

Comparative Study of Alternative and Traditional Fungicides Combinations in Management of Banana Root Rot Disease

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Abstract

Field study was conducted during two successive seasons of Williams banana Cv., grown in loam soil under surface flood irrigation system, located at Al-Daqahlia and Domietta Governorates, and in sandy soil at Beheira Governorate in Wadi Alnatroon, under drip irrigation system in Egypt. Our study investigated the relationship between application of alternative fungicides and chemical fungicides alone and combined on the severity of fungal root rot disease and their effects on banana trees growth. Disease reducing was observed by using inducer compounds i.e ascorbic acid, citric acid and salicylic acid and fertilizers i.e., potassium and copper combined with protective fungicide (copper oxychloride). The chemical fungicide of copper oxychloride, followed by the organic acids and nutrients fertilizers reflected the higher reduction in the percentages of frequency occurrences of fungal pathogens it reveals 10%, 5%, 0% and 0% frequency of *Fusarium oxysporum*, *Macrophomina phaseolina*, *Verticillium Sp.* and *Botrydipodia sp.* respectively. Though, the alternative fungicides in combined with protective fungicide is safety strategy for control banana root rot disease, which will defense banana trees against fungal root rot disease infection. On the other hand, the economic aspect plays the major role in this respect.

Keywords: Banana; inducer compounds; chemical fungicides; roots rot and nutrient fertilizers.

1. Introduction

Banana (*Musa* sp.) is one of the most economic tropical fruit crops in the world. Bananas grow in a wide variety of soils. In Egypt 2021, bananas production reached to 1.29 million tons **World Data Atlas, 2021** Bananas subjected to infection by various pests and diseases, particularly Fusarium wilt. The disease is caused by the soil borne fungus *Fusarium oxysporum* f. sp. *cubense*, which is one of the major constraints to banana production worldwide [Dita *et.al.*,2018]. Infects the xylem through the roots, and causes extensive necrosis leading to plant deaths [Dita *et.al.*,2018]. Currently, management of the Fusarium wilt disease mainly involves rotation, selection of resistant varieties; and chemical or biological methods. Nonetheless, Fusarium wilt of banana has not effective control, as evidenced by the continuous spread among continents, countries and regions [Getha and Vikineswary 2002 and Wang b, *et.al* 2013]. *Macrophomina phaseolina* (Tassi) Goid is one of the most damaging seed and soil borne pathogen infecting about 500 plants species in more than 100 families throughout the world [Mihail and Taylor 1995]. Under favorable conditions, it causes many diseases like damping off, seedling blight, collar rot, stem rot, charcoal rot and root rot in various economically important crops.

Nowadays, there is a widespread use of natural and safety substances such as antioxidants particularly, ascorbic acid, citric acid, oxalic acid and salicylic acid for enhancing or improving growth and productivity of many crops for the aim of

reducing health hazards. Since, antioxidants have synergistic effect on growth, yield and chemical composition under favorable and unfavorable environmental conditions. These compounds non enzymatic material have beneficial effect on catching the free radical or the active oxygen, superoxide anion, hydrogen peroxide, hydroxyl radicals and ozone that producing during photosynthesis and respiration process (Zhang and Klessing, 1997). Leaving these free radicals without chelating or catching leads to lipids oxidation and the loss of plasma membrane permeability and the death of cell within plant tissues. They also have an auxinic action (Prusky, 1988; Elade, 1992; Raskin 1992 and Buehala and Schmid, 1997)

Alternative control strategies such as potassium nutrition and /or bio-control agents are needed. Potassium is considered one of the three major soil nutrients. Its indirect effects can be variable for inducing plant resistance, directly stimulating or inhibiting the penetration, multiplication, survival and establishment of a pathogen (Amtmann *et al.*, 2008). So, this work aimed to study the effect of potassium fertilizer levels alone on performance of banana Williams cv. and controlling the fungal root rot. In the present investigation, the potential of organic acids, amino acids and nutrient fertilizers were tested against fungicides in reducing disease incidence of soil born fungi of banana and reduce using chemical fungicides for health and environment.

2. Material and Methods

Field experiment:

This investigation was conducted on banana plants (*Musa cavendishii* L.) Was applied in two private orchard locations (Aldaqahlia and Domietta Governorates)

Transplants were grown at 3.5 x 4 m in loamy soil irrigated by flooding system. Four transplants were used for each treatment and transplant was cultivated per hole. All recommended horticultural practices were made and alternative fungicides were carried out within horticultural practices (table, 1) as recommended dose from April to the first of July season 2019/2020 (6 doses) as once / two weeks compared with chemical fungicide. These doses were treated to banana by drenching and spraying.

On the other hand, the second season 2021/2022 at the third Governorate Beheira in Wadi Alnatroon, was conducted during two locations in banana plants (*Musa cavendishii* L.), grown in a private orchard. Transplants were grown at 5 x 4 m. The soil texture was sandy, under drip irrigation system. All recommended horticultural practices were made. During April, one transplant per hole was planted and, 4 transplants as replicates for each treatment were made. Fungicidal application (copper oxychloride and azoxystrobin), as a therapeutically treatment and chemical compounds including 6 organic acids 2 amino acids and 7 fertilizers used as alternative fungicides were applied (table: 1) as recommended dose once after 15 days periodically for 6 weeks in case of treatments alone at the first location. On the other hand there were combined treatments between alternative fungicides and chemical fungicides at Wadi Alnatroon in the second location.

In this respect, we added the first dose of alternative fungicides with recommended doses in April, then the second addition after one month in May, whereas the third dose applied after one month in June. After one week from the alternative fungicides we added the copper fungicide (protective fungicide) as the first dose, as recommend

dose, then after two weeks we added Azoxystrobin (systemic fungicide) as second dose. Finally, after two weeks we add the last dose as mixed the two fungicides. All treatments were done as soil drench, as recommended dose. After, a week we added the last dose from alternative fungicides. After one month, around the fourth week of August, the disease severity percent had been recorded. In this respect, one successor for each replicate had been removed for isolation and length of root, beside disease severity percentage recorded. Fungi pathogen was isolated pathogen from roots and recorded numbers of new roots.

Isolation and identification of causal organisms

In this study samples were taken from banana trees in various stages in two locations at Wadi Alnatroon showing symptoms of root rot disease. Samples of root collected from banana plantations were put in plastic bags kept in a refrigerator and transferred to the laboratory. In the laboratory samples were first washed under running tap water then cut into small pieces and surface sterilized with 5% sodium hypochlorite for 2 minutes; washed in sterilized water and then dried between two sterilized filter papers. The surface sterilized pieces were transferred individually to Petri dishes, each containing 20 ml of sterilized PDA medium (Nelson *et al.*, 1983). After 3 days of incubation at 28°C, developed fungi were identified using hyphal tips or single spore technique adopted by (Dhingra and Sinclair 1995). The purified fungi were verified and identified by the author according to references in Microscopic features of conidiophores, shape, and size of conidia, colony characters and growth rate following (Nelson *et al.*,

1983). Frequency of fungi isolated from diseased banana root samples was calculated according to the following equation: % Frequency = (No. of colonies for each fungus)/Total No. of fungal colonies × 100

Disease assessments

The subcrown internodes were excised and rated for extent of dark brown to black discoloration, indicative of diseased tissue, using a 0–3 scale as follows: 0 = no discoloration, 1 = 1–25% of the total surface area discolored (slight), 2 = 26–50% (moderate) and 3 = >50% (severe). The percentage of plants in the moderate or severe discoloration category, or the severe category only, was determined for each plot based on the total number of

subcrown internodes collected and assessed from the plot.

Disease severity of sub crown discoloration = (category value x plants in category)/total number of plants sampled x 100 (**Zadoks et al. 1974**)

Efficacy % were determined according to the following equation

$$\text{Efficacy \%} = \frac{C-T}{C} \times 100$$

Where C = Control, T = Treatment

Statistical analysis:

The obtained data were statistically analyzed according to **Snedecor and Cochran (1980)**

Table 1: Fungicides and alternative fungicides used in the experiments.

	Treatments	Compound Type	Active ingredient	Dose
1	Citric Acid	Organic acids	Acetone	100mg/l
2	Ascorbic Acid	Organic acids	Vitamin C	150mg/l
3	Salicylic Acid	Organic acids	Hydroxyl acid	150mg/l
4	Humic Acid	Organic acids	Humic acid	0.3g/l
5	Boric acid	Organic acids	Boric acid	0.5 g/l
6	Arginine	amino acid	Guanidine group	0.1g/l
7	Glycine	amino acid	Amino group	0.1g/l
8	Ascobain	Organic acids	38% Citric Acid+ Ascorbic Acid	2g/1.5L
9	Potassium Disulphide	Fertilizer	Potassium	8 kg/fadan
10	Magnesium Disulphide	Fertilizer	Magnesium	1.5kg/fadan
11	Ammonium Sulphate	Fertilizer	Ammonium	1.5kg /fadan
12	Zinc Sulphate	Fertilizer	Zinc	250gm/fadan
13	Manganese Sulphate	Fertilizer	Manganese	250gm/fadan
14	Ferro Sulphate	Fertilizer	Ferro	150gm/fadan
15	Copper Sulphate	Fertilizer	Copper	200gm/fadan
16	Copper Oxychloride WP	Fungicide	Copper Oxychloride50%	2gm/L
17	AzoxystrobineSC	Fungicide	Azoxystrobine25%	80-100ml/L
18	Control		without any treatments	

3. Results

Data in table 2 showed ,that there was a significant differences between all treatments and control also there was a significant differences between treating by foliar and by drench in soil ,hence all applied by drench gave high effect in compare with foliar by reducing disease severity at two governorates . Data also recorded, the most effective treatment by

copper sulphate fertilizer (70%) in Aldaqahlia in compare with treatment with fungicide (copper oxychloride) which recorded 80% efficacy. in Domietta data recorded humic acid and coppersulphate as the best treatment they were close to the fungicide Azoxystrobin they recorded (65%,70 and70) respectively. The others treatments were close to each other in reducing %disease severity.

Table 2: Comparative effectiveness of two fungicides and alternative fungicides on root rot disease in banana in two Governorates season 2019/2020.

Treatments	% mean disease severity in Aldaqahlia		% Efficacy		% mean disease severity in Domietta		% Efficacy	
	foliar	drench	foliar	drench	foliar	drench	foliar	drench
Citric Acid	70	45	30	55	72	68	28	32
Ascorbic Acid	70	50	30	50	75	73	25	27
Salicylic Acid	60	40	40	60	60	60	40	40
Humic Acid	30	35	70	65	35	30	65	70
Boric acid	75	80	25	20	70	65	30	35
Arginine	60	60	40	40	60	60	40	40
Lysine	65	65	35	35	65	65	35	35
Ascopine	65	67	35	33	63	60	37	40
Potassium Disulphide	60	40	40	60	55	50	45	50
Magnesium Disulphide	60	65	40	35	53	50	47	50
Ammonium Sulphate	70	60	30	40	75	72	25	28
Zinc Sulphate	100	67	0	33	100	93	0	7
Manganese Sulphate	80	60	20	40	75	70	25	30
Ferro Sulphate	50	40	50	60	55	53	45	47
Copper Sulphate	45	30	55	70	40	35	60	65
Copper Oxychloride	20	20	80	80	///////	////////	///////	////////
Azoxystrobin	///////	///////	////////	////////	30	30	70	70
Control	100	100	///////	///////	100	100	///////	///////
L.S.D 5%	4.604	3.731	////	////	3.598	2.880	///////	///////

Data in table 3: in the second season 2021-2022 all treatments were applied by drench as it gave the best method in the first season. There is a significant differences

between alternative fungicides and two fungicides alone or combined under field condition at two different locations, hence we found that the most effective treatments at first

location were fertilizing with copper sulphate and the two fungicides (copper oxychloride and azoxystrobine 62.5% & 80% and 60%) respectively, In case of using alternative compounds combined with fungicides we found that citric acid, Ascorbic acid, salicylic acid, humic acid as organic acids and argenine

and glycine as amino acids and fertilizers potassium disulphide and copper sulphate their efficacy in controlling root rot and wilt diseases were increased, data indicated that combined treatments gave high efficacy in compare with treatments alone.

Table 3: Comparative effectiveness of two fungicides and alternative fungicides on root rot disease in banana at two locations in Wadi Alnatroon season 2021-2022

Treatments	% mean disease severity		% Efficacy	
	first location alone treatments	second location combined treatments	first location alone treatments	second location combined treatments
Citric Acid	50	25	50	75
Ascorbic Acid	50	20	50	80
Salicylic Acid	62.5	15	37.5	85
Humic Acid	57.5	35	42.5	65
Boron	72.5	70	27.5	30
Arginine	60	30	40	70
Glycine	62.5	20	37.5	80
Ascopine	65	56	35	44
Potassium Disulphide	52.5	40	47.5	60
Magnesium Disulphide	65	50	35	50
Ammonium Sulphate	92.5	60	7.5	40
Zinc Sulphate	80	80	20	20
Manganese Sulphate	80	80	20	20
Ferro Sulphate	65	50	35	50
Copper Sulphate	37.5	20	62.5	80
Copper Oxychloride	20	20	80	80
Azoxystrobine	40	30	60	70
Control	100	100	////	/////
L.S.D 5%	6.486	3.313	////	/////

The diseased plants were stimulated to produce new roots as shown in table (4), the number of new roots in case of each treatment, it is obvious that the high levels were in combined treatments hence all treatments were

very close and there is not significant differences between them in compare with treatment alone but of course there were significant differences between all treatments alone or combined and control.

Table 4: effect of treatment with alternative fungicides and two fungicides alone and combined on rate of renew rootless at three governorates.

Treatments	Rate of renew rootless			
	Aldaqahlia	Domietta	WadiAlnatroon alone treatments	WadiAlnatroon combined treatments
Citric Acid	3	3	2	4
Ascorbic Acid	5	4	2	5
Salicylic Acid	3	3	3	5
Humic Acid	3	3	4	4
Boron	1	2	2	5
Arginine	4	3	5	5
Glycine	5	4	5	5
Ascopine	5	5	4	5
Potassium Disulphide	3	5	4	5
Magnesium Disulphide	2	3	3	5
Ammonium Sulphate	3	5	4	5
Zinc Sulphate	1	0	2	4
Manganese Sulphate	0	0	0	2
Ferro Sulphate	3	2	3	5
Copper Sulphate	3	5	4	5
Copper Oxychloride	5	5	5	5
Azoxystrobine	4	4	4	5
Control	0	0	0	0

Macrophominaphaseolina 25%, *Botrydiplodiasp* 15% and *Verticillum Sp.* 10%. we found that, the most effective treatment which reducing % frequency was the fungicide copper oxychloride 15% , copper sulphate 20% and citric acid 25% the lowest treatment was ascorbic acid it was 40% equal with control that was recorded with *Fusarium sp.* In case of *Macrophomina phaseolina* the most treatment reducing frequency were copper oxychloride and potassium disulphide 10% in addition to the two fungi

Botrydiplodia sp and *Verticillum Sp* were already reducing with all treatments significantly .**Table 5** and Fig.2 recorded in the second location (combined treatments) that *Fusarium oxysporum* was the most prevalent fungus 50% followed by, *Macrophomina phaseolina* 35%, *Botrydiplodiasp* 10% and *Verticillum Sp.* 5%. Copper oxychloride was the highest effective in compare with all treatments hence there was no significant difference between others.

Table 5: Frequency occurrence of some soil borne fungi isolated from roots and crown banana plantlets cultivation from two locations in Wadi Alnatroon.

Treatments	%Frequency <i>Fusarium oxysporum</i>		(%)Frequency <i>Macrophomina phaseolina</i>		(%)Frequency <i>Verticillium Sp.</i>		(%)Frequency <i>Botrydiplodia sp.</i>	
	first location	second location	first location	second location	first location	second location	first location	second location
Citric Acid	25	20	25	15	10	0	5	0
Ascorbic Acid	40	25	30	25	5	0	5	0
Salicylic Acid	30	25	25	10	5	0	5	0
Humic Acid	30	15	25	0	0	0	0	0
Boron	30	25	25	20	0	5	0	0
Arginine	35	5	20	10	0	0	10	0
Lysine	35	10	25	5	0	0	5	0
Ascopine	35	10	20	0	0	5	5	0
Potassium Disulphide	25	15	10	10	0	0	10	0
Magnesium Disulphide	30	20	25	15	5	0	10	0
Ammonium Sulphate	40	25	25	20	5	5	10	0
Zinc Sulphate	35	15	20	10	0	5	10	5
Manganese Sulphate	35	20	25	5	5	5	10	0
Ferro Sulphate	35	20	25	5	5	5	5	0
Copper Sulphate	20	10	15	5	5	5	5	5
Copper Oxychloride	15	10	10	0	0	0	5	5
Azoxystrobine	35	15	15	10	5	0	5	0
Control	40	50	25	35	10	5	15	10

(Table 5). *Fusariumoxysporum* (Fig. 1) was the most prevalent fungus 40% followed by, Figure (1): Frequency occurrence of some soil borne fungi isolated from roots and

crown banana plantlets cultivation from first location (alone treatments) in Wadi Alnatroon.

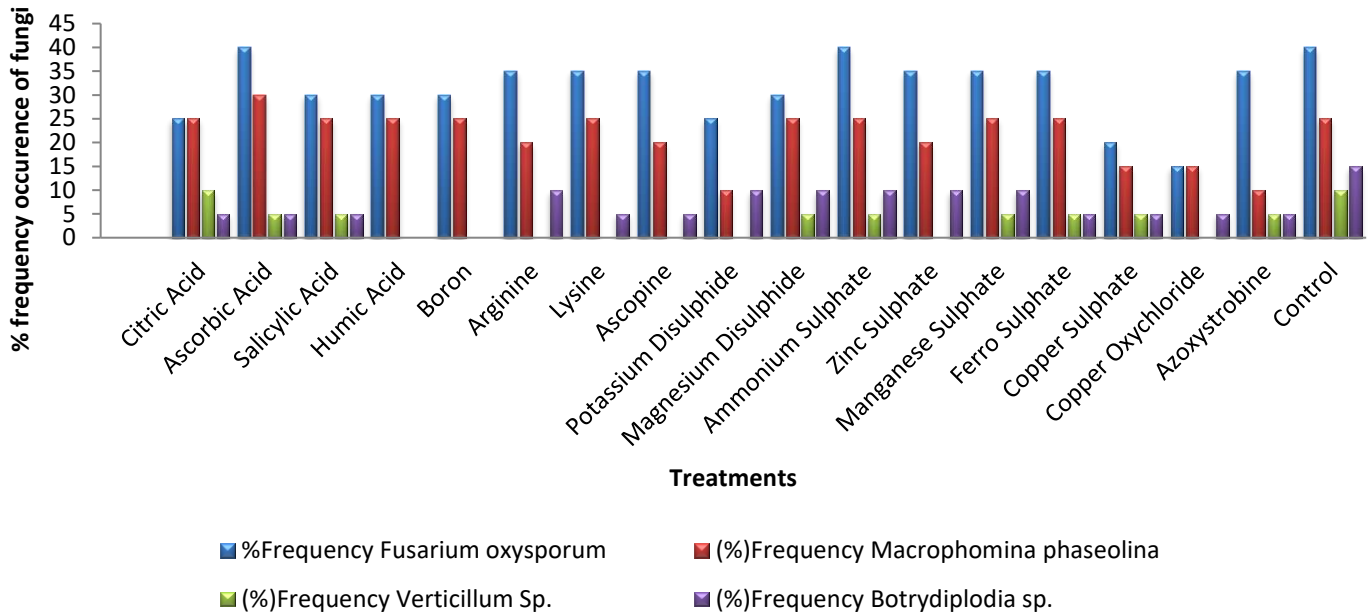
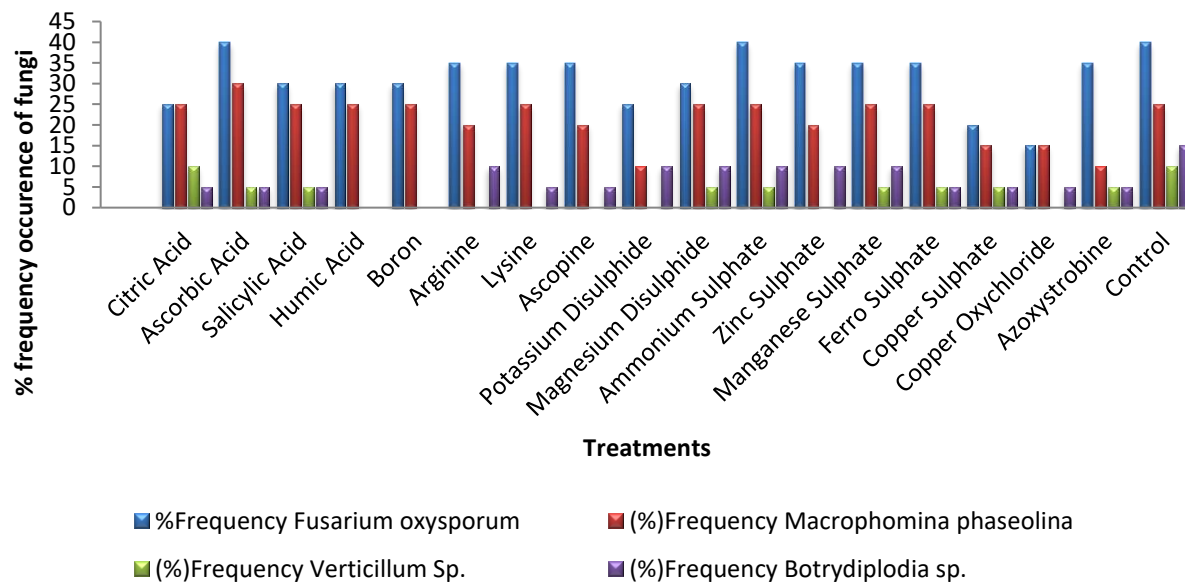


Figure (2): Frequency occurrence of some soil borne fungi isolated from roots and crown banana plantlets cultivation from second location (combined treatments) in WadiAlnatroon.



4. Discussion

Results indicated that all tested antioxidants reduced damping-off, root rot and the fertilizers with the soil born fungi;

Generally, for the tested antioxidants citric acid was better than the other antioxidants to reduced damping-off, root rot, similar results were reported by Galal and Abdou (1996)

found that application of salicylic or ascorbic acid as a soil drenching was better than foliar application to control fusarial diseases of cowpea. Abdel-Monaim (2008) showed that soaking lupine seeds in antioxidant solutions (reduced the damping-off and root rot diseases caused by *Fusarium solani* and *Macrophomina phaseolina*). The antioxidants mode of action was reported in many host-pathogen interactions *i.e.* many oxidative enzymes such as peroxidase, catalase, ascorbate oxidase and polyphenol oxidase were detected as a result of infection with many pathogens (Clark *et al.*, 2002) or as a result of treatments with various antioxidants (Takahama and Oniki, 1994, El-Khallal, 2007b and Abdel-Monaim, 2008). Moreover, Lyon and McGill (1989) reported that the phenolic acids benzoic, ferulic, coumaric and protocatechoic acid inhibit *in-vitro* activity of polygalacturonase and polygalacturonic acid lyase from *Erwinia carotovora*. Chen *et al.* (1993) reported that SA binds inhibits catalase. antioxidants might be right being regulating plant growth by increasing enzyme activity as α -amylase and nitrate reductase, which accelerate the sugar translocation from the leaves to developing fruit (Sharma *et al.*, 1986). In addition, application of SA inhibits ethylene production leading to an increase in fruit number and consequently increases fruit yield per plant (Leslie and Romani, 1986). Abdel-Monaim (2008) found that lupine seed soaking in antioxidant solutions increases of chlorophyll and carotenoids content in leaves and this reflects the health condition of the plant.

The management of controlling these diseases by using chemical fungicides is not recommended because of risks to humans and the environment. Thus, alternative control strategies such as potassium nutrition was successfully used in controlling the fungal soil borne pathogens. (Generalao and Davide, 1995; Esnardet *al.*,

1998 and Butt *et al.*, 2001). Moreover, results of the present study showed that fungi isolated from naturally rotted roots and wilt roots of banana plants collected from two locations in WadiALnattroon, Egypt (represented to *Fusarium* genus (*F. oxysporum*), followed by *Macrophomina phaseolina*, *Botrydiplodia sp.* and *Verticillium Sp.* Data are in accordance with those reported by This result was in agreement with that reported in Wardlaw (1961 and 1972); Stover (1972); Ploetz *et al.*, (1994); Jones (2000); Sing (2000). in this respect, potassium is considered one of the three major soil nutrients. Its indirect effects can be variable for inducing plant resistance, directly stimulating or inhibiting the penetration, multiplication, survival and establishment of a pathogen (Amtmann *et al.*, 2008). these results were agree with this study hence potassium disulphide was the best treatment in controlling root rot also it reducing the %occurrence frequency of isolated fungi this fertilizer gave results very close to the fungicides copper oxychloride and azoxystrobin.

Conclusion

using some organic acids *i.e.* Ascorbic acid, citric acid and salicylic acid and fertilizers nutrients *i.e.* potassium disulphide and copper sulphate in combined with protective chemical fungicide (copper oxychloride) gave the best treatment to control root rot in banana also reducing %the frequency occurrence of pathogen fungi. This strategy reducing the cost of chemical fungicides and was co-friendly with environment, also using protective fungicides avoiding the risks of residual effect of systemic fungicides in fruits.

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