Synthesis, Characterization and Antifungal Activity of Different Nanomaterials Against Phytopathogenic Fungi

Presented by

Shimaa Ahmed Zaki

A Thesis Submitted

To

Faculty of Science

In Partial Fulfillment of the Requirements for the Degree of PhD of Science (Microbiology)

Botany and Microbiology Department
Faculty of Science
Cairo University

(2021)

تصنيع وتوصيف بعض مضادات الفطريات النانوية واستخدامها ضد الفطريات الممرضة للنبات

إعداد

شيماء أحمد زكى

رسالة مقدمة

إلي

كلية العلوم

كجزء من متطلبات الحصول علي درجة الدكتوراه في فلسلفة العلوم (ميكروبيولوجى)

قسم النبات والميكروبيولوجي كلية العلوم جامعة القاهرة

(7.21)

CONTENTS

1.	Abstract	1
2.	Introduction	3
3.	Review of literature	6
3	3.1. cotton plant	
3	3.2. Phytopathogenic fungi causing damping-off of cotton plant	6
	3.2.1. Rhizoctonia solani	6
	3.2.1.1. Variability of Rizoctonia solani	7
	3.2.2. Fusarium	8
	3.2.3. Macrophomina phaseolina	10
3	3.3. Disease management	12
	3.3.1. Management via biocontrol agents	12
	3.3.2. Management through chemicals	14
	3.3.3. Integrated management	16
3	3.4. Nanotechnology	18
	3.4.1. Methods for nanoparticles synthesis	18
	3.4.1.1. chemical methods	18
	3.4.1.1.1. Wet impregnation	18
	3.4.1.1.2. co-precipitation method	19
	3.4.1.1.3. Precipitation-Deposition	19
	3.4.1.1.4. Microemulsions	19
	3.4.1.1.5. Photochemistry	20
	3.4.1.1.6. Chemical Vapour Deposition	20
	3.4.1.1.7. Electrochemical Reduction	20
	3.4.1.2. Physical methods	21
	3.4.1.2.1. Sonochemistry	21
	3.4.1.2.2. Microwave Irradiation	21
	3.4.1.2.3. Laser Ablation	21
	3.4.1.2.4. Supercritical fluids	22
	3.4.1.2.5. Plasma	22
	3.4.1.3. Biological synthesis	22
	3.4.1.3.1. Bacterial synthesis	22
	3.4.1.3.2. Fungal synthesis	23
	3.4.1.3.3. Plant-mediated synthesis	23
	3.4.2. Characterization of nanoparticles	24

3.4.2.1. Morphological characterizations	24
3.4.2.2. Structural characterizations	2 4
3.4.2.3. Particle size and surface area Characterization	25
3.4.2.4. Optical characterizations	25
3.4.3. Nanoparticles and phytopathogenic fungi	26
3.4.3. 1. Nanoparticles and Rhizoctonia solani	20
3.4. 3. 2. Nanoparticles and Fusarium spp	28
3.4.3. 3. Nanoparticles and Macrophomina phaseolina	29
3.4.4. Mode of action of nanoparticles against pathogens	31
3.4.4.1. Mode of Action of AgNPs Against Microorganisms	31
3.4.4.2. Mode of action of other NPs towards microorganisms	32
3.4.4. 3. Effect of Nanoparticles on DNA Damage	32
4. Materials and Methods	
4.1. Isolation, purification and identification of the isolated fungi	33
4.1.1. Preparation of fungal inocula	33
4.2. Pathogenicity test	33
4.3. Effect of <i>Trichoderma</i> isolates on (Rs9), (F10) and (M4)	34
4.3.1. Preparation of Trichoderma isolates culture	
4.3.2. In vitro antagonism of Trichoderma isolates against (Rs9), (F10) and (M4)	34
4.3.3. Efficiency of fungicides, Trichoderma isolates and their combinations in co	
damping off of cotton seedlings under greenhouse conditions	35
4.3.3.1. Chemical fungicides and Trichoderma isolates used to control the pathogen	nic fungi
	35
4.3.3.2. Evaluation of sensitivity of <i>Trichoderma</i> isolates to the chemical fungicides	35
4.3.3.3. Efficiency of fungicides, Trichoderma isolates and their combinations in co	ntrolling
damping off of cotton seedlings under greenhouse conditions	36
4.4. Effect of <i>Trichoderma</i> isolates on germination of cotton seeds	37
4.4.1. In vitro effect of culture filtrates of Trichoderma isolates on germination of cot	ton seeds
and elongation of radical	37
4.5. synthesis of nanoparticles by <i>Trichoderma</i> isolates and their bio-efficacy evaluation	n against
R. solani (RS9), Fusarium sp. (F10) and M. phaseolina (M4)	38
4.5.1. Preparation of cultural extract	38
4.5.2. 4.5.2. Synthesis of silver nanoparticles	
4.5.3. 4.5.3. Synthesis of zinc oxide nanoparticles	
4.5.4. Preparation of ZnO NPs and ZnO-Chitosan nanocomposites	
4.5.5. 4.5.5.Characterization of nanoparticles	
4.5.5.1. Ultraviolet-visible spectrophotometer analysis	

4.5.5.	2. Transmission electron microscopy (TEM)	40
4.5.5.	.3. X-ray diffraction (XRD)	40
4.5.5.	.4. Dynamic Light Scattering (DLS)	40
4.5.5.	.5. Zeta potential	41
4.5.5.	.6. Fourier Transform Infrared Spectroscopy (FTIR)	41
4.5.5.	7. Scanning electron microscope (SEM)	41
4.5.5.	.8. Energy dispersive X-ray (EDX) spectroscopy	41
4.5.6.	Antifungal activity of synthesized nanoparticles	41
4.5.6.	.1. In vitro antifungal activity of synthesized nanoparticles	41
4.5.6.	2. Antifungal activity of synthesized nanoparticles under greenhouse conditions	42
4.6. Statis	stical analysis	43
5. Experi	mental results	44
5.1. Isola	tion, purification and identification of the isolated fungi	44
5.2. Path	ogenicity test	44
5.3. Effec	et of Trichoderma isolates on (Rs9), (F10) and (M4)	52
5.3.1.	In vitro antagonism of Trichoderma isolates against (Rs9), (F10) and (M4)	52
5.3.2.	Efficiency of fungicides, Trichoderma isolates and their combinations in cont	rolling
	damping off of cotton seedlings under greenhouse conditions	55
5.3.2	1. Evaluation of sensitivity of <i>Trichoderma</i> isolates to the chemical fungicides	55
5.3.2	2. Efficiency of fungicides, Trichoderma isolates and their combinations in cont	rolling
	damping off of cotton seedlings under greenhouse conditions	58
5.4. Effec	ct of <i>Trichoderma</i> isolates on germination of cotton seeds	68
5.4.1.	In vitro effect of culture filtrates of Trichoderma isolates on germination of cotto	n seeds
	and elongation of radical	68
5.4.2.	Effect of selected Trichoderma isolates on germination of cotton seeds and gro	wth of
	seedlings under greenhouse conditions	71
5.5. Silve	r nanoparticles	73
5.5.1.	Characterization of synthesized silver nanoparticles	73
5.5.1.	.1. UV-Visible Spectral Analysis of AgNPs	- 73
5.5.1.	2. Dynamic light scattering (DLS) of AgNPs	74
5.5.1.	.3. Zeta Potential analysis of AgNPs	74
5.5.1.	4. Transmission Electron Microscope analysis(TEM) of AgNPs	75
5.5.1.	.5. Scanning Electron Microscope analysis(SEM) of AgNPs	75
5.5.1.	.6. Energy Dispersive X-Ray Diffractive (EDX) analysis of AgNPs	76
5.5.2.	In vitro antifungal activity of synthesized silver nanoparticles against R. solani	(RS9),
	Fusarium (F10) and M. phaseolina (M4)	77

5.5.3. Effect of silver nanoparticles against damping-	off disease caused by F10, Rs9, and M4
under greenhouse conditions	
5.6. Zinc oxide nanoparticles	83
5.6.1. Characterization of synthesized ZnO nanopart	icles 83
5.6.1.1. UV-Visible Spectral Analysis of ZnONPs	83
5.6.1.2. X-Ray Diffraction (XRD) Analysis of ZnONF	's 83
5.6.1.3. Zeta Potential analysis of ZnONPs	84
5.6.1.4. Transmission Electron Microscope analysis(7	TEM) of ZnONPs 84
5.6.1.5. Scanning Electron Microscope analysis(SEM) of ZnONPs 85
5.6.1.6. Energy Dispersive X-Ray Diffractive (EDX)	analysis of ZnONPs 86
5.6.1.7. Fourier Transforms Infrared Spectrosco	oy (FTIR) Analysis of ZnONPs
	86
5.6.2. In vitro antifungal activity of synthesized Zi	ONPs nanoparticles against R. solani
(RS9), Fusarium (F10) and M. phaseolina (M4)	87
5.6.3. Effect of ZnONPs nanoparticles against damp	ing-off disease caused by F10, Rs9, and
M4 under greenhouse conditions	88
5.7. ZnO-chitosan nanocomposites	
5.7.1. Characterization of synthesized ZnO-chitosan	nanocomposites 93
5.7.1.1. UV-Visible Spectral Analysis of ZnO-chitosa	n nanocomposites 93
5.7.1.2. X-Ray Diffraction (XRD) Analysis of ZnO-ch	itosan nanocomposites 93
5.7.1.3. Transmission Electron Microscope a nanocomposites	nnalysis (TEM) of ZnO-chitosan
5.7.1.4. Scanning Electron Microscope analysis of Zn	O-chitosan nanocomposites 95
5.7.1.5. Energy Dispersive X-Ray Diffractive nanocomposites	•
5.7.2. In vitro antifungal activity of synthesized Z	no-chitosan nanocomposite against R .
solani (RS9), Fusarium (F10) and M. phaseolina	<i>i</i> (M4) 96
5.7.3. Effect of Zno-chitosan nanocomposite against of	lamping-off disease caused by F10, Rs9,
and M4 under greenhouse conditions	
6. Discussion	
7. Summary	
8. Conclusion	
0. Defenences	122

List of Tables

Table 1. Common and trade names, formulations, rates of applications of fungicides and a
biocide used in the present study 35
Table2. Fungicides used in evaluating sensitivity of <i>Trichoderma</i> isolates to chemical fungicides 36
Table3. Fungicides, Trichoderma isolates and their combinations in controlling damping off of
cotton seedlings under greenhouse conditions 37
Table4. Fungicides and synthesized nanoparticles in controlling damping off of cotton seedlings under greenhouse conditions
Table5. isolation frequency of fungi isolated from cotton seedlings showing typical damping-off
symptoms growing on soil samples collected from Giza governorate 44
Table6. Analysis of variance of effect of some fungal isolates, cultivars, and their interaction on some growth variables of cotton seedlings grown under greenhouse conditions 45
Table 7. Effect of some fungal isolates, cultivars, and their interaction on Pre-emergence damping-
off percentage of cotton seedlings grown under greenhouse conditions 47
Table8. Effect of some fungal isolates, cultivars, and their interaction on Post-emergence
damping-off percentage of cotton seedlings grown under greenhouse conditions 48
Table9. Effect of some fungal isolates, cultivars, and their interaction survival percentage of
cotton seedlings grown under greenhouse conditions 49
Table10. Analysis of variance of effect of the most pathogenic fungal isolates, cultivars, and their
interaction on plant height and dry eight of cotton seedlings grown under greenhouse
conditions 50
Table11. Effect of the most pathogenic fungal isolates, cultivars, and their interaction on plant
height and dry weight of cotton seedlings grown under greenhouse conditions 51
Table 12. Analysis of variance of effect of some Trichoderma isolates, fungal pathogen
(F10,RS9 and M4), and their interaction on linear growth of fungal pathogen
52
Table13. Effect of some Trichoderma isolates, fungal pathogen (F10,RS9 and M4), and their
interaction on linear growth of fungal pathogen 53
Table14. Analysis of variance of effect of some fungicides, concentrations, and their interaction on
linear growth some <i>Trichoderma</i> isolates (T ₂₈ , T ₃₄ and T _{vivi}) 55
Table15. Effect of some fungicides, concentrations, and their interaction on linear growth some
Trichoderma isolates (T_{28}) 56
Table16. Effect of some fungicides, concentrations, and their interaction on linear growth some
<i>Trichoderma</i> isolates (T ₃₄) 57

Table 17. Effect of some fungicides, concentrations, and their interaction on linear growth some
Trichoderma isolates (T _{vivi}) 57
Table 18. Analysis of variance of effect of some treatments, cultivars, and their interaction on some
growth variables of cotton seedlings grown in soil infested with Fusarium sp. (F10)
under greenhouse conditions 58
Table19. Effect of some treatments, cultivars, and their interaction on survival percentage of
cotton seedlings grown in soil infested with Fusarium (F10) under greenhouse
conditions 59
Table 20. Effect of some treatments, and cultivars on plant height of cotton seedlings cultivated in
infested soil with Fusarium (F10) under greenhouse conditions 60
Table21. Effect of some treatments, cultivars, and their interaction on dry weight of cotton
seedlings grown in soil infested with Fusarium (F10) under greenhouse conditions 60
Table 22. Analysis of variance of effect of some treatments, cultivars, and their interaction on some
growth variables of cotton seedlings grown in soil infested with R. solani (Rs9) under
greenhouse conditions 61
Table23. Effect of some treatments, cultivars, and their interaction on survival percentage of
cotton seedlings grown in soil infested with R. solani (Rs9)under greenhouse conditions
62
Table24. Effect of some treatments, and cultivars on plant height of cotton seedlings cultivated in
infested soil with R. solani (Rs9) under greenhouse conditions 63
Table 25. Effect of some treatments, and cultivars on dry weight of cotton seedlings cultivated in
infested soil with R. solani (Rs9) under greenhouse conditions 64
Table 26. Analysis of variance of effect of some treatments, cultivars, and their interaction on
some growth variables of cotton seedlings grown in soil infested with M. phaseolina
(M4) under greenhouse conditions 65
Table 27. Effect of some treatments, and cultivars on survival percentage of cotton seedling
cultivated in infested soil with M. phaseolina (M4) under greenhouse conditions 6
Table 28. Effect of some treatments, and cultivars on plant height of cotton seedlings cultivated in
infested soil with M. phaseolina (M4) under greenhouse conditions 6'
Table 29. Effect of some treatments, and cultivars on dry weight of cotton seedlings cultivated in
infested soil with M. phaseolina (M4) under greenhouse conditions 68
Table 30. Analysis of variance of effect of culture filtrates of Trichoderma isolates, cotton
cultivars, and their interaction on some growth variables of cotton seedlings grown in
vitro 69
Table 31. Effect of culture filtrates of <i>Trichoderma</i> isolates, cultivars and their interaction on
survival percentage of cotton seeds and radical length in vitro 70

Table 32. Analysis of variance of effect of some <i>Trichoderma</i> isolates, cultivars, and their
interaction on some growth variables of cotton seedlings grown under greenhouse
conditions 71
Table 33. Effect of some <i>Trichoderma</i> isolates, cultivars, and their interaction on survival
percentage of cotton seedlings grown under greenhouse conditions 72
Table 34. Effect of some <i>Trichoderma</i> isolates, cultivars, and their interaction on plant height of
cotton seedlings grown under greenhouse conditions 72
Table 35. Effect of some <i>Trichoderma</i> isolates, cultivars, and their interaction on dry weight of
cotton seedlings grown under greenhouse conditions 73
Table 36. Analysis of variance of the antifungal effects of different concentrations of silver NPs
and against the linear growth of Fusarium sp.(F10), R. solani (Rs9) and M.
phaseolina(M4) 77
Table 37. Antifungal effects of different concentrations of silver NPs and against the linear growth
of Fusarium sp.(F10), R. solani (Rs9) and M. phaseolina(M4) 78
Table 38. Analysis of variance of effect of some fungi, treatments, and their interaction on some
growth variables of cotton seedlings of Giza90 grown in infested soil under greenhouse
conditions 78
Table 39. Effect of some fungi, treatments, and their interaction on survival percentage of cotton
seedlings of Giza90 grown in infested soil under greenhouse conditions 79
Table 40. Effect of some fungi, treatments, and their interaction on plant height of cotton
seedlings of Giza90 grown in infested soil under greenhouse conditions 80
Table 41. Effect of some fungi, treatments, and their interaction on dry weight of cotton seedlings
of Giza90 grown in infested soil under greenhouse conditions
Table 42. Analysis of variance of effect of some fungi, treatments, and their interaction on some
growth variables of cotton seedlings of Giza94 grown in infested soil under greenhouse
conditions 80
Table 43. Effect of some fungi, treatments, and their interaction on survival percentage of cotton
seedlings of Giza94 grown in soil infested under greenhouse conditions
Table 44. Effect of some fungi, treatments, and their interaction on plant height of cotton
seedlings of Giza94 grown in soil infested under greenhouse conditions
Table 45. Effect of some fungi, treatments, and their interaction on dry weight of cotton seedlings
of Giza94 grown in infested soil under greenhouse conditions
Table 46. Functional Group present in the Trichogenic ZnONPs analyzed by FTIR 87
Table 47. Analysis of variance of effect of some fungi, treatments, and their interaction on some
growth variables of cotton seedlings of Giza90 grown in soil infested under greenhouse
conditions 88

Table 48. Effect	of some fungi, treatments, and their interaction on survival percentage of cotton
seedli	ngs of Giza90 grown in soil infested under greenhouse conditions 89
Table 49. Effec	et of some fungi, treatments, and their interaction on plant height of cotton
seedli	ngs of Giza90 grown in soil infested under greenhouse conditions 89
Table 50. Effect	of some fungi, treatments, and their interaction on dry weight of cotton seedlings
of Giz	za90 grown in soil infested under greenhouse conditions 90
Table 51. Analy	sis of variance of effect of some fungi, treatments, and their interaction on some
growt	th variables of cotton seedlings of Giza94 grown in soil infested under greenhouse
condi	tions 90
Table 52. Effect	of some fungi, treatments, and their interaction on survival percentage of cotton
seedli	ngs of Giza94 grown in soil infested under greenhouse conditions 91
Table 53. Effec	et of some fungi, treatments, and their interaction on plant height of cotton
seedli	ngs of Giza94 grown in soil infested under greenhouse conditions 91
Table 54. Effect	of some fungi, treatments, and their interaction on dry weight of cotton seedlings
of Giz	za94 grown in soil infested under greenhouse conditions 92
Table 55. Analy	sis of variance of effect of some fungi, treatments, and their interaction on some
growt	th variables of cotton seedlings of Giza90 grown in soil infested under greenhouse
condi	tions 97
Table 56. Effect	of some fungi, treatments, and their interaction on survival percentage of cotton
seedli	ngs of Giza90 grown in infested soil under greenhouse conditions 97
Table 57. Effec	t of some fungi, treatments, and their interaction on plant height of cotton
seedli	ngs of Giza90 grown in infested soil under greenhouse conditions 98
Table 58. Effect	of some fungi, treatments, and their interaction on dry weight of cotton seedlings
of Giz	za90 grown in infested soil under greenhouse conditions 98
Table 59. Analy	sis of variance of effect of some fungi, treatments, and their interaction on some
growt	th variables of cotton seedlings of Giza94 grown in infested soil under greenhouse
condi	tions 99
Table 60. Effect	of some fungi, treatments, and their interaction on survival percentage of cotton
seedli	ngs of Giza94 grown in infested soil under greenhouse conditions 99
Table 61. Effec	t of some fungi, treatments, and their interaction on plant height of cotton
seedli	ngs of Giza94 grown in infested soil under greenhouse conditions 100
Table 62. Effect	of some fungi, treatments, and their interaction on dry weight of cotton seedlings
of Giz	za94 grown in infested soil under greenhouse conditions 100

List of Figures

Figure 1. Effect of some Trichoderma isolates on linear growth of F10 53
Figure 2. Effect of some <i>Trichoderma</i> isolates on linear growth of Rs9 54
Figure 3. Effect of some <i>Trichoderma</i> isolates on linear growth of M4 54
Figure 4. Effect of culture filtrates of <i>Trichoderma</i> isolates on survival percentage of Giza 90 seeds
and radical length in vitro 70
$ \textbf{Figure 5. Effect of culture filtrates of } \textbf{\textit{Trichoderma}} \ \ \textbf{isolates on survival percentage of Giza 94 seeds} $
and radical length in vitro 71
Figure 6. UV-Vis spectrum of AgNPs produced by Tvivi, T34 and T28 after 2, 4 and 7 days after
synthesis 74
Figure 7. (7A). Nanoarticle size distribution of AgNPs solution synthesized by Tvivi, (7B) Zeta
potential analysis of synthesized AgNPs and the potential value was found to be -25.1
mV 74
Figure8. Transmission electron microscopy (TEM) image of synthesized ZnO-NPs; the inset
shows the corresponding particle size distribution and shape on the left , and selected
area electron diffraction(SAED) pattern of the sample on the right 75
Figure 9. Scanning electron microscope micrographs at different magnifications, 10, 5, 1 μm and
500 nm) of AgNPs 76
Figure 10. (A). EDX spectrum and elemental analysis of EDX spectrum of synthesized silver
nanoparticles.(B).Screened area for EDX spectrum of synthesized silver nanoparticles -
76
Figure 11. view of synthesized AgNPs on the left. Culture matt of fungus Trichoderma in liquid
media(1) T28, (2)Tvivi, and (3)T34 on the right 77
Figure 12. In vitro effect of silver nanoparticles against damping-off disease caused by F10(A),
Rs9(B), and M4(C) 78
Figure 13. Cotton Seedlings cultivars Giza 90 and Giza 94 obtained by sowing uncoated cotton
seeds in sterilized soil infested with three fungal pathogens including, Fusarium, R.
solani, and M. phaseolina as a negative control, uncoated seeds sown in sterilized soil as
a positive control, treated seeds with two fungicides (Maxim XL, Moncut) sown in
infested soil, and coated seeds with AgNPs (100,200μg/mL) in infested soil 82
Figure 14. UV-Vis spectrum of Trichogenic ZnONPs produced by Tvivi, T34 and T28 after 2, 4
and 7 days after synthesis 83
Figure 15. (A) Zeta potential analysis of synthesized ZnONPs. Trichoderma-mediated ZnONPs
were spherical and rod-shaped and the potential value was found to be $-24.0 mV$. (B) X-
ray diffraction pattern of ZnONPs. All peaks reveal the purity and crystalline nature.
No traces of other impurity phases were detected 84

Figure 16. (A) Transmission electron microscopy (TEM) image of synthesized ZnO-NPs	
shows the corresponding particle size distribution and shape. (B): Selection	
Electron Diffraction (SAED) of ZnONPs	
Figure 17. Scanning electron microscope micrographs at different magnifications, 10, 5,	•
500 nm) of ZnONPs	85
Figure 18. Elemental and energy dispersive X-ray spectroscopic analysis of ZnONPs, and	d
Screened area for EDX spectrum of synthesized zinc oxide nanoparticles	86
Figure 19. The inhibitory effect of mycelia growth on F10(A), Rs9(B), M4(C)on potate	o dextrose
agar medium containing ZnONPs at concentrations: Control, (C1) 20, (C2	2) 40, and
(C3) 100 μg/ml during 7 days	87
Figure 20. Cotton Seedlings cultivars Giza 90 and Giza 94 obtained by sowing uncoa	ted cotton
seeds in sterilized soil infested with three fungal pathogens including, Fu	sarium, R.
solani and M. phaseolina as a negative control, uncoated seeds sowed in sterili	zed soil as
positive control, treated seeds with two fungicides (Maxim XL, Moncut)	sowed in
infested soil, and coated seeds with ZnONPs (100,200 μ g/ml) in infested soil.	Photo was
taken after 45 days under standard growth conditions in greenhouse conditio	ns
	92
Figure 21. UV-Vis spectrum of synthesized ZnO-chitosan nanocomposites after 2, and 4	days after
synthesis	93
Figure 22. XRD pattern of synthesized ZnO-chitosan nanocomposites	93
Figure23.Transmission electron microscope images of synthesized ZnO-chitosan nanoc	
	94
Figure 24. (A) Selected Area Electron Diffraction(SAED) pattern of the sample	, and (B)
Histogram for calculating average particle size for ZnO-chitosan nanocompos	sites94
Figure 26. EDX spectrum and screened area for EDX spectrum of synthesized zinc ZnO)-chitosan
nanocomposites	95
Figure 27. Elemental analysis of EDX spectrum of ZnO-chitosan nanocomposites	96
Figure 28. The inhibitory effect of mycelia growth on F10(A), M4 (B), Rs9 (C)on potat	
agar medium containing Zno-chitosan NPs at concentrations: Control, (C1) 2	
and (C3) 100μg/mL after 7days	
Figure 29. Cotton Seedlings cultivars Giza 90 and Giza 94 obtained by sowing uncoa	
seeds in sterilized soil infested with three fungal pathogens including, Fun	
solani and M. phaseolina as a negative control, uncoated seeds sowed in sterili	,
positive control, treated seeds with two fungicides (Maxim XL, Moncut)	
infested soil, and coated seeds with Z/cNPs (100,200μg/ml) in infested soil.	
taken after 45 days under standard growth conditions in greenhouse condition	

7-Summary

- 1. Samples of seedlings infected with damping-off or root rot of adult plants were obtained from different locations at Giza governorate. The samples yielded 25 fungal isolates. Isolates were identified as *Rhizoctonia solani* (44%), *Fusarium* spp. (44%) and *Macrophomena phasolina* (12%).
- 2. In pre-emergence stage of cotton seedlings, twenty four fungal isolates were pathogenic on Giza90, while all fungal isolates were pathogenic on Giza94 compared to the control. On Giza90, *Fusarium* isolate F10 (80.000%), *Rhizoctonia solani* RS9 (100.000%), *Macrophomena phasolina* M4, and M12 (34.000%) were the most pathogenic isolates, while on Giza 94, *Fusarium* isolate F10 (100.000%), *R. solani* RS9 (100.000%), *M. phasolina* M4 (64.000%) were the most pathogenic isolates.
- 3. In post-emergence stage of Giza 90, *Fusarium* isolate F1 (22.000%), *R. solani* RS11 (18.000%), *M. phasolina* M4, and M12 (48.000%) were the most pathogenic isolates, while on Giza 94, *Fusarium* isolate F1 (16.000%), *R. solani* RS4 (14.000%), and *M. phasolina* M4 (24.000%) were the most pathogenic isolates.
- 4. All fungal isolates were pathogenic and decreased survival percentage on the two cultivars. On Giza 90, *Fusarium* isolate F10 (00.000%), *R. solani* RS9 (00.000%), and *M. phasolina* M4 (18.000%) were the most effective isolates in decreasing survival, while on Giza 94, *Fusarium* isolate F10 (00.000%), *R. solani* RS9 (00.000%) group, and *M. phasolina* M4 (16.000%) were the most effective isolates in decreasing survival.
- 5. The most effective fungal isolates that decreased plant height and dry weight for both cultivars were F10, RS9 and M4.
- 6. All tested *Trichoderma* isolates were effective and caused inhibition of linear growth of all pathogenic fungal isolates F10, Rs9, and M4 under in vitro conditions. *Trichoderma* T_{vivi} was the most effective isolate against F10 as it inhibited growth by 85.9%, while against (Rs9), *Trichoderma* T₂₈ was the most effective one as it inhibited growth by 52.6%. Against *M. phasolina* (M4), *Trichoderma* T₃₄ was the most effective one as it inhibited growth by 70.46%.
- 7. All concentrations of Maxim XL were effective in decreasing the linear growth of *Trichoderma* T₂₈ in vitro compared to the control, and concentration 25% was the least effective one in decreasing the linear growth as it decreased linear growth to 4.917cm compared to control (9 cm). All concentrations of Moncut were effective in decreasing the linear growth of *Trichoderma* T₂₈ compared to the control, and concentration 50% was the

- least effective one in decreasing the linear growth as it decreased linear growth to 2.917cm compared to control (9 cm). On Eleven, all concentrations were equally effective in decreasing the linear growth of *Trichoderma* T₂₈ compared to the control, and decreased the linear growth to 1cm compared to control (9 cm).
- 8. All concentrations of Maxim XL were effective in decreasing the linear growth of *Trichoderma* T₃₄ in vitro compared to the control, and concentration 25% was the least effective one in decreasing the linear growth as it decreased linear growth to 3.750cm compared to control (9 cm). on Moncut, all concentrations were effective in decreasing the linear growth of *Trichoderma* T₃₄ compared to the control, and concentration 25% was the least effective one in decreasing the linear growth as it decreased linear growth to 7.667cm compared to control (9cm). On Eleven, all concentrations were effective in decreasing the linear growth of *Trichoderma* T₃₄ compared to the control, and concentration 25% was the least effective one in decreasing the linear growth as it decreased linear growth to 2.000cm compared to control (9 cm).
- 9. All concentrations of Maxim XL were effective in decreasing the linear growth of *Trichoderma* T_{vivi} in vitro compared to the control, and concentration 25% was the least effective one in decreasing the linear growth as it decreased linear growth to 5.250 cm compared to control(9cm). On Moncut, all concentrations were equally effective in decreasing the linear growth of *Trichoderma* T_{vivi} compared to the control, and concentration 50% was the least effective one in decreasing the linear growth as it decreased linear growth to 6.333cm compared to control (9 cm). On Eleven, all concentrations were effective in decreasing the linear growth of *Trichoderma* T_{vivi} compared to the control, and decreased the linear growth to 1.000cm compared to control (9 cm).
- 10. Combinations of seed dressing fungicides and *Trichoderma* isolates were evaluated as to their effects on susceptibility of cotton cultivars to *F. fujikuroi*(F10) under greenhouse conditions. From the practical point of view, using of biocide *Trichoderma* T_{vivi} (Treatment 16) the best one for both cultivars and it did not include chemical fungicides. The maximum value of plant height for both cultivars was moncut(0.5g) + T34 (7g) and represented by treatment 11 (20.174cm). The maximum value of dry weight for both cultivars was moncut(0.5 g)+T_{vivi}(7g) and represented by treatment 10 (1.822 g).
- 11. As to *R. solani* (RS9), using of biocide *Trichoderma* T28 (Treatment 18) was a good one for both cultivars as it did not include chemical fungicides, and the difference between it and the best treatment, which include chemical fungicide (Treatment13) was non-significant. The

- maximum value of plant height for both cultivars was Maxim xl (0.5 ml) and represented by treatment 14 (21.670cm). The maximum value of dry weight for both cultivars was Maxim xl(0.5ml)+T28(7g) and represented by treatment 15(1.897g).
- 12. For *M. phasolina* (M4) using of biocide *Trichoderma* T34 (Treatment 17), which increased was a good one for both cultivars. as it did not include chemical fungicides. The maximum efficiency for plant height in controlling the disease was T_{vivi}(7g) and represented by treatment 16(22.737cm). The maximum efficiency for dry weight in controlling the disease was T34 (7g) and represented by treatment 17 (2.632g).
- 13. The effect of culture filtrates of *Trichoderma* isolates on survival and radical length of cotton cultivars in vitro was evaluated. Most of *Trichoderma* isolates were pathogenic or highly pathogenic on both cotton cultivars, five isolates were non pathogenic and did not show significant difference from control on Giza90. Only *Trichoderma* T_{vivi} increased survival percentage significantly on Giza94. Eleven *Trichoderma* isolates decreased radical length significantly.
- 14. Under greenhouse conditions, the effect of the non pathogenic five isolates in vitro on Giza90 was tested and *Trichoderma* T₄₇ increased the survival to 93.334% followed by T₂ which increased survival to 91.667% on both cultivars. The most effective *Trichoderma* isolates on plant height was *Trichoderma* T₄₇. The effect of *Trichoderma* isolates in vitro and under greenhouse conditions were not the same and the response of the cotton cultivars was also different.
- 15. AgNPs was biosynthesized from *Trichoderma* extract of Tvivi, T34 and T28 by green synthesis using *Trichoderma* without using any harmful reducing agents such as sodium borohydride and any other capping or dispersing agent.. Characterization of the synthesized nanoparticles by Tvivi, operated via UV-Visible Spectral, Dynamic light scattering (DLS), Zeta Potential analysis, Scanning electron microscopy (SEM), Transmission electron microscopy (TEM) and Energy dispersive x-ray (EDX) revealed that the particles were AgNPs with a spherical shaped particles. The nanoparticles were individuals and agglomerated in clusters. Particle size given by DLS was at average 52.34 nm and 0.559 PdI value. Particle size was within the range 6-15nm under TEM. and zeta potential of -25.1.mV.
- 16. All concentrations of AgNPs(20, 40, and 100 μg/mL) were effective in decreasing the linear growth of all fungi compared to the control, and concentration AgNPs100μg/mL was the most effective one in decreasing the linear growth as it decreased linear growth to 4.000, 2.250 and 4.167cm compared to control (9 cm) for F10, Rs9 and M4,respectively. On both

- cotton cultivars, all treatments were effective in controlling disease and increasing survival regardless of fungus, however, treatment AgNPs ($100\mu g/mL$) was the least effective treatment, under greenhouse conditions.
- 17. ZnO-NPs was synthesized from *Trichoderma* extract of Tvivi, T34 and T28, which has advantages such as inexpensive, simple work-up, costly and safe method. Characterization of the synthesized nanoparticles by Tvivi operated via UV-Visible Spectral, X-Ray Diffraction (XRD), Zeta Potential analysis, Scanning electron microscopy (SEM), Transmission electron microscopy (TEM), Fourier Transforms Infrared Spectroscopy (FTIR) Analysis and Energy dispersive x-ray (EDX) revealed that the particles were ZnO-NPs with a mixture of hexagonal, spherical, and rod shaped particles with crystalline structure. The nanoparticles were individuals and agglomerated in clusters. Particle size was within the range 8- 23nm and zeta potential of -24.0mV.
- 18. Three concentrations (20, 40, and 100 μg/ml) of the synthesized Zno nanoparticles were evaluated in vitro against *R. solani* (RS9), *F. fujikuroi*(F10) and *M. phasolina* (M4), the mycelial diameter was completely reduced by 100% in all tested concentrations for all tested fungi. All treatments were effective in increasing survival and controlling disease regardless of fungus on Giza90, however Zno NPs(100μg/ml) showed the least efficiency in controlling disease. All treatments were effective in increasing survival and controlling disease regardless of fungus on Giza94 except Zinc(100μg/ml) was ineffective in controlling disease. Moncut(2g) showed the maximum efficiency in controlling disease regardless of fungus (88.889% survival) followed by Maxim XL(2ml) and Zno NPs(200μg/ml).`
- 19. ZnO-Chitosan nanocomposites was synthesized and characterized by UV-Visible Spectral, X-Ray Diffraction (XRD), Scanning electron microscopy (SEM), Transmission electron microscopy (TEM) and Energy dispersive x-ray (EDX) revealed that the particles were ZnO-Chitosan nanocomposites with a mixture of hexagonal and spherical shaped particles with crystalline structure. The nanoparticles were individuals and agglomerated in clusters. Particle size was within the range 6-18nm.
- 20. Three concentrations (20, 40, and 100 μg/ml) of the synthesized nanocomposites were evaluated in vitro against *R. solani* (RS9), *F. fujikuroi* (F10) and *M. phasolina* (M4), the mycelial diameter was completely reduced by 100% in all tested concentrations for all tested fungi. On Giza90, all treatments were equally effective in increasing survival and controlling disease regardless of fungus. On Giza94, All treatments were effective in controlling the disease, treatment Z/C (100μg/ml) was the least effective treatment while other treatments were equally effective.