Molecular characterization of *Trichoderma* isolates as biological control agent

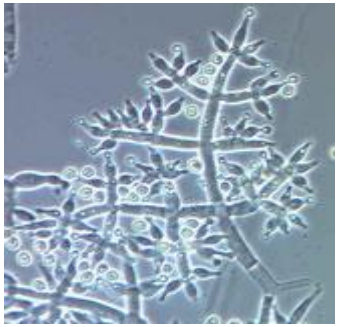
Khalid Abdel-lateif
Menoufia University, Egypt

Why we need biological control?

- Chemical pesticides
  - Implicated in environmental and human health problems
  - Require yearly treatments and expensive
  - Toxic to both beneficial and pathogenic species
- Biological control agents
  - Non-toxic to human and animal
  - Not polluted
  - Host specific
    - Only effect one or few species

Trichoderma spp.

- *Trichoderma spp.* are present in nearly all agricultural soils
- Agriculturally used as biocontrol agent and as a plant growth promoter
- *Trichoderma* species exhibited effective antagonism of different degrees against wide range of soilborne plant-pathogenic fungi such as *Pythium*, *Rhizoctonia*, *Fusarium* and *Sclerotina*



Mechanisms of *Trichoderma* as biological control agent

- Mycoparasitism

  ➡ ***Trichoderma*** **penetrates into pathogen mycelium by degradation of its cell wall**

- Antibiosis

  ➡ **By secretion of antibiotics**

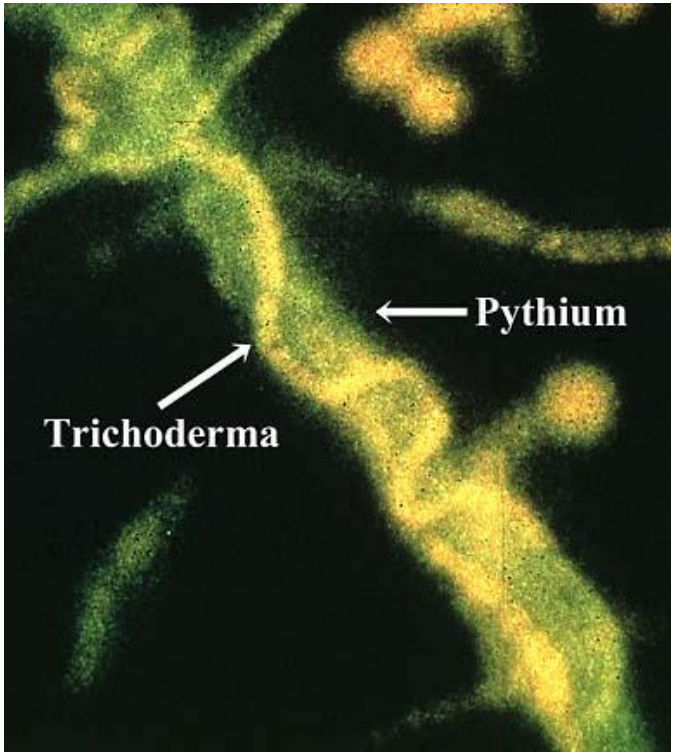
- Competition for nutrients or space

- Induced resistance

- Inactivation of the pathogen's enzymes

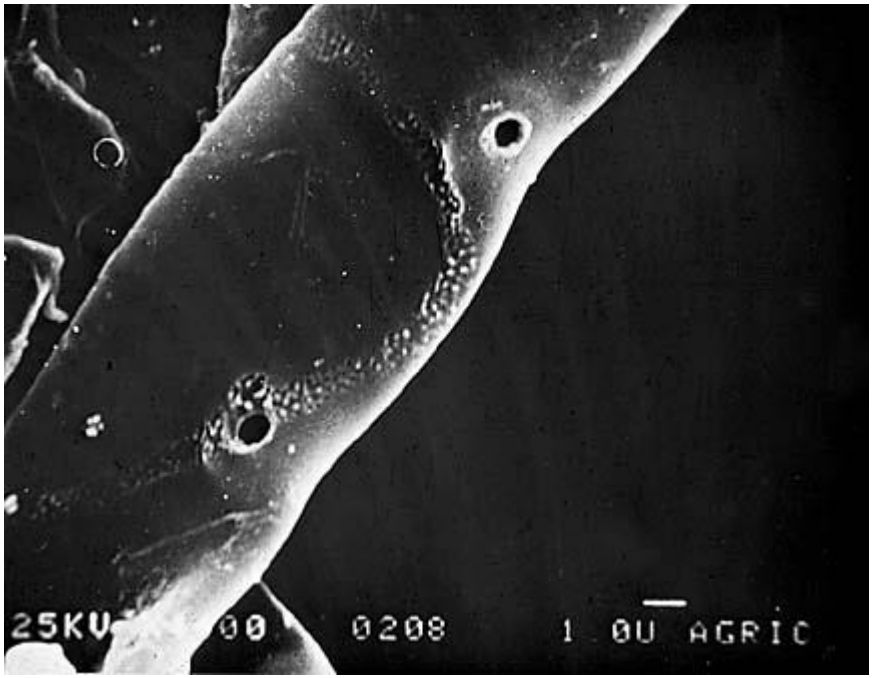
Action of *Trichoderma* against pathogenic fungi

1- Attachment to the host hyphae by coiling



(Hubbard et al., 1983. Phytopathology 73:655-659).

2- Penetrate the host cell walls by secreting lytic enzymes: Chitinases, Proteases, Cellulases



(Ilan Chet, Hebrew University of Jerusalem).

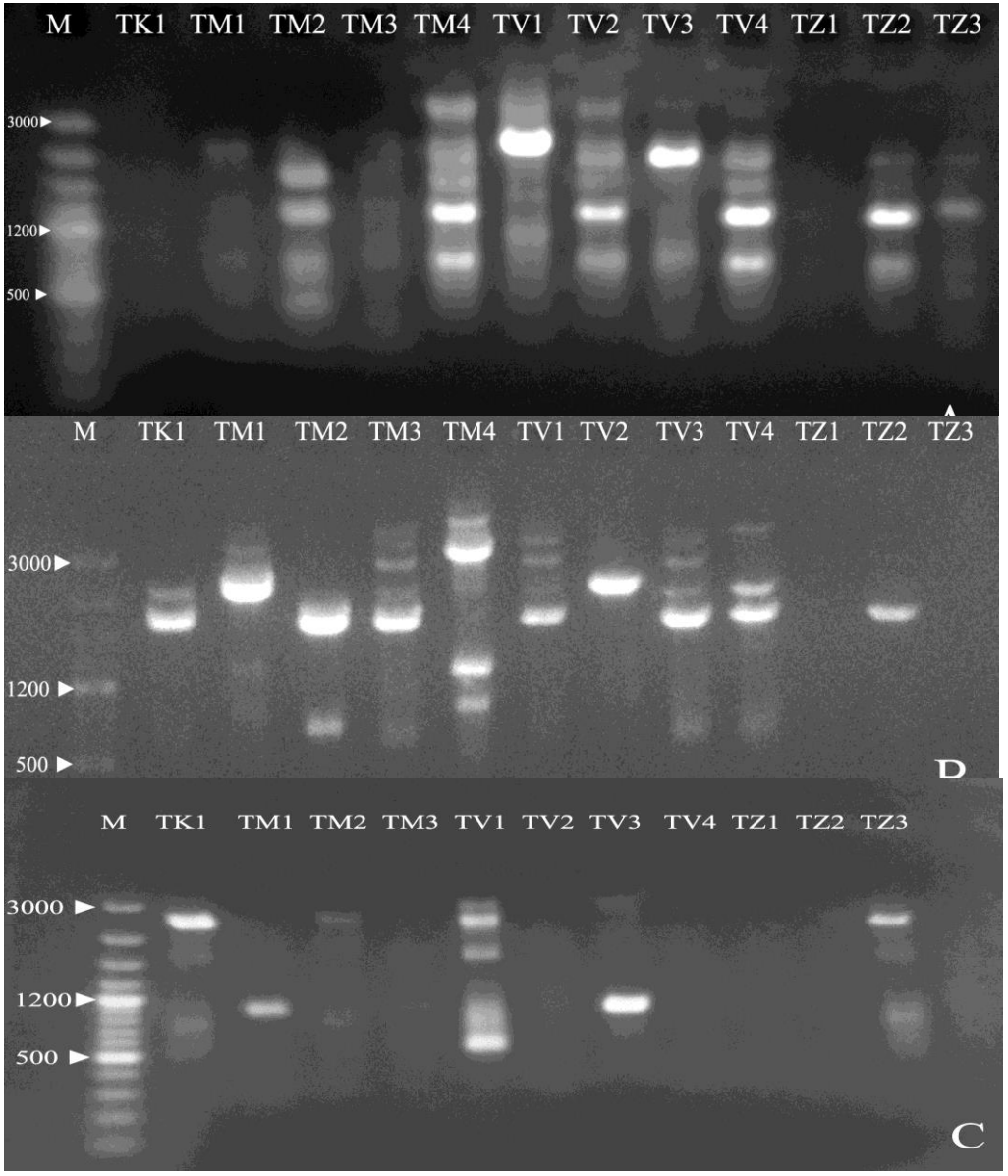
Isolation and identification of *Trichoderma* isolates

Trichoderma isolates	Code	Isolation source
T. koningii	TK	Ismailia governorate
T. hamatum1	TM1	Menoufia governorate
T. hamatum2	TM2	Menoufia governorate
T. hamatum3	TM3	Gharbia governorate
T. hamatum4	TM4	Sharkya governorate
T. viride 1	TV1	Gharbia governorate
T. viride 2	TV2	Kafer El-shikh governorate
T. viride 3	TV3	Sharkya governorate
T. viride 4	TV4	Menoufia governorate
T. harzianum 1	TZ1	Sharkya governorate
T. harzianum 2	TZ2	Ismailia governorate
T. harzianum 3	TZ3	Menoufia governorate

Isolation according to Elad *et al*, 1981
identification according to Barnett (1998) and Bissett (1991a,b,c)

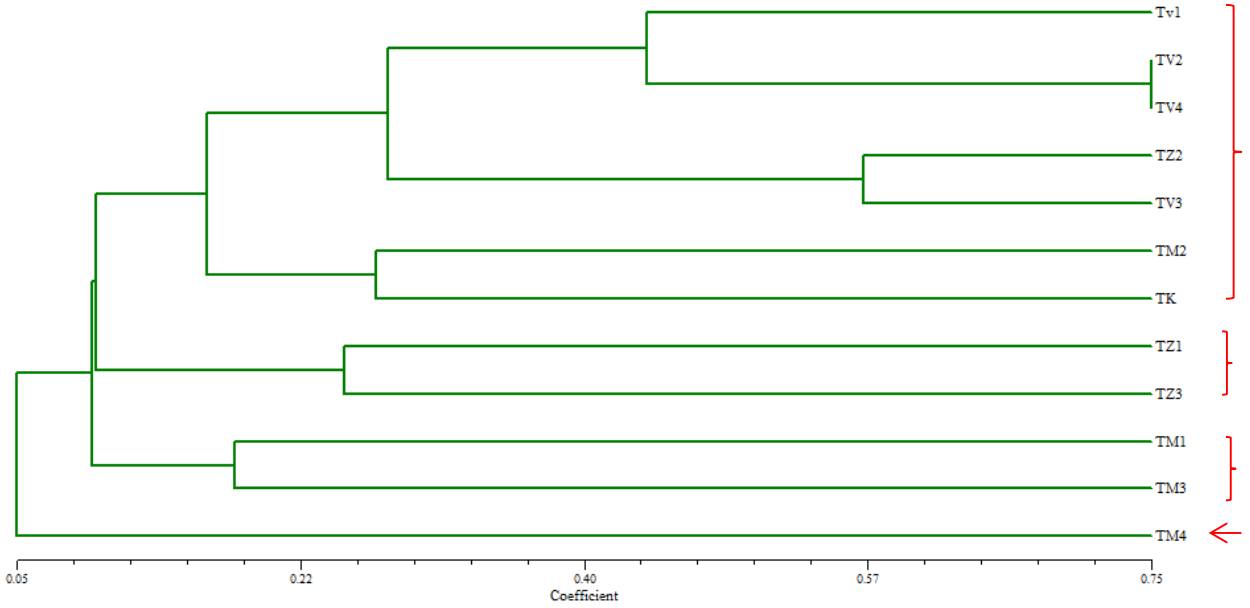
Genetic diversity based PCR-RAPD

Primer	Sequence
OPA-02	5′- TGCCGAGCTG-3′
OPB-07	5′- GGTGACGCAG-3′
OPB-08	5′- GTCCACACGG-3′
OPB-09	5′-TGGGGGACTC-3′
OPB-18	5′-CCACAGCAGT-3′
OPB-19	5′-ACCCCCGAAG-3′
OPG-04	5′-AGCGTGTCTG-3′
OPG-07	5′-GAACCTGCCC3-3′
OPE-04	5′- GTGACATGCC-3′
OPF-06	5′-GGGAATTCCC-3′



Isol	TK1	TM1	TM2	TM3	TM4	TV1	TV2	TV3	TV4	TZ1	TZ2	TZ3
TK	1.00											
TM1	0.08	1.00										
TM2	0.26	0.16	1.00									
TM3	0.12	0.18	0.17	1.00								
TM4	0.10	0.00	0.04	0.00	1.00							
TV1	0.09	0.18	0.08	0.09	0.00	1.00						
TV2	0.30	0.00	0.18	0.06	0.26	0.37	1.00					
TV3	0.11	0.10	0.19	0.14	0.12	0.11	0.50	1.00				
TV4	0.25	0.00	0.15	0. 07	0.14	0.50	0.75	0.30	1.00			
TZ1	0.06	0.10	0.24	0.23	0.00	0.11	0.07	0.27	0.08	1.00		
TZ2	0.08	0. 14	0.07	0.18	0.00	0.16	0.33	0.57	0.25	0.22	1.00	
TZ3	0.20	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.25	0.00	1.00

The similarity matrix



Evaluation of *Trichoderma* Antagonism against some soil porne pathogens

- ***Pythium aphanidermatum:***

  - Cellulose is the main cell wall component

  - a wide host range and cause damping off (kill seeds or seedlings before or after they germinate)

- ***Rhizoctonia solani:***

  - Chitin is the main cell wall component

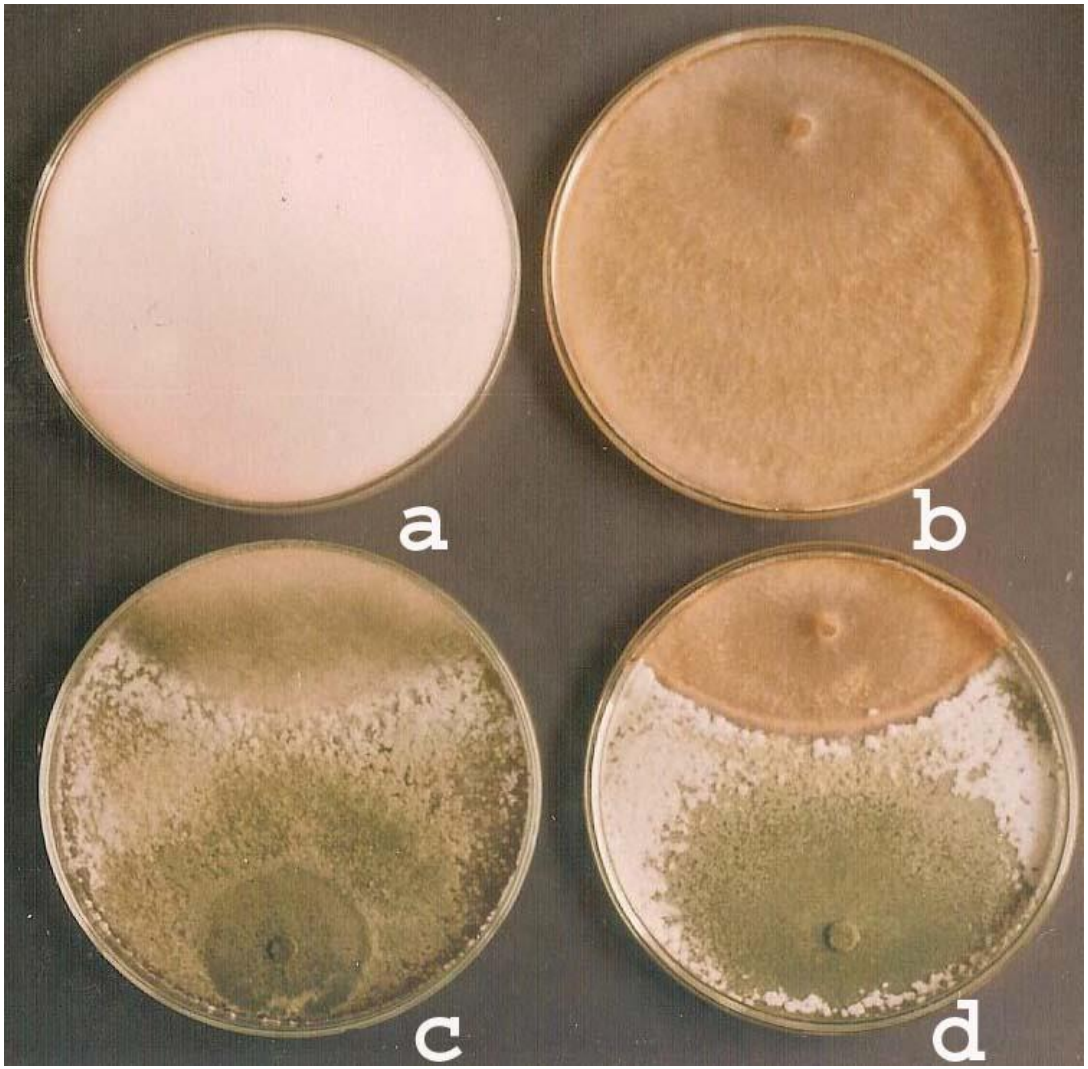
  - Cause various plant diseases such as collar rot and damping off .

Evaluation of *Trichoderma* Antagonism against *P. aphanidermatum* and *R. solani*

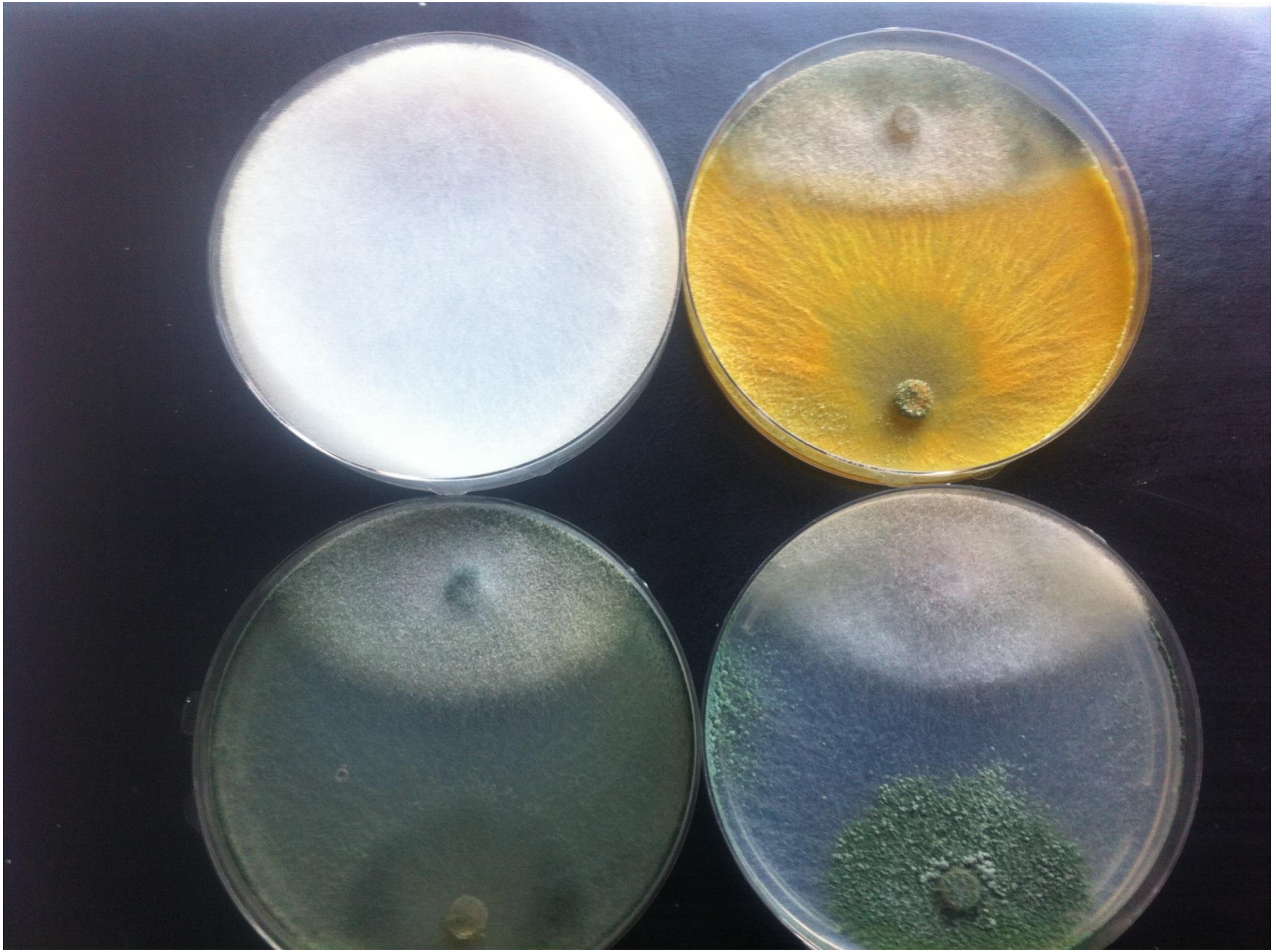
The antagonism of *Trichoderma* was evaluated by determined three criteria:

- Radial growth inhibition by comparing radial growth of *Trichoderma* against growth of pathogen (R1 - R2)/ R1 $\times$ 100
- Over growth ability was calculated as mycelial growth of *Trichoderma* over the pathogen
- Inhibition zone was measured by observation the clear zones formed between *Trichoderma* and pathogen as result of antibiotic secretion

Inhibition of *P. aphanidermatum* and *R. solani* growth in presence of *Trichoderma*



a; *Pythium aphanidermatum*, b; *Rhizoctonia Solani*, c; *Trichoderma* against *Pythium aphanidermatum*, *d; Trichoderma* against *Rhizoctonia Solani*



	Radial growth inhibition(%)		Over growth (mm)		Inhibition zone	
Isolates	*P. aphnidermatum*	*R. solani*	*P. aphnidermatum*	*R. solani*	*P. aphnidermatum*	*R. solani*
TK	$64.4^{d} \pm 1.1$	$60.7^{e} \pm 1.5$	$17.7^{bcd} \pm 1.5$	N	N	+
TM1	$71.4^{b} \pm 0.0$	$67.6^{abc} \pm 0.6$	$17.3^{cd} \pm 0.6$	N	N	+
TM2	$67.4^{c} \pm 1$	$64.7^{d} \pm 0.6$	$18^{bc} \pm 1$	N	N	+
TM3	$66.9^{c} \pm 1.3$	$55^{f} \pm 1$	$18^{bc} \pm 1.5$	N	N	+
TM4	$70.4^{b} \pm 1.7$	$70.4^{ab} \pm 1.7$	$20^{a} \pm 1$	N	N	+++
TV1	$71.2^{b} \pm 0.2$	$68.1^{abc} \pm 0.2$	$18^{bc} \pm 0.6$	N	N	++
TV2	$71.3^{b} \pm 0.2$	$69.3^{ab} \pm 0.6$	$18^{bc} \pm 0$	N	N	+++
TV3	$66.6^{c} \pm 0.7$	$66.^{3c} \pm 0.6$	$17^{cd} \pm 1.7$	N	N	+
TV4	$72.1^{b} \pm 1.8$	$69^{ab} \pm 1$	$18^{bc} \pm 0$	N	N	++
TZ1	$72.2^{b} \pm 1.8$	$67.2^{bc} \pm 0.8$	$19.3^{ab} \pm .2$	N	N	++
TZ2	$78^{a} \pm 1.5$	$73^{a} \pm 1$	$16^{d} \pm 2$	N	N	+++
TZ3	$72.3^{b} \pm 1.6$	$67.7^{abc} \pm 1.2$	$17.3^{cd} \pm 0.6$	N	N	++

- No over growth of *trichoderma* on *R. Solani*
  No inhibition zones were observed between *trichoderma* and *P. aphnidermatum*

Conclusion

- PCR-based RAPD techniques are useful tool to cluster, verify, and prove taxonomy morphology-based data
- The isolates of *Trichoderma harizianum* and *Trichoderma viride* were the most active against *R.Solani* and *P. aphnidermatum*

Future working

- *Trichoderma* contain a large number of genes which allow biocontrol to occur
- Trying cloning certain genes from *Trichoderma* and introduce them into plants to obtain crops resistant to plant diseases

THANK YOU