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Araştırma Makalesi

Effect of Nano Technology in Combination with Soil Solarization to Control Panama Disease of Banana in Jordan Valley

Ahmad Mohamad ALMOMANY*, Nida SALEM, Monther Mohumad TAHAT

The University of Jordan, School of Agriculture, Department of Plant Protection, 11942, Amman, Jordan

*Corresponding author email: momanyah@ju.edu.jo

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Abstract: Soil solarization was applied for a field which was planted with infected banana seedlings. Spreading transparent plastic sheets of 80µ thickness was done from August, 1 until October 12, 2017 at the University Farm in Jordan Valley. Soil solarization was effective in elimination of all phytopathogenic propagules at 30 cm depth. Then clear plastic sheets were removed and the solarized land was planted with healthy banana seedlings cultivar Grand Naine. Banana plantlets were chemically treated as soil drench with Nanoparticle solution of 200 and 400 ppm of Silver Nanoparticles (AgNPs), two fungicides, Revanol and Tachigaren, Sodium Hypochlorite in addition of irrigation of one treatment with treated wastewater. Biological control included three treatments; Endomycorrhiza, *Trichoderma* as a commercial product (BioHealth) and plant growth promoting rhizo-bacteria. Fresh chicken and sheep manure was added to two treatments. Twelve treatments were distributed randomly in randomized complete block design. Endomycorrhizal inoculation with *Glomus mosseae*, 200 ppm of AgNPs, Revanol and Tachigaren treatments of banana seedlings were the most effective in completely protecting banana plants from Fusarium wilt during the whole experimental period. Several applications of *Trichoderma*, wastewater and 400 ppm of AgNPs were effective in maintaining some infected banana seedlings nine months after planting very healthy. Sheep and chicken manure treatments resulted with 60 and 40% disease incidence with Fusarium wilt respectively and 20% of disease incidence in Hypex, PGPR and control treatments. Wastewater, Nanoparticles 200 ppm and endomycorrhizal treatments gave the highest ratio of sword sucker development. We recommend soil solarization and use of integrated program to control Panama disease of banana in Jordan.

Ürdün Vadisinde Yetiştirilen Muzlarda Panama Hastalığının Kontrolünde Toprak Solarizasyonu ile Kombine Nano-Teknolojinin Etkisi

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Anahtar kelimeler

AgNPs,
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Öz: Toprak solarizasyonu, enfekte muz fidelerinin dikildiği parselde uygulanmıştır. 80µ kalınlığında şeffaf plastik örtülerin yayılması, 1 Ağustos - 12 Ekim 2017 tarihleri arasında Jordan Valley'deki Üniversite Çiftliği'nde yapılmıştır. Bütün fitopatojenik propagüllerin 30 cm derinlikte giderilmesinde toprak solarizasyonu etkili olmuştur. Daha sonra şeffaf plastik örtüler kaldırılmış ve solarize edilmiş toprağa sağlıklı Grand Naine çeşidi muz fideleri ile dikilmiştir. Muz bitkileri, arıtılmış atık su ile sulanmasının yanı sıra 200 ila 400 ppm Gümüş Nanopartikül (AgNP), iki fungusit, Revanol ve Tachigaren, Sodyum Hipoklorit, Nanopartikül çözeltisi ile toprağa kimyasal olarak muamele edilmiştir. Biyolojik kontrol üç tedavi içermektedir; Endomikorrhiza, Ticari bir

Panama hastalığı,
Toprak solarizasyonu,
Trichoderma

ürün olarak *Trichoderma* (BioHealth) ve rizo-bakterileri teşvik eden bitki büyümesi. İki uygulamaya taze tavuk ve koyun gübresi eklenmiştir. On iki uygulama tam tesadüfi blok deneme deseninde rastgele dağıtılmıştır. *Glomus mosseae* endomikhriza, 200ppm AgNPs, Revanol ve Tachigaren muz fidesi uygulamaları ile yapılan aşılama, muz bitkilerinin *Fusarium solgunluğu* tüm deney süresince tamamen korumada en etkili olmuştur. Bazı *Trichoderma* uygulamaları, atık su ve 400 ppm AgNP'ler, enfekte olmuş muz fidelerinin dikimden dokuz ay sonra korunmasında etkili olmuştur. Koyun ve tavuk gübresi uygulamaları, *Fusarium solgunluğu* ile sırasıyla % 60 ve% 40, Hypex, PGPR ve kontrol tedavilerinde % 20 ve% 40 hastalık oluşumu ile sonuçlanmıştır. Atık su, Nanopartiküller 200 ppm ve endomikorhizal işlemler en yüksek piç oranını vermiştir. Toprak solarizasyonunu ve Ürdün'deki Panama muz hastalığını kontrol etmek için entegre program kullanmanızı önerilmektedir.

1. Introduction

Banana (*Musa* spp.) is an important crop in Jordan, comprising about 4 % of total fruit production (Ministry of Agriculture, Jordan, 2016). Bananas are an excellent source of potassium, magnesium, phosphorus, vitamins (B6, A, C, D) and carbohydrate (Mohapatara et al., 2010). Banana cultivars vary greatly in plant and fruit size, plant morphology, fruit quality and disease resistance (Khalequzzaman et al., 2009). Banana production is threatened by biotic and abiotic constraints (Arvanitoyannis et al., 2008). The major biotic factors responsible for yield reduction are fungal, nematode, bacterial and viral pathogens (Stover and Simmonds, 1987). There are different fungal diseases that can cause serious damage for banana plantation, and the most important one is Panama disease caused by the soil borne fungus *Fusarium oxysporum* f. sp. *cubense* (Foc.) (Ploetz, 2006; Butler, 2013). Four races of Foc. have been recognized in the world (Ploetz et al., 2015). The pathogen was first reported from Panama as early as 1890 and commonly known as Panama disease (Stover, 1962). Banana plantation in Jordan is threatened by abiotic problems also such as water requirements, poor soil fertility and soil nutrients depletion. Recently Foc. in Jordan, as tropical race 4 was reported affecting Cavendish cultivar and 80% of the Jordan Valley production area was affected by *Fusarium wilt* (Garcia et al., 2014). Disease symptoms in Jordan Valley are severe in July till September during the hottest time of the year and during fruiting period. No effective control measure for *Fusarium wilt* has been found other than the use of resistant cultivars in newly planted areas (Ploetz and Churchill, 2011). It is a soil borne fungus, survives up to 30 years in the absence of banana and spreads to new fields with infected suckers, soil and water. The practices of growing banana regularly in infested fields have assisted the movement of the pathogen. The fungus infects banana through the root system, colonizes the rhizome and eventually blocks the vascular system (Stover, 1962). Wilting symptoms begin as a yellowing from the margins of the lower leaves that die first and later continued to the upper plant parts until the entire plant is killed. Internal symptoms include the yellow to reddish brown discoloration of the rhizome and the vascular bundles of the pseudostem (Su et al., 1986).

Control strategies such as breeding for disease resistance varieties, soil fumigation (Herbert and Marx, 1990) and fungicides (Nel et al., 2007) were used in the management of Panama disease of banana. Biological control of *Fusarium wilt* has become popular disease management consideration in many countries by isolation of nonpathogenic *F.oxysporum* isolates from the rhizosphere of healthy banana plants (Nel et al., 2006). Various biocontrol agents have been tried for suppressing Panama disease (Akila et al., 2011; Thangavelu and Mustafa, 2012). *Trichoderma* and endophytic microbes have been shown to suppress *Fusarium* growth in the laboratory and greenhouse experiments (Saravanan et al., 2003; Lian et al., 2009) but they were unable to control the disease under field conditions. Plant-growth promoting bacteria as *Bacillus chitenosporus* slightly reduced the growth of *Fusarium in vitro*, but there was no evidence that this bacterium prevents disease development *in vivo* (Visser and Bezuidenhout, 1996). *Streptomyces violaceusniger* strain G10 was used for the biological control of Foc. race 4 by producing an antibiosis effect in soil (Getha and Vikinesway, 2002). Peroxidase and polyphenol oxidase activity increased two folds on 8 days in roots treated with *Pseudomonas fluorescens* and challenged with Foc. and the same trend of enzymes were noticed in *Trichoderma viride* and *T. harzianum* inoculated roots (Saravanan et al., 2004; Nel et al., 2006).

Endomycorrhiza represent a group of fungi that are associated with most agricultural crops and provide biological protection against soil borne diseases (Smith and Read, 2008). Arbuscular mycorrhizal fungi (AMF) are the symbiotic fungi that predominate in the root and soils of agricultural crop plants. AMF were used widely against various soil borne pathogens as biocontrol agents. Many workers have observed an antagonistic effect of AMF against some fungal pathogens (Tahat et al., 2010).

Lakshmanan et al., (1987) in India reported a dramatic decrease in disease incidence by injecting rhizomes with carbendazim. Soil solarization resulted in a marked reduction of Fusarium wilt of bananas in South Africa (Herbert and Marx, 1990). Solarization alone was ineffective, whereas fumigation reduced the disease for at least two years. After this period, the disease became even more severe than it was before fumigation.

Silver nanoparticles (AgNPs) are increasingly used as antimicrobials in consumer products (Gil-Allué et al., 2015). Silver is a widely distributed metal in the environment originating from its different forms of application as metal, salt and nanoparticle (Boenigk et al., 2014). Nanoparticles can be produced from nearly any chemical; most currently in use today nanoparticles have been made from transition metals, silicon, carbon and metal oxides. These particles are different chemically from the original parent material and they have different properties that might make them more harmful. Therefore in-depth research should be conducted on them (Benedicta and Ertel, 2008; Theodore and Kunz, 2005). Because of the limited information about banana decline problem in Jordan, this study was conducted to evaluate the different control measures including biological, chemical and physical methods to manage Fusarium wilt of banana.

Materials and Methods

Soil solarization was used to eliminate the large amount of Fusarium propagules from the heavily infested soil at the University Farm in Jordan Valley which was previously planted with heavily infected banana seedlings for the first time. The farm was severely diseased where 64% of banana plants and area was infected. Soil samples were taken at 5, 10 and 30 cm depth and tested for Fusarium infestation by both direct planting and dilution method by using potato dextrose agar (PDA) media amended with 600 mgL⁻¹ of Lincocin (lincomycin hydrochloride) as an antibiotic. The soil was ploughed after removal of heavily infected banana plants and addition of sheep manure. Drip irrigation tubes were applied at 2m distance between each line and from the beginning of August clear plastic sheets of 80µ thickness and 3m wide were perfectly fasten over the soil surface by making ditches of 20cm depth and fasten one edge of the plastic sheet and stretched gently to avoid any air pockets and put the second edge of the plastic in the opposite furrow. Irrigation was done every three days for 5 hours until the end of the solarization period which lasted until October 12, 2017.

Soil samples were taken after soil solarization at three depths and tested for Fusarium infestation as explained previously. Banana seedling cultivar Grand Naine were brought from private nursery and tested individually to be sure that all seedlings were healthy and the infected seedlings were excluded. Healthy seedlings were planted at 2m distance in each row and 2m between rows from October 12, 2017 till August 12, 2018 next year and treated on the first day with all treatments. Each treatment composed of five banana seedlings and twelve treatments were distributed by randomized complete block design (RCBD). The solarized area was 750 m². Chemical treatments included Revanol-SL (active ingredient 8-Hydroxyquinoline sulphate 500g L⁻¹) 20cm³/seedling, Tachigaren (a. i. Hymexazol 41.5%) 30 cm³ and hypex as sodium hypochloride 20 cm³/seedling. Biological control treatments included endomycorrhizal inoculation in pots 20 cm depth containing 300 chlamydospores from local isolate of *Glomus mosseae* two weeks prior transplantation and application of the commercial product BioHealth as *Trichoderma* by dissolving 100 g/20 Liter of water per seedling at every application time. Plant growth promoting rhizo-bacteria (PGPR) was used by applying 400 cm³ of broth solution/seedling (10⁸ CFU/MI⁻¹). AgNPs solution was used as 80 cm³ /seedling from AgNPs 200 ppm and 160 cm³ from AgNPs 400 ppm. Chicken and sheep manure was used by applying 4 kg /seedling for one time at the planting date. As physical control method, treated wastewater was used by applying 20 liter/seedling each application time. Control treatment received normal irrigation water. All materials were dissolved in 20 liter irrigation water and applied to each seedling as indicated in Table 1. The first application was done at the planting date and disease

incidence was calculated as the percentage of infected plants among the total number of banana plants. Leaf sampling for *Fusarium* infectivity was started three months after planting banana and disease incidence was calculated as the percentage of infected plants among the whole number of banana plants. Counting number of new suckers was done five months after planting as well as plant growth over a scale from 0-5 where 5 was the highest banana plant and 1 was the smallest or shortest plant and 0 means dead plant. The number of new suckers and plant growth were statistically analyzed by one-way analysis of variance (ANOVA) and grouping information using the Tukey method and 95% confidence. Correlations between plant growth and number of new suckers were conducted by Pearson correlation.

Table 1. Date of treatments application (wastewater, Revanol, Tachigaren, hypex, *Glomus mosseae*, *Trichoderma*, PGPR, AgNPs, Chicken and sheep manure).

No. of application	Date of application
First	12 October 2017
Second	16 November 2017
Third	16 January 2018
Fourth	15 March 2018
Fifth	15 May 2018
Sixth	12 July 2018

3. Results and discussions

3.1. *Fusarium* propagules

Soil samples were taken before and after soil solarization at different depths to find out the effectiveness of the accumulated heat created by plastic sheets on number of fungal propagules. Data are present in Table 2. The number of fungal propagules for each soil sample was estimated by using two PDA plates from both direct plating and dilution methods for accurate estimations. Infested soil contained from 16 to 55 CFU/g oven dry soil and after soil solarization, the number of propagules was zero at 5, 10 cm and at 30 cm depth declined to 2 CFU/g oven dry soil. Dilution method to estimate the number of pathogenic propagules for solarized soil was more accurate than direct plating due to low propagule content. Fresh chicken and sheep manure was added to raise the soil organic matter content as well as for biological fermentation by inducing heat to minimize the number of *Fusarium* propagules. Previous studies reported that organic matter content was negatively correlated to disease incidence and to the relative abundance of *Fusarium* (Shen et al., 2018).

Table 2. Effect of soil solarization on *Fusarium oxysporum* f.sp. *cubense* propagules

Soil Depth	<i>Fusarium</i> propagules after soil solarization CFU/g dry soil by		<i>Fusarium</i> propagules before soil solarization CFU/g dry soil by	
	Dilution Method	Direct plating	Dilution Method	Direct plating
5 cm	0	0	33	22
5 cm	0	0	55	16
10 cm	0	0	33	16
10 cm	0	0	40	30
30 cm	2	0	44	27
30 cm	2	0	54	55

3.2. Disease incidence of banana

Plant samples were taken from all banana plants from the field by cutting the leaf blades arbitrary and plating the vessels from the midrib of the leaf over PDA plates and tested microscopically for the presence of fungal colonies. Fungi were identified morphologically and certified by molecular biotechnology as shown in Table 3. Microconidia were thick with single cell and two celled macroconidia of white color mycelium. Soil solarization reduced the inoculum density of *Fusarium* which let most of the treatments were effective in disease control and these findings were

in consistent with other results in South Africa (Herbert and Marx, 1990; Viljoen, 2002) but alone was ineffective. The best treatments were Mycorrhiza, AgNPs 200 ppm, Tachigaren and Revanol where disease incidence was zero even nine months after planting (Table 4). Frequent applications of *Trichoderma*, AgNPs 400 ppm and wastewater even nine months after planting were also able to control some infected transplants.

Table 3. The maximum nucleotide identity (BLASTn) between Jordanian isolates of fungi (J-isolates) amplified with ITS1/ITS4 and TEF1/TEF2 sets of primers and the most closely species/subspecies.

Sample No.	Region	Fungal color	Source	Closely related species/subspecies
266	South Shunah	White	Seedling	<i>Fusarium oxysporum</i> f.sp. <i>cubense</i>
405	Wady Alrayan	Pink	Leaves	<i>Fusarium equiseti</i>
412	Wady Alrayan	Purple	Pseudostem	<i>Fusarium oxysporum</i> f.sp. <i>cubense</i>
421	Alramah	Purple	Leaves	<i>Gibberella moniliformis</i> voucher
429	South Shunah	Purple	Leaves	<i>Fusarium verticillioides</i>
571	University Farm	White	Pseudostem	<i>Fusarium oxysporum</i> f.sp. <i>cubense</i>

Sheep and chicken manure was not significantly different from each other and from the control. Disease incidence of control plants were 20% by low soil inoculum of *Fusarium* from previously solarized soil and shallow root system by smaller plants. Hypex as well as the local isolate of PGPR were not effective in controlling *Foc.* as reported earlier (Xue et al., 2015; Lian et al., 2008).

Table 4. Disease incidence of banana treated with different treatments at different dates.

Treatment	% of Infected plants at different dates (Disease incidence)				
	16/1/2018	8/2/2018	15/3/2018	15/5/2018	12/7/2018
Endomycorrhiza <i>Glomus</i> spp.	0*	0	0	0	0
<i>Trichoderma</i> spp.	0	40	0	0	0
Wastewater	0	20	20	0	0
Hypex	0	20	0	20	20
AgNPs 200ppm	0	0	0	0	0
AgNPs 400ppm	0	0	20	0	0
Sheep manure	0	40	0	40	60
Chicken manure	0	0	0	40	40
Revanol	0	0	0	0	0
Tachigaren	0	0	0	0	0
PGPR	0	20	0	20	20
Control	0	0	0	20	20

*Average of five banana plants.

3.3. Banana growth

Growth of banana plants was evaluated over a scale from 0-5 where 0 means dead plants and 5 the longest as shown in Table 5. Endomycorrhiza and wastewater treatments gave the highest growth during the first two evaluation dates due to its high content of nitrogen and beneficial microorganisms, same findings were recorded by controlling *Fusarium oxysporum* of tomato and corn by irrigating treated wastewater (AlMomany et al., 2014), it was followed by Endomycorrhiza and Revanol (Schliemann et al., 2008). Nine months after planting, AgNPs, wastewater, hypex, Revanol and Endomycorrhiza treatments showed the longest plants while both manures, Tachigaren, *Trichoderma* and PGPR were not significantly different from the control. Slight suppression of *Fusarium oxysporum* f.sp. *cubense* was observed *invitro* tested with two *Trichoderma* isolates (Nel et al., 2006). The best growth was associated with strong and more suckers development. The lowest growth was found in PGPR treated plants. This disease is very difficult to control once it entered the soil, our results were in agreements with many findings (Ploetz and Churchill, 2011).

Table 5. Growth of banana plants treated with different treatments at different dates

Treatment	Plant height (m)		
	15/3/2018	15/5/2018	12/7/2018
Endomycorrhiza	3.6* abc	5** a	4.0***abcd
Trichoderma	3 abc	4.4 ab	3.6 abcd
Wastewater	4.2 a	5 a	4.6 ab
Hypex	3.2 abc	4.2 ab	4.6 ab
AgNPs 200ppm	2.4 bc	4 abc	4.8 a
AgNPs 400ppm	2 c	2.6 c	4.4 abc
Sheep manure	2.4 bc	4.2 ab	3.4 bcd
Chicken manure	2.4 bc	3.6 abc	3.2 cd
Revanol	3.8 ab	4.8 ab	4.2 abc
Tachigaren	3.2 abc	4.2 ab	3.6 abcd
PGPR	2.4 bc	3.4 bc	2.8 d
Control	2.4 bc	3.8 abc	3.4 bcd

Average of five plants and means with same letters are not significantly different from each other using Tukey test and 95% confidence. *Scale 5 was 1.4 meter height, ** Scale 5 means 2.4 meter height, *** Scale 5 means 4.0 meter height.

3.4. Development of suckers

New suckers were counted as parameter for banana development as shown in Table 6. Wastewater treatment gave the highest number of new suckers at early growth stages followed by Revanol and Tachigaren. At the second evaluation date, PGPR, AgNPs and *Trichoderma* produced the lowest number of new suckers. From different commercially available PGPR tested, only *Bacillus chitenosporus* slightly inhibited the growth of *Fusarium invitro* but not *invivo* (Visser and Bezuidenhout, 1996; Saravanan et al., 2004). Other treatments were not significantly different from each other. Nine months after planting, wastewater, AgNPs 200 ppm and endomycorrhiza treatments showed the highest ratio of good sucker development which reflects higher and early yield. Very few or no sword suckers were found in sheep manure, PGPR and control treatments. The high dose of AgNPs resulted with low ratio of good suckers to small from 1.28 to 0.6 as well as small growth at the first two evaluations. The lowest ratio of good suckers was observed in treatments where infected plants were detected.

Table 6. Number of suckers per mother corm of banana treated with different treatments at different dates.

Treatment	15/3/2018	15/5/2018	12/7/2018		Ratio A:B
			Sword suckers (A)**	Smal suckers (B)	
Endomycorrhiza	0.2* c	8.2 ab	**2.8 abc	2.4 c	1.16
Trichoderma	0.6 bc	6.6 ab	2.8 abc	3.4 abc	0.82
Wastewater	3.2 a	7.8 ab	4.6 a	2.4 c	1.91
Hypex	0.4 bc	9 a	2.6 abc	4.0 abc	0.65
AgNPs 200 ppm	0.2 c	5.2 b	3.6 ab	2.8 bc	1.28
AgNPs 400 ppm	0 c	6 ab	2.4 abc	4.0 abc	0.6
Sheep manure	0 c	7.2 ab	1.0 c	6.2 a	0.16
Chicken manure	0 c	7 ab	3.0 abc	3.4 abc	0.88
Revanol	1.6 b	8.4 ab	3.2 abc	5.4 ab	0.59
Tachigaren	1.6 b	7.6 ab	3.4 abc	3.8 abc	0.89
PGPR	0 c	6 ab	1.2 bc	4.6 abc	0.26
Control	0 c	8 ab	1.6 bc	6.0 a	0.26

*Average of five banana plants and means with same letters are not significantly different from each other using Tukey method and 95% confidence. A:B = Ratio of Sword suckers to small suckers.** Suckers with more than 10 cm in diameter in addition to mother sucker

4. Conclusion

Soil solarization for two months during the hottest period of the year was highly effective in completely elimination of fungi from the upper soil layers and minimizing the fungal propagules of infested deep soil layers properly. Endomycorrhizal inoculation with *Glomus mosseae*, 200 ppm of AgNPs, Revanol and Tachigaren treatments of banana seedlings were the most effective treatments by completely protecting banana plants from *Fusarium* infection during the whole experimental period. Several applications of *Trichoderma*, wastewater and 400 ppm of AgNPs were effective in maintaining some infected banana seedlings nine months after planting very healthy. We recommend soil solarization during Summer time and use of integrated program to control Panama disease of banana in Jordan Valley.

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