fungus	*F. oxysporum*	*R. solani*	*Aspergillus* spp.	*Penicillium* spp.	Others
Frequency (%)	21.4	24.6	26.7	18.8	8.3

According to the cultural and microscopical characteristics, it was found that the fungal isolates of *R. solani* and *F. oxysporum* were identical. These isolates were tested for ability to cause disease infection on banana plantlets under greenhouse conditions (Table 2).

Table 2. Root rot and wilt disease complex on banana plantlets cultivated in culture soil artificially infested with *R. solani* and *F. oxysporum* under greenhouse conditions

Tested fungus	Disease symptom	Disease incidence (%)	Healthy plantlets (%)
R. solani	Root rot	72 b *	28 b
F. oxysporum	Wilt	68 b	32 b
R. solani + *F. oxysporum*	Root rot and wilt disease complex	84 c	16 c
Check	Healthy seedlings	0.0 a	100 a

* Figures with the same letters are not significantly different (P=0.05).

Data presented in Table (2) reveal that the tested isolates were able to induce root rot and/or wilt as well as disease complex on banana plantlets. Data also show that there was no significant difference between the two tested isolates when used individually, while both isolates together differed significantly in the percentage of disease incidence. The obtained results are in accordance with those reported by Roy *et al.* (1998), Peng *et al.* (1999) and Kung's *et al.* (2001).

Determination of some culture soil characteristics:

Soil texture, pH and water holding capacity of the mixture culture soil taken from cultivated pots with banana plantlets during acclimatization and breeding stages are presented in Table (3). Measures concerning soil culture under cultivated banana plantlets recorded level of pH (3.4) lower than the optimal recommended one (between 7.5-8.5). Opposite feature was observed for the high record of water holding capacity (99.3%) comparing with the recommended level (between 50-70%) for optimal plant growth. These measures are referred to the optimal soil conditions for growing agricultural crops, *i.e.* soil type, soil physical texture, pH, organic matter and available dissolving nutrients, as reported by Jackson (1971).

Table 3. Determination of some culture soil characteristics under cultivated banana plantlets

Soil characteristic		Measured level	Optimal level
Soil texture	Sand (%)	29.4	----
	Silt (%)	13.5	----
	Peat-moss (%)	42.9	----
	Vermiculite (%)	14.3	----
PH		3.4	7.5-8.5
Water holding capacity		99.3	50-70

Sensitivity test against fungicides:

Laboratory test of the fungicidal effect against *R. solani* and *F. oxysporum* is a simple approach for understanding a small sector of chemical system on root rot and wilt complex disease of banana plantlets. Data in Table (4) reveal that the reduction in the growth of both tested fungi was correlated to the increasing of either Rizolex-T or Topsin-M concentrations in the medium. Complete growth inhibition was recorded for *R. solani* and *F. oxysporum* at 200 ppm of Rizolex-T and Topsin-M, respectively. Data also indicate that the two tested fungi varied in their sensitivity against the fungicides used. The growth of *R. solani* showed more positive response to Rizolex-T than Topsin-M concentrations, while an opposite trend was observed in the growth of *F. oxysporum*. Similar results concerning the response of Rizolex-T and Topsin-M at different concentrations were also reported by Abdel-Kader (1997 and 1999) and Ragab *et al.* (1999).

Table 4. *In vitro* growth reduction of *R. solani* and *F. oxysporum* affected by different concentrations of Rizolex-T and Topsin-M

Fungicide	Concentration	Growth reduction (%) of	
		R. solani	*F. oxysporum*
Rizolex-T	25	53.2 b*	28.3 a
	50	68.4 ab	35.4 a
	100	86.7 bc	52.6 b
	200	100 d	76.4 c
Topsin-M	25	34.6 a	64.2 ab
	50	51.7 b	78.3 c
	100	69.8 ab	86.8 bc
	200	79.4 c	100 d
Check	0	----	----

* Figures with the same letters are not significantly different (P=0.05).

Disease control:

In vitro treatments of Rizolex-T and Topsin-M at 200 ppm caused complete inhibition to root and wilt complex disease pathogens. Therefore, control scheme, as irrigation of the diseased banana plantlets alternatively each five days with either Rizolex-T or Topsin-M at the rate of 2 g/l water and one day-off between the two fungicides, was used. The fungicidal application was repeated twice with an interval of five days.

Data in Table (5) show obviously that the used fungicidal control scheme improved the percentage of recovered banana plantlets grown in both acclimatization and breeding greenhouses especially when the irrigation time was minimized to 2 hr/day. Data also indicate that the percentage of survival banana plants in the acclimatization greenhouse reached 59.3%, which is not significantly as high as 78.6% that recorded with plants in breeding greenhouse. This observation could be attributed to the advanced development of the damage in the plant tissue of juvenile plantlets due to disease infection which had weak ability to rebuild up and recovery.

Table 5. Recovery (%) of diseased banana plantlets affected with fungicidal treatment in acclimatization and breeding greenhouses

Greenhouse	No. of diseased seedlings	No. of recovered seedlings	Recovery (%)
Acclimatization	300	178	59.3 a
Breeding	300	236	78.6 b

* Figures with the same letters are not significantly different (P=0.05).

Present results proved that the main source of *R. solani* and *F. oxysporum*, the causal pathogens of root rot and wilt complex disease, was the used soil culture medium in some surveyed nurseries. In addition, measures of soil pH and water holding capacity of cultivated soil under banana revealed the records of 3.4 and 99.3%, respectively. These unfavourable growth conditions affected negatively on the plant which became more susceptible to attack by soilborne pathogens. In this regard, Peng *et al.* (1999) reported that the high water content more than 80% and low level of soil pH enhanced Fusarium wilt incidence in banana. Moreover, Aguilar *et al.* (1998) stated that high soil moisture and low pH level increase susceptibility to pathogen infection. They added that high level of water content around banana roots caused lake aeration and O_2 deficiency which reflected on the earliest root functions that responsible for net nutrient transfer into the vascular tissue. Furthermore, low level of soil pH caused lake availability of nutrient absorption (*e.g.* phosphorus) by plant roots which resulted in a weak structure of the plant tissues.

Supervisors in some nurseries could neglect the importance of environmental conditions role in plant production, especially in wide prevalence agricultural private sectors, which reflected negatively on plant growth and provide suitable conditions for several plant diseases incidence. Therefore, the optimal environmental conditions for plant growth should be taken in consideration.

Indicating to the represented results in this study, it could be concluded that to avoid the incidence of root rot and wilt complex disease of banana plantlets in such nurseries, it is strongly recommended to use suitable mixture culture medium for cultivation. The recommended mixture culture medium should contain, peat-moss; vermiculite and sand as 3:1:3 w/w/w and calcium carbonate at the rate of 8% of the soil mixture weight, in addition to fungicides Rizolex-T and Topsin-M at the rate of 3 g/kg of the soil mixture. Moreover, soil moisture must not exceed 80% WHC (Peng *et al.*, 1999).

It could be also suggested that application of irrigation scheme with Rizolex-T and Topsin-M alternatively for five days each at the rate of 2 g/l irrigation water and the minimization of the irrigation time to 2 hr/day at the first emergence of disease symptoms might be successfully used as a control measure for root rot and wilt complex disease of banana plants in production nurseries.

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الفطريات الممرضة وظروف التربة المسببة لحدوث مرض عفن الجذور والذبول خلال أقلمة نبتات الموز المنتجة بواسطة زارعة الأنسجة

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تم عزل فطريات الريزوكتونيا سولانى وفيوزاريوم اوكسيسبوريوم من نباتات موز ناتجة من خلال تقنيات زراعة الأنسجة حيث ظهرت عليها أعراض الإصابة بمرض عفن الجذور والذبول.. كما تم أيضا عزل فطريات من مخلوط التربة المستخدم لزراعة نبتات الموز ووجد أنها متطابقة في الصفات المز رعية والميكروسكوبية للفطريات السابق عزلها من النباتات المصابة.

أثبتت الفطريات المعزولة قدرتها على إحداث مرض عفن الجذور والذبول مع ظهور نفس الأعراض السابق تسجيلها على نباتات الموز وذلك تحت ظروف الصوبة.

أثبتت الاختبارات أن استخدام المبيدات الفطرية ريزولكس-ت وتوبسن-م بتركيز ٢٠٠ جزء فى المليون عمل على إحداث تثبيط كامل للمو الفطريات ريزوكتونيا سولانى وفيوزاريو اوكسيسبوريوم تحت الظروف المعملية.

تشير النتائج المسجلة في هذه الدراسة أن المصدر الرئيسي للفطريات المسببة لمرضى عفن الجذور والذبول هو مخلوط التربة المستخدمة في الزراعة ، كما وجد أن تركيز الأس الأيدروجينى لهذه التربة هو ٤ ر٣ وكذلك نسبة الرطوبة الأرضية تصل إلي ٣ر٩٩ % من السعة المائية .. وهذه الظروف غير الملائمة للنمو أثرت إيجابيا على تهيئة نباتات الموز بحيث أصبحت معرضة للإصابة بهذه الفطريات.

بناء على ما تقدم إيضاحه يمكن التوصية بإضافة المبيدات الفطرية ريزولكس-ت و توبسين-م بتركيز ٢ جم/لتر إلي ماء الري بالتبادل لمدة ٥ أيام لكل منهما مع تقليل زمن الري/ يوم عند بداية ظهور الأعراض المرضية في الصوب المنتجة لنباتات الموز بطريقة مزارع الأنسجة ، وهذه الطريقة من الممكن أن تستخدم بنجاح في مكافحة مرض عفن الجذور والذبول في نباتات الموز في مشاتل الإنتاج.