Role of Endophytic Fungi for Sustainable Agriculture

Sopialena

"Any organisms occurring within plant tissues"

Bary (1866)

"Mutualists, those fungi that colonize aerial parts of living plant tissues and don not cause symptoms of disease"

Carroll (1977)

"All organisms inhabiting plant organs tha at some time in their life, can colonize internal plant tissues without causing apparent harm to the host"

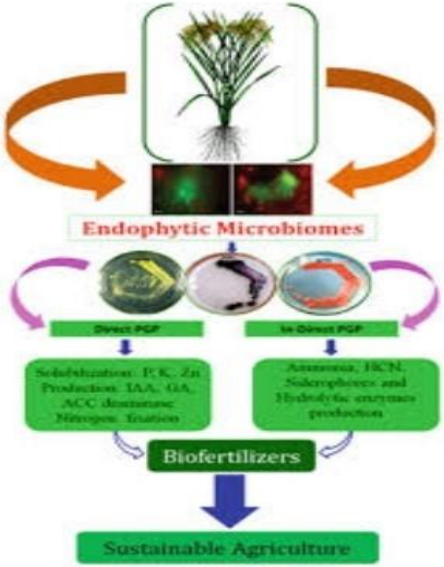
Petrini (1991)

"A group that colonize living, internal tissues of plants without causing any immediate, over negative effects"

Hirsch and Braun (1992)

"Fungi as colonizers of the living interna tissues of their plant host"

Rollinger and Langenheim (1993)



"Infection strategy is regarded as important in the definition of the term endophyte"

Wilson (1995)

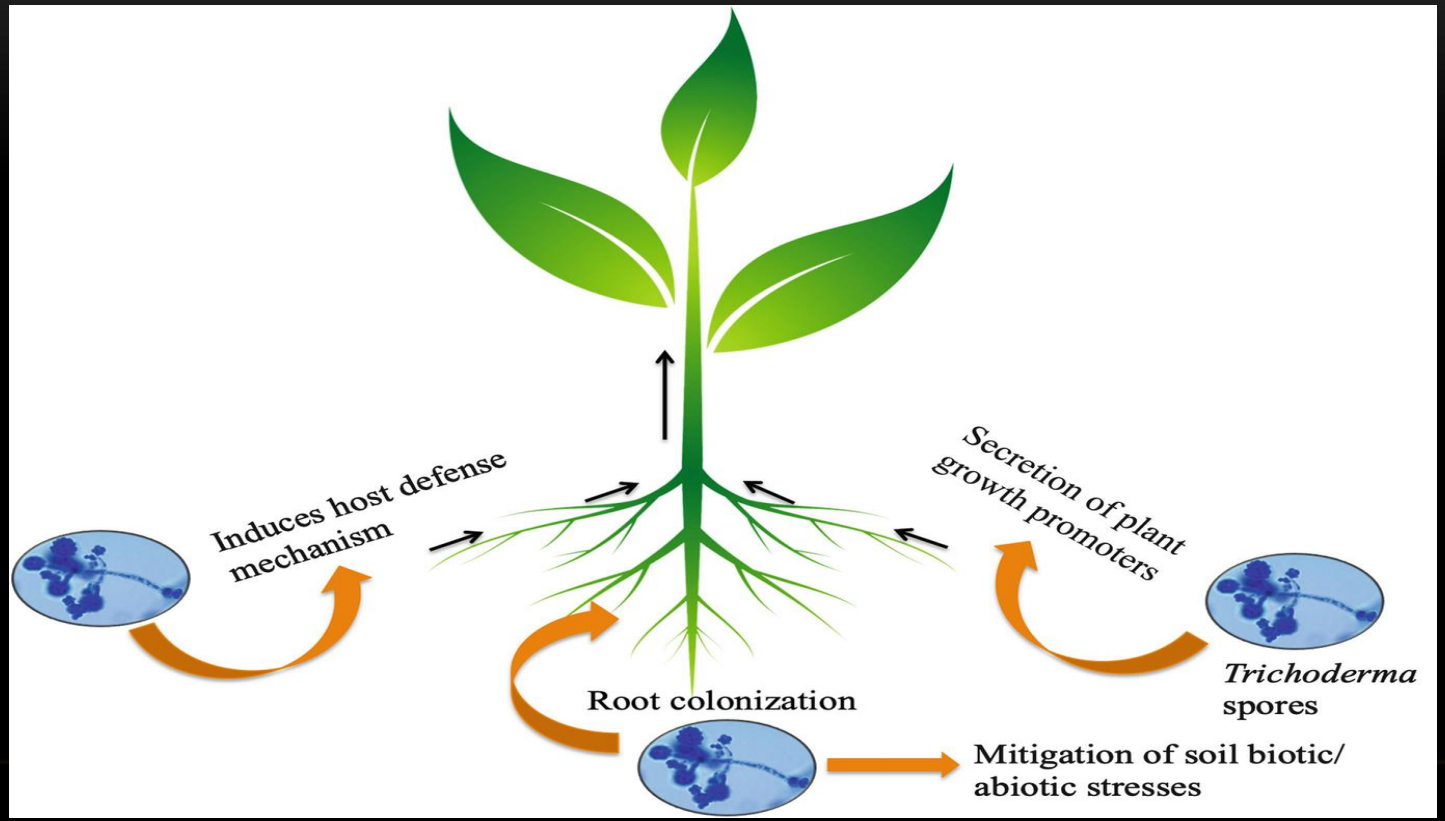
"True endophytes– fungi whose colonization never results in visible disease symptoms"

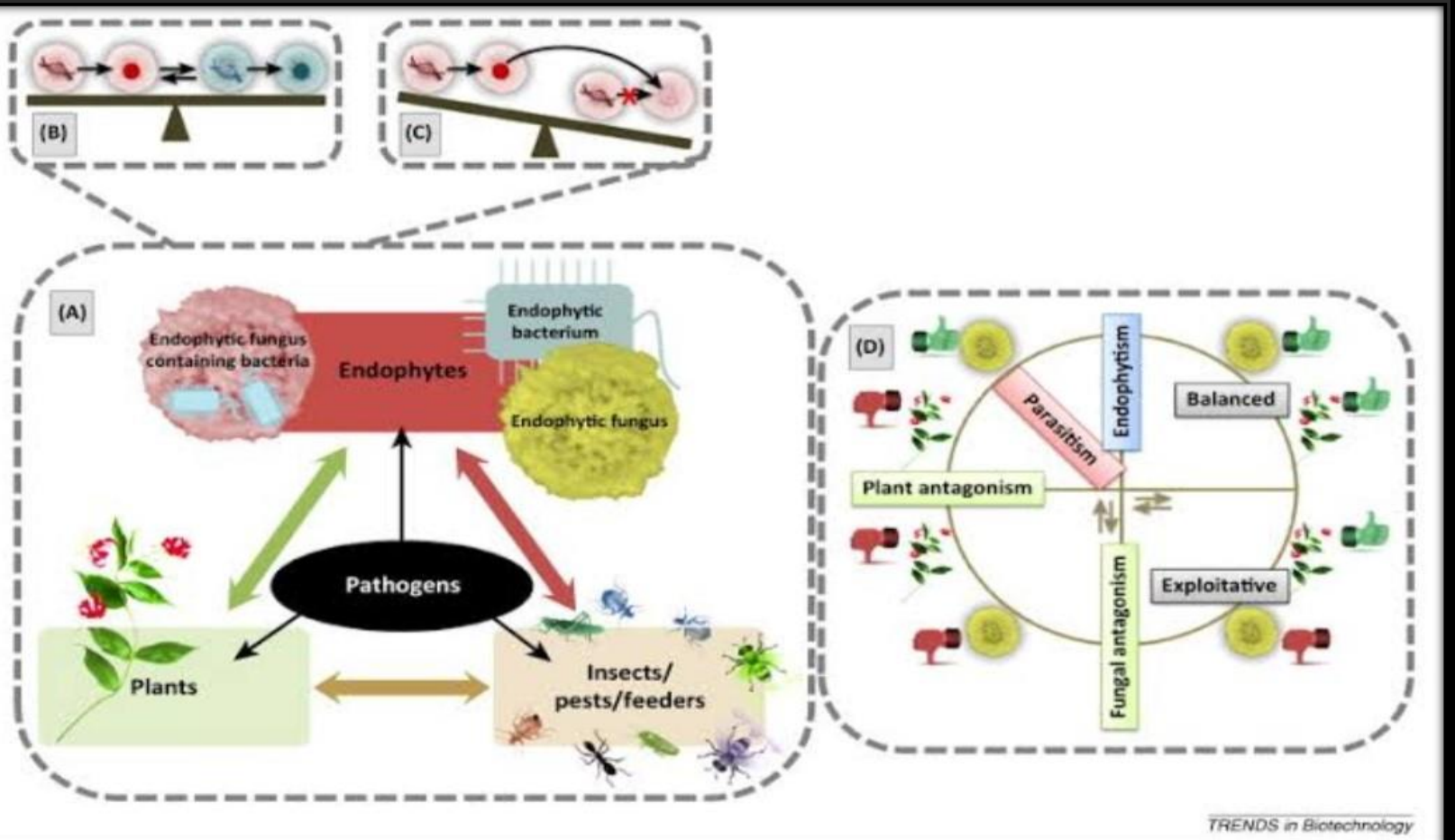
Mostert et al. (2000)

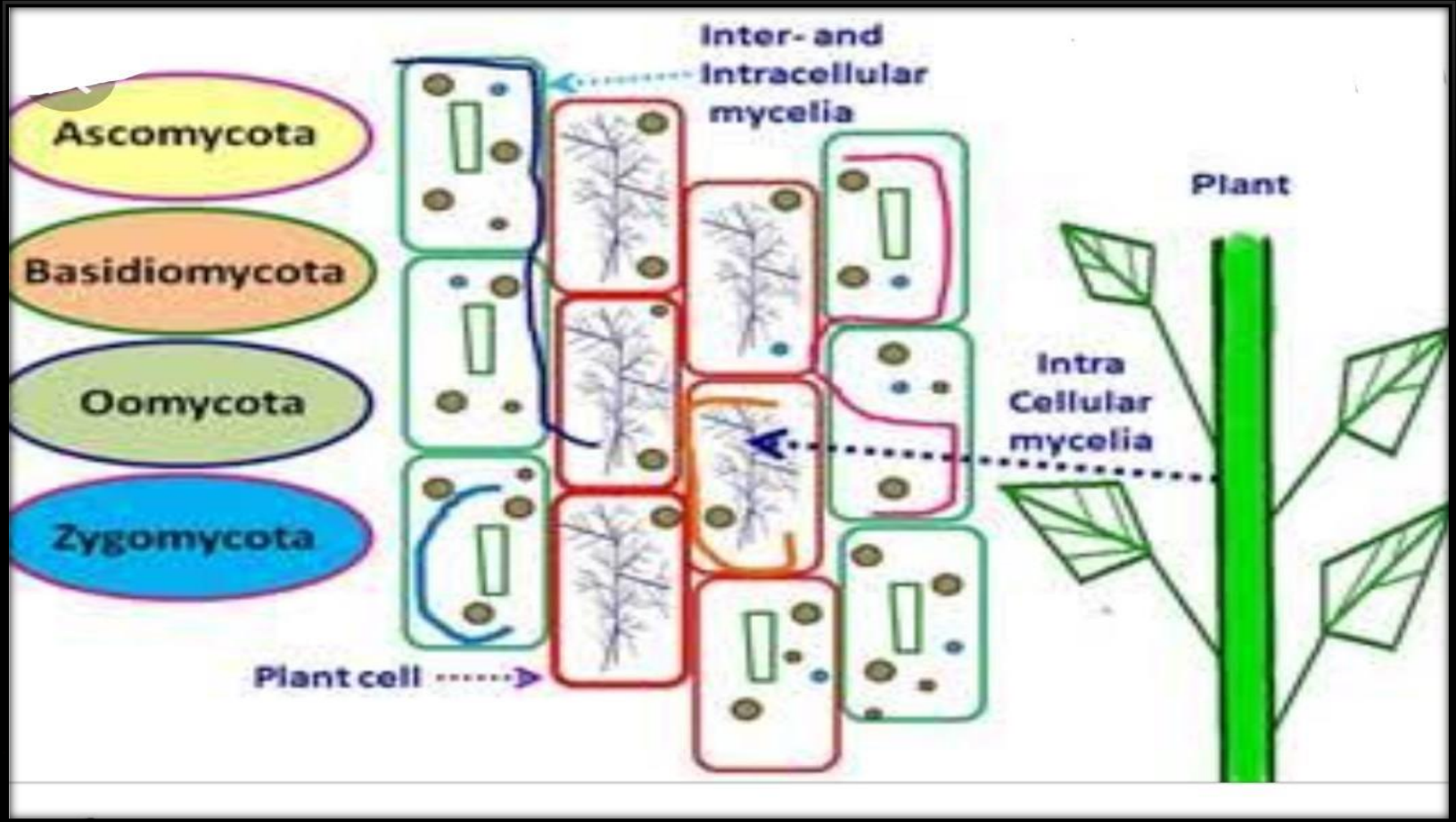
"Fungi that colonize a plant without causing visible disease symptoms at any specific moment"

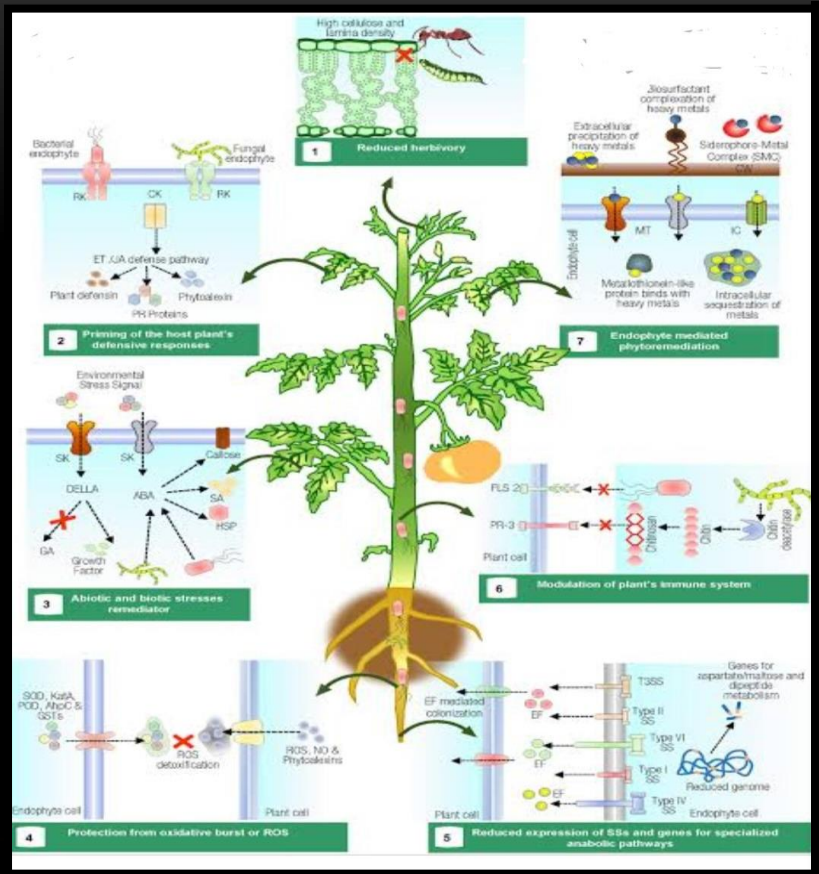
Schulz and Boyle (2005)

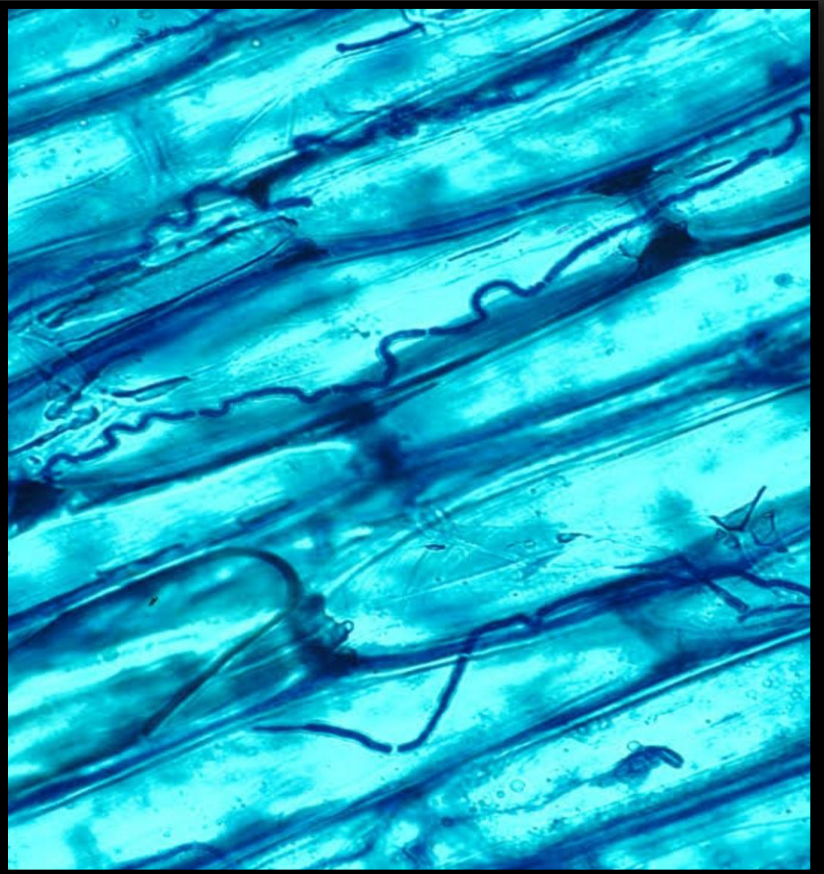
THE ENDHOPHYT-HOST INTERACTION



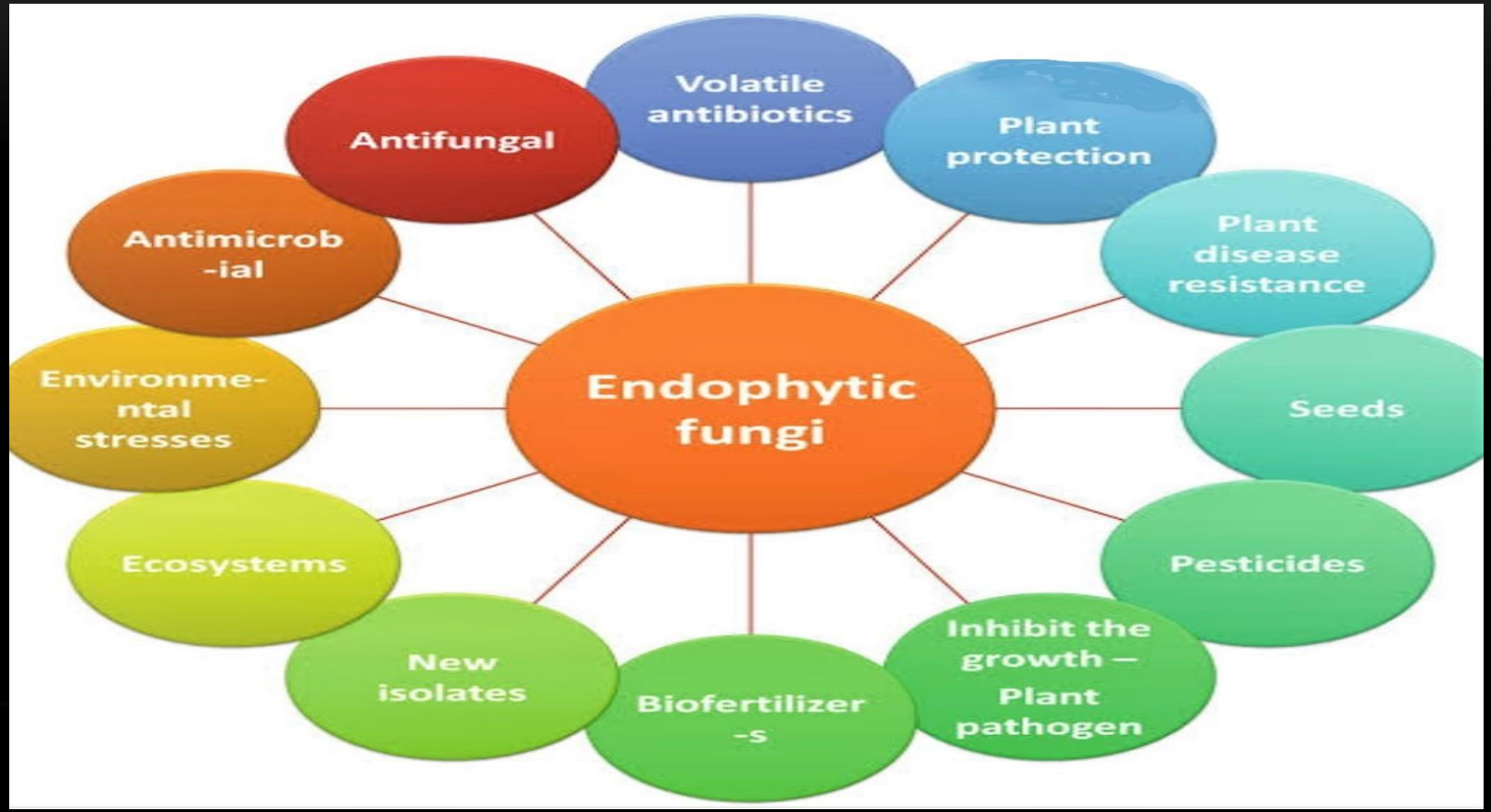

(B)
(C)
(A)
Endophytic fungus containing bacteria
Endophytes
Endophytic bacterium
Endophytic fungus
Pathogens
Plants
Insects/ pests/feeders
(D)
Endophytism
Parasitism
Balanced
Plant antagonism
Exploitative
Fungal antagonism
TRENDS in Biotechnology


Ascomycota
Basidiomycota
Oomycota
Zygomycota
Inter- and
Intracellular
mycelia
Plant
Intra
Cellular
mycelia
Plant cell

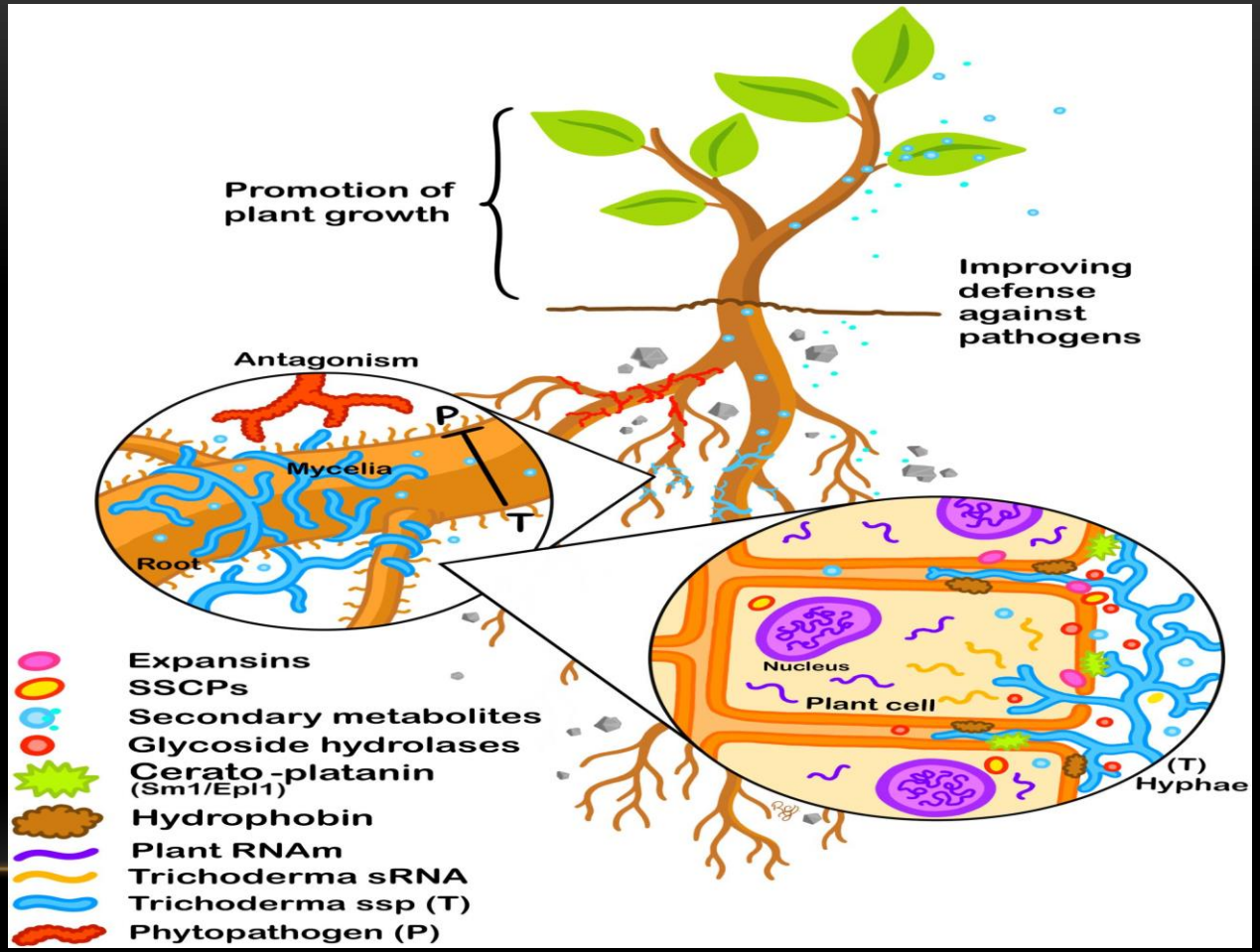

High cellulose and lamina density
1 Reduced herbivory
Bacterial endophyte
Fungal endophyte
RK
CK
RK
ET /JA defense pathway
Plant defensin
Phytoalexin
PR Proteins
2 Priming of the host plant's defensive responses
Environmental Stress Signal
SK
SK
Callose
DELLA
ABA
SA
HSP
GA
Growth Factor
3 Abiotic and biotic stresses remediator
Biosurfactant complexation of heavy metals
Extracellular precipitation of heavy metals
Siderophore-Metal Complex (SMC)
MT
IC
Endophyte cell
Metallothionein-like protein binds with heavy metals
Intracellular sequestration of metals
7 Endophyte mediated phytoremediation
FLS 2
PR-3
Chitinase
Chitin
Chitin deacetylase
Plant cell
6 Modulation of plant's immune system
SOD, KatA, POD, AhpC & GSTs
ROS detoxification
ROS, NO & Phytoalexins
Endophyte cell
Plant cell
4 Protection from oxidative burst or ROS
EF mediated colonization
EF
T3SS
Type II SS
Type VI SS
Type I SS
Type IV SS
Genes for aspartate/maltose and dipeptide metabolism
Reduced genome
Endophyte cell
Plant cell
5 Reduced expression of SSs and genes for specialized anabolic pathways

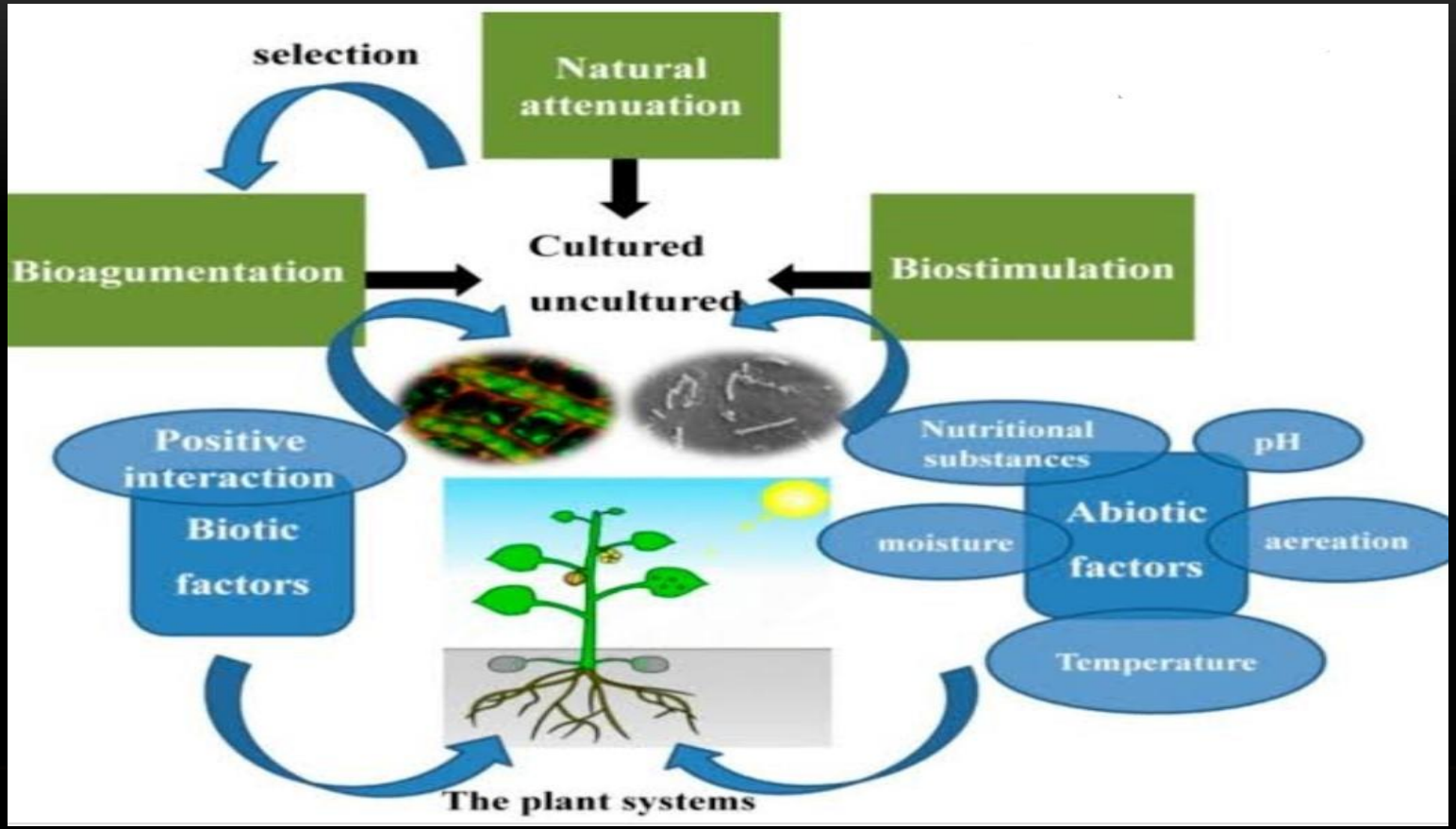


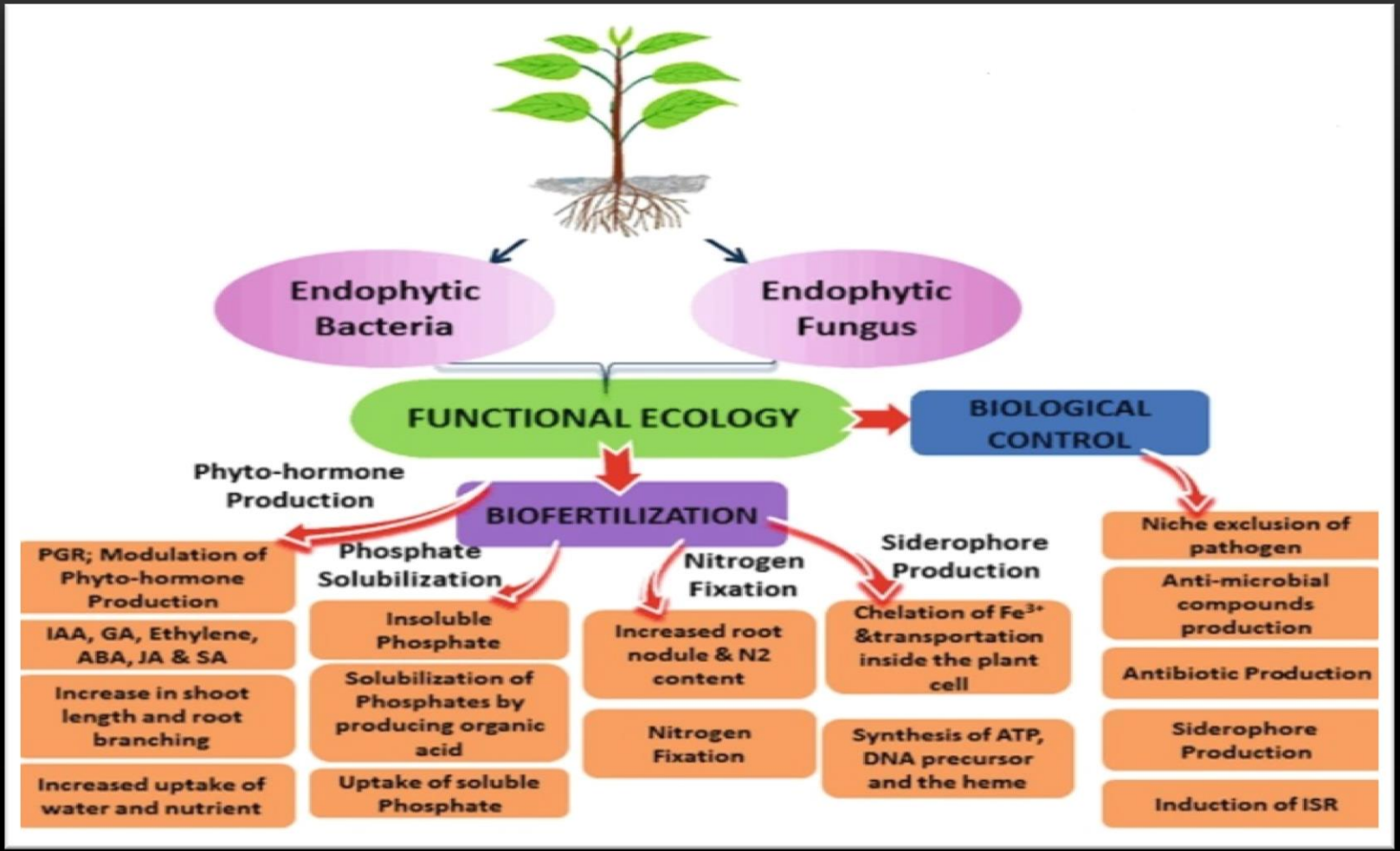
ROLE OF ENDOPHYTIC FUNGI

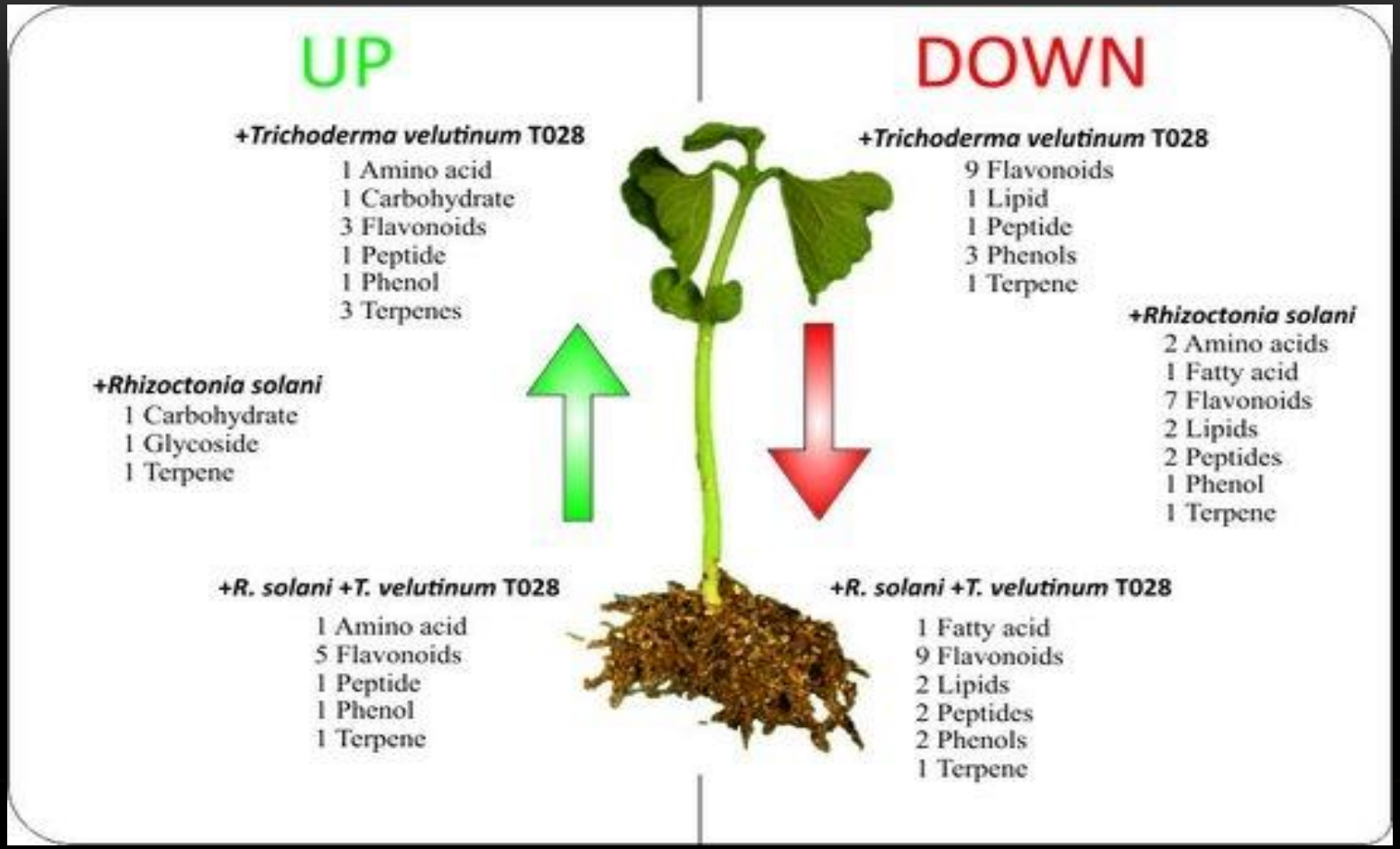


Role Of Endophytic Fungi




selection
Natural attenuation
Bioagumentation
Cultured
uncultured
Biostimulation
Positive interaction
Biotic factors
Nutritional substances
pH
Abiotic factors
moisture
aereation
Temperature
The plant systems


Endophytic Bacteria
Endophytic Fungus
FUNCTIONAL ECOLOGY
BIOLOGICAL CONTROL
BIOFERTILIZATION
Phyto-hormone Production
PGR; Modulation of Phyto-hormone Production
IAA, GA, Ethylene, ABA, JA & SA
Increase in shoot length and root branching
Increased uptake of water and nutrient
Phosphate Solubilization
Insoluble Phosphate
Solubilization of Phosphates by producing organic acid
Uptake of soluble Phosphate
Nitrogen Fixation
Increased root nodule & N2 content
Nitrogen Fixation
Siderophore Production
Chelation of Fe3+ &transportation inside the plant cell
Synthesis of ATP, DNA precursor and the heme
Niche exclusion of pathogen
Anti-microbial compounds production
Antibiotic Production
Siderophore Production
Induction of ISR


UP
DOWN
+Trichoderma velutinum T028
1 Amino acid
1 Carbohydrate
3 Flavonoids
1 Peptide
1 Phenol
3 Terpenes
+Trichoderma velutinum T028
9 Flavonoids
1 Lipid
1 Peptide
3 Phenols
1 Terpene
+Rhizoctonia solani
1 Carbohydrate
1 Glycoside
1 Terpene
+Rhizoctonia solani
2 Amino acids
1 Fatty acid
7 Flavonoids
2 Lipids
2 Peptides
1 Phenol
1 Terpene
+R. solani +T. velutinum T028
1 Amino acid
5 Flavonoids
1 Peptide
1 Phenol
1 Terpene
+R. solani +T. velutinum T028
1 Fatty acid
9 Flavonoids
2 Lipids
2 Peptides
2 Phenols
1 Terpene

To Controll Rice Plant Diseases

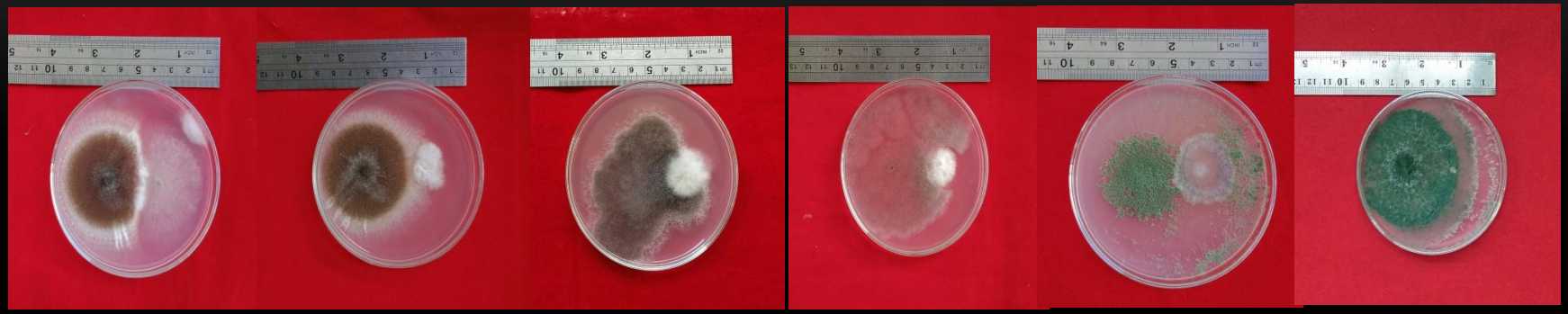
Antagonism of Endophytic Fungi to *Cercospora* sp.

Treatment	Repitition					MEAN
	1	2	3	4	5	
A1	84,00	73,68	82,35	79,16	75,00	78,84 a
B1	63,00	37,50	66,00	44,00	80,00	58,10 b
C1	53,33	41,76	55,00	75,00	35,00	52,02 b
Mean	66,78	50,98	67,78	66,05	63,33	62,99

Antagonism of Endophytic Fungi to *Rizhoctonia* sp.

Treatment	Repetition					MEAN
	1	2	3	4	5	
A2	33,00	66,00	85,00	42,00	81,00	61,40 a
B2	45,45	72,72	33,33	71,42	61,53	56,89 a
C2	95,00	85,00	91,00	88,00	87,00	89,20 b
Mean	57,82	74,57	69,78	67,14	76,51	69,16

Antagonistic Mechanism

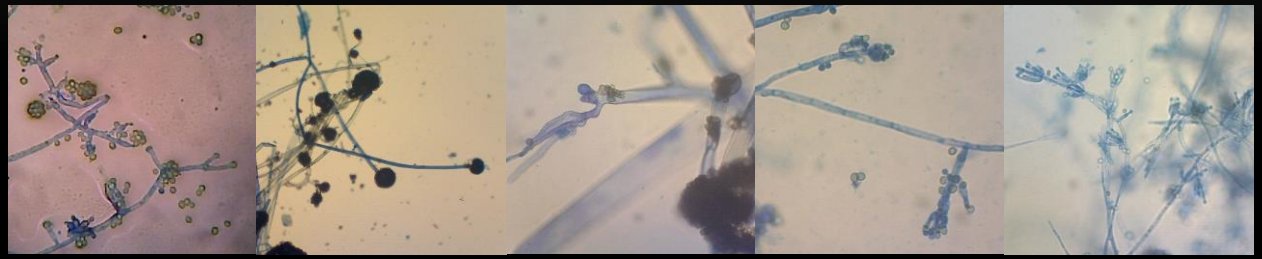


a b c d e f

(a). *A. Niger* vs *Cercospora* sp.
(b) *A. Niger* vs *Rhizoctonia* sp.
(c) *Mucor* sp. vs *Cercospora* sp.

(d) *Mucor* sp vs *Rhizoctonia* sp.
(e)*Trichoderma* sp. vs *Cercospora* sp .
(f) *Trichoderma* sp. vs *Rhizoctonia*

Endophytic Fungi in Rice Plant



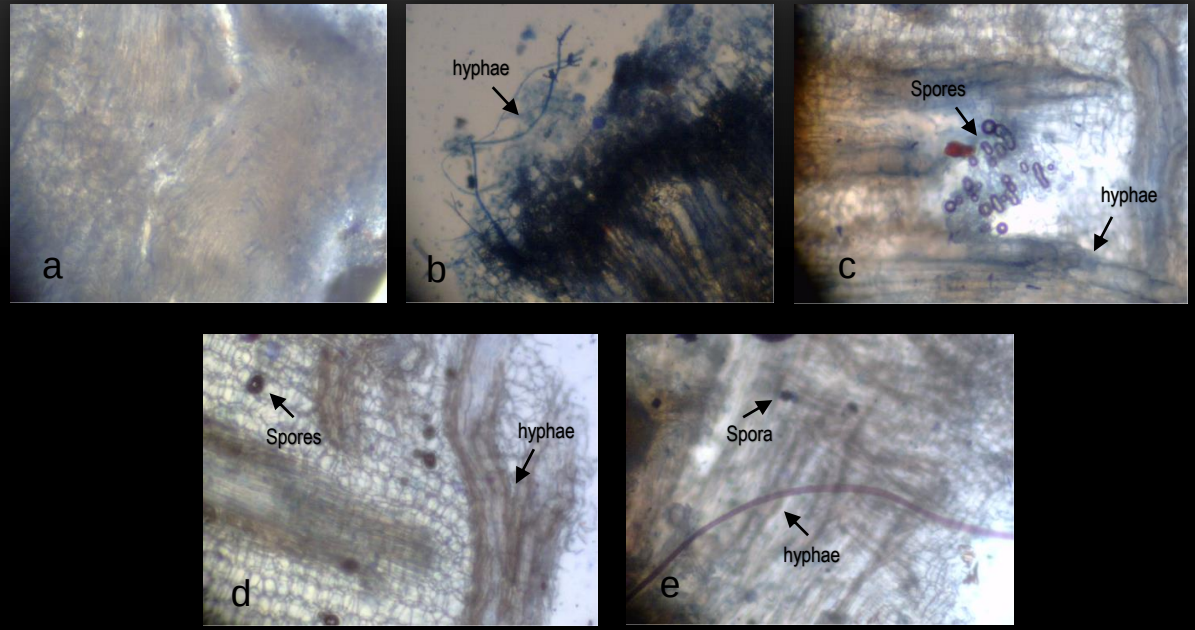
a b1 b2 c d

Trichoderma sp.
(b1) Rhizopus sp.
(b2) Rhizoid of Rhizopus sp.

(b)

(c) Gliocladium sp.
(d) Penicillium sp.

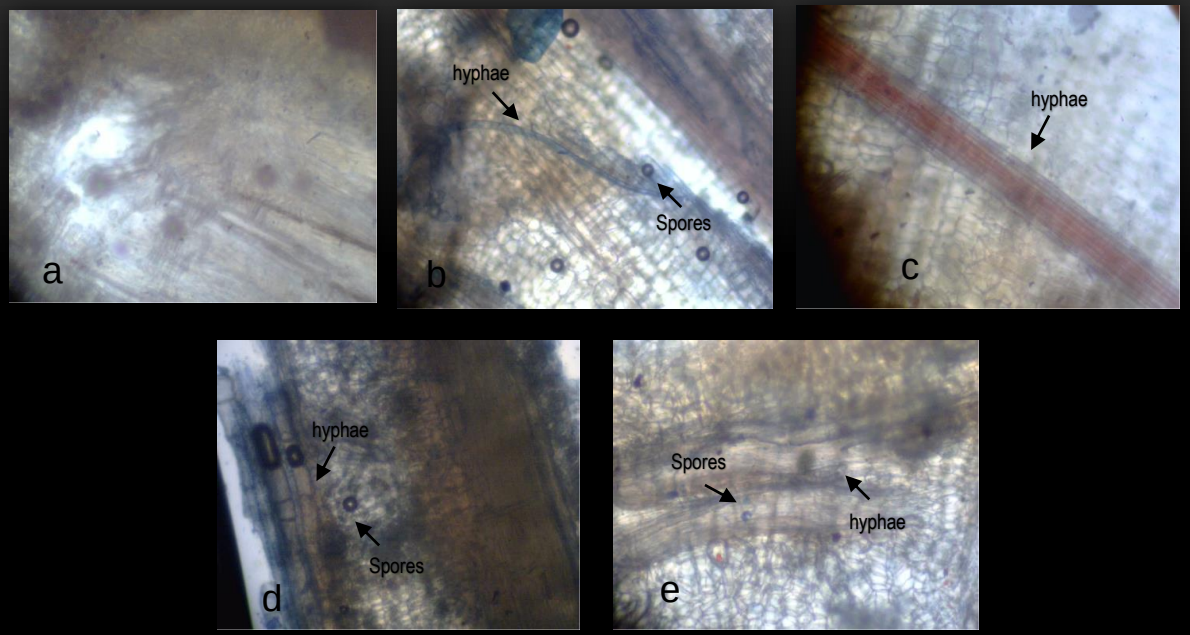
Endophytic Fungus Association on Rice Roots of Ciherang Variety



Control (No Treatment)

b. *Trichoderma* sp.
c. *Rhizopus* sp.
d. *Gliocladium* sp.
e. *Penicillium* sp.

Endophytic Fungus Association on Rice Roots of Kambang Variety



Control (No Treatment)

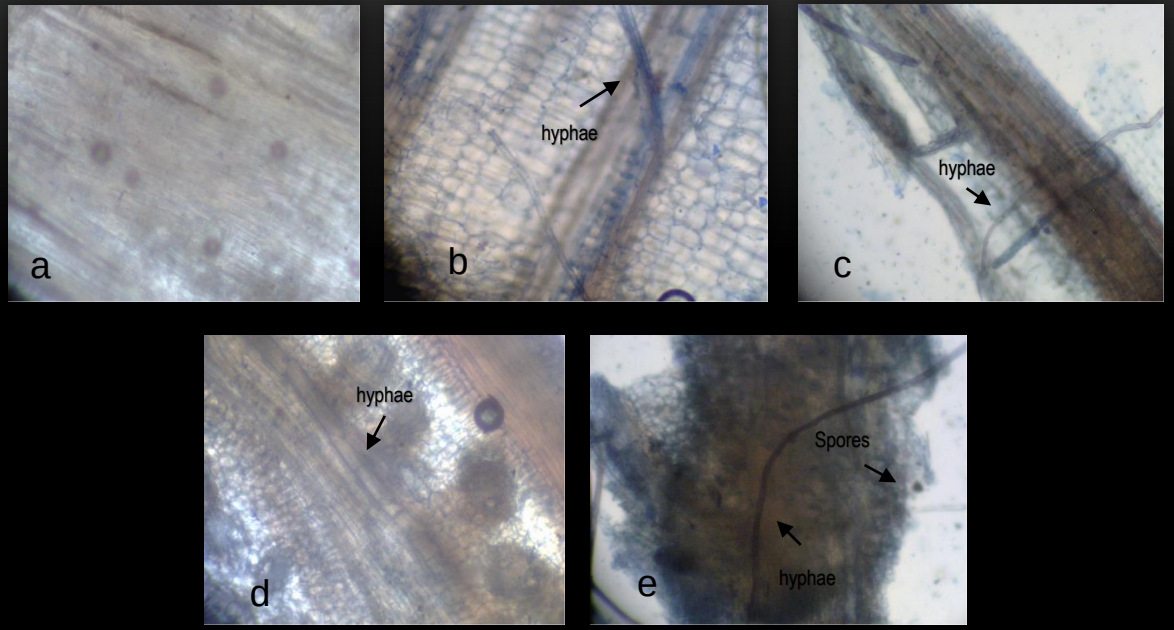
b. *Trichoderma* sp.

c. *Rhizopus* sp.

d. *Gliocladium* sp.

e. *Penicillium* sp.

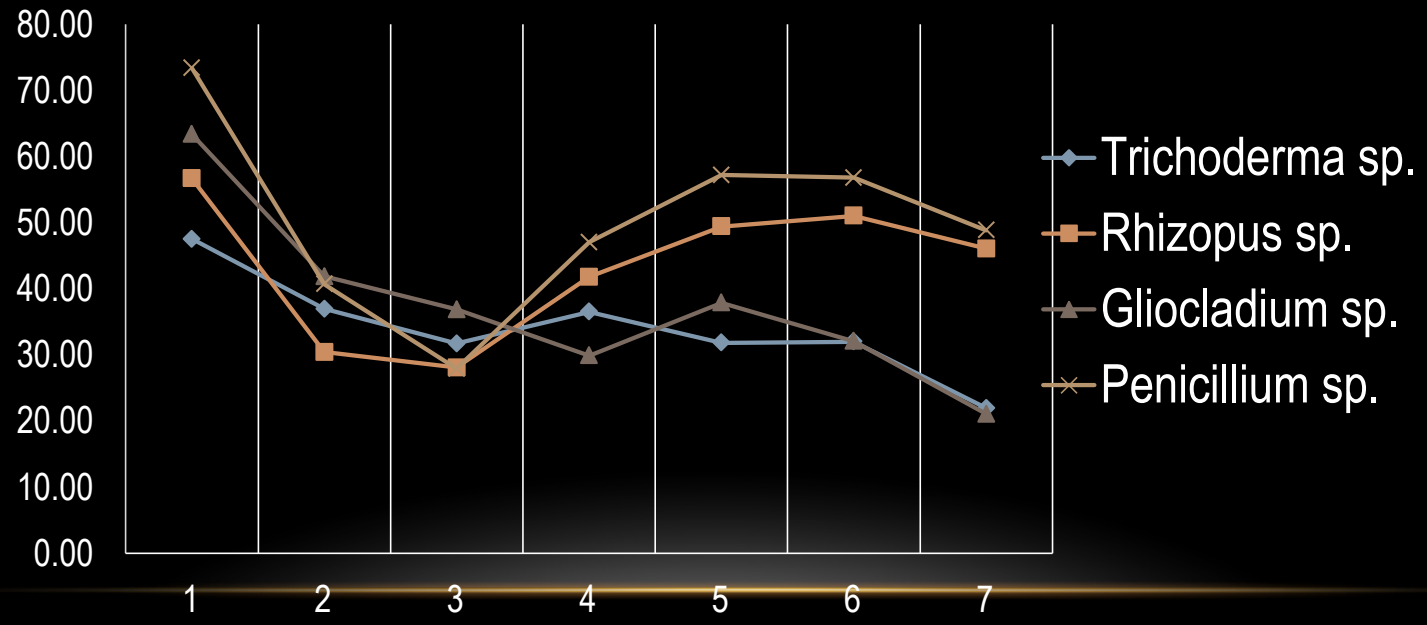
Endophytic Fungus Association on Rice Roots of Pandan Ungu Variety



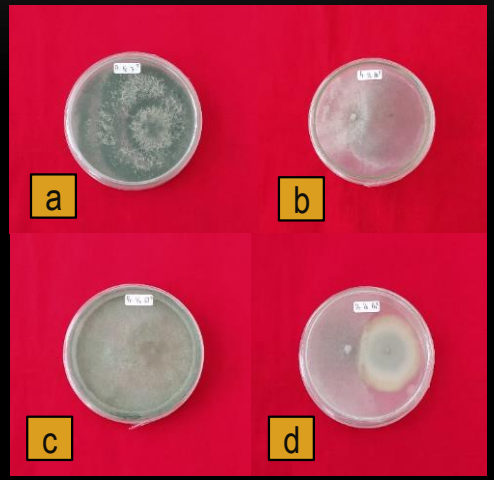
Control (No Treatment)

b. *Trichoderma* sp.
c. *Rhizopus* sp.
d. *Gliocladium* sp.
e. *Penicillium* sp.

Antagonistic of Endophytic Fungi to *Pyricularia oryzae* Cav.



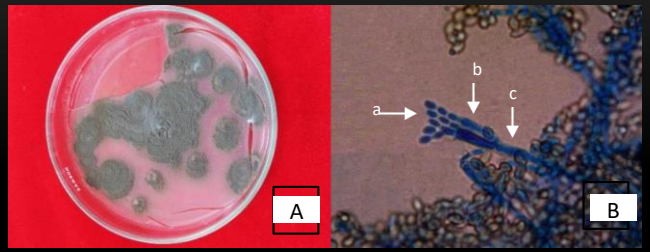
Perlakuan	Competition	Antibiosis	Parasitism
P.oryzae vs *Tricho* sp.	+	-	+
P.oryzae vs *Rhizopus* sp.	+	-	-
P.oryzae vs *Gliocladium* sp.	+	-	+
P.oryzae vs *Penicillium* sp.	-	+	-



(a) *P.oryzae* vs *Trichoderma* sp.
(b) *P.oryzae* vs *Rhizopus* sp.
(c) *P.oryzae* vs *Gliocladium* sp.
(d) *P.oryzae* vs *Penicillium* sp.

( ) tidak terjadi mekanisme antagonis

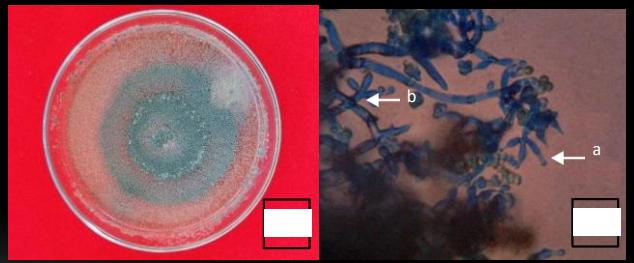
CENDAWAN ENDOFIT PADA PADI



Metarhizium sp. (A)Colony (B)(a)Conidia (b)Phyalid (c)Conidiophore

Penicillium sp. (A)Colony (B)(a)Conidia (b) Phyalid (c)Conidiophore

Aspergillus sp. (A)Colony (B)(a)Conidia (b)Conidiophore



Trichoderma sp. (A)Colony (B)(a)Conidia (b)Conidiophore

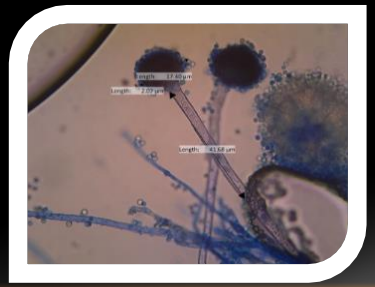
To Controll Chilli’s Diseases

Endophytic Fungi in Chilli's Plant

Trichoderma sp.

Penicillium sp.

Aspergillus sp.



Rhizopus sp.

The Ability of Endophytic Fungi to Supress Pathogen Growth *in vitro* (*Cercospora* sp.; *Colletotrichum capsici*; dan *fusarium* sp.)

Trichoderma sp.	: >55%
Aspergillus sp.	: 45-53%
Rhizopus sp.	: 44-51%
Penicillium sp.	: 41-43%

Ability to Suppress the Growth of *Cercospora* sp.

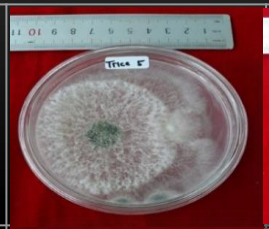
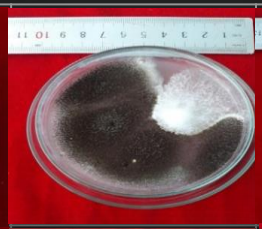
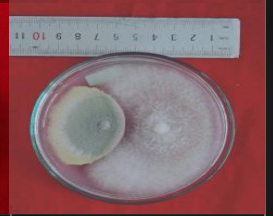
Treatment	Repetition					Mean
	1	2	3	4	5	
Trichoderma	53,33	62,5	62,5	57,14	53,33	$57,76_{a}$
Aspergillus	33,33	43,33	46,66	44	50	$43,46_{bc}$
Rhizopus	53,12	50	50	44,44	60,66	$51,64_{ab}$
Penicillium	42,85	33,33	42,85	53,48	33,33	$41,17_{c}$
Mean	45,6575	47,29	50,5025	49,765	49,33	48,51

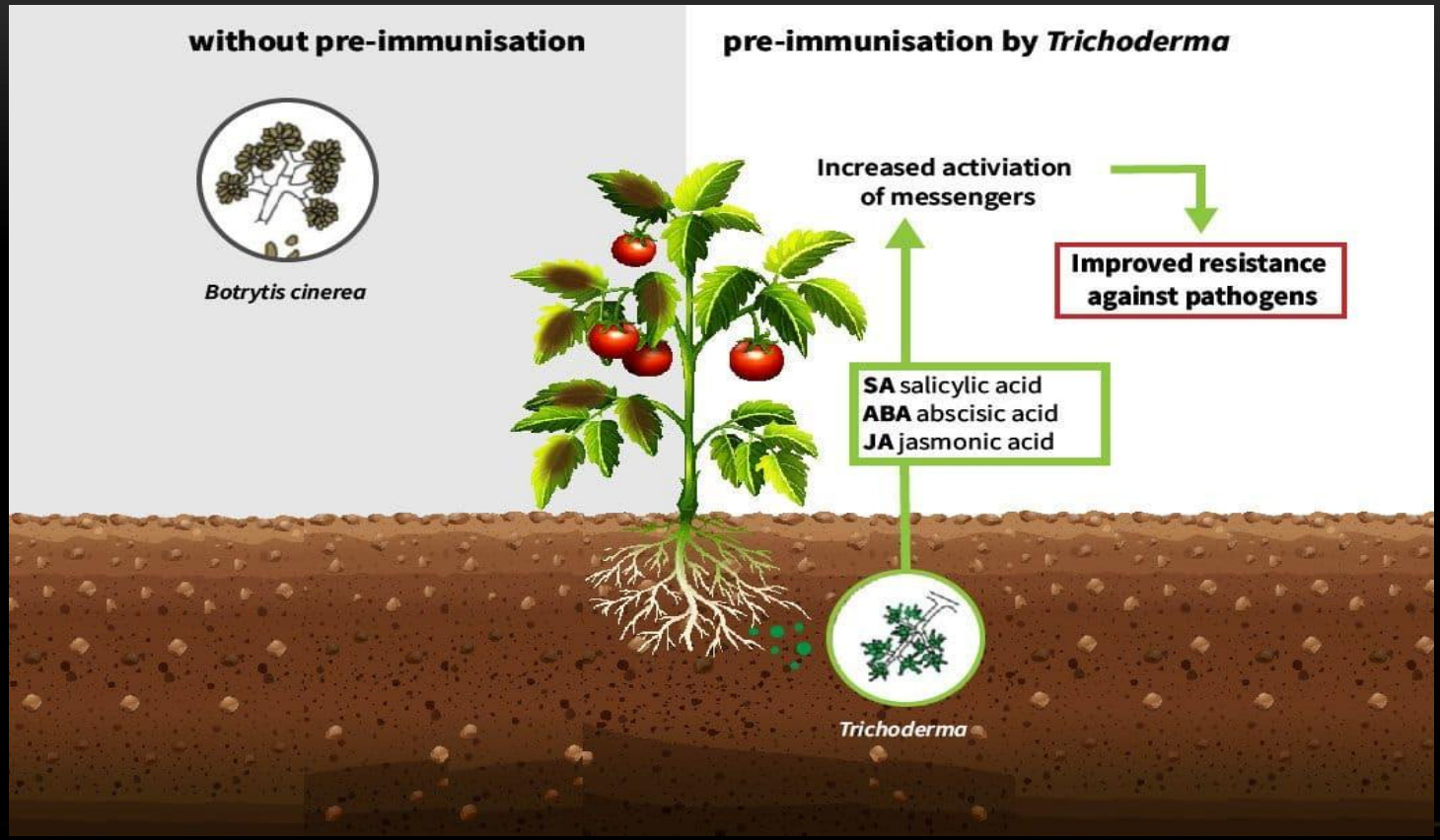
Ability to Suppress the Growth of *Colletotrichum capsici*

Treatmen	Repetition					Mean
	1	2	3	4	5	
Trichoderma	53,33	63,84	57,05	53,66	61,53	$57,882_{a}$
Aspergillus	59,45	57,14	65,11	46,66	50	$55,672_{a}$
Rhizopus	45	33,33	53,48	42,85	46,66	$44,264_{b}$
Penicillium	33,33	53,48	42,85	42,85	33,33	$41,168_{b}$
Mean	47,777	51,947	54,622	46,505	47,880	49,746

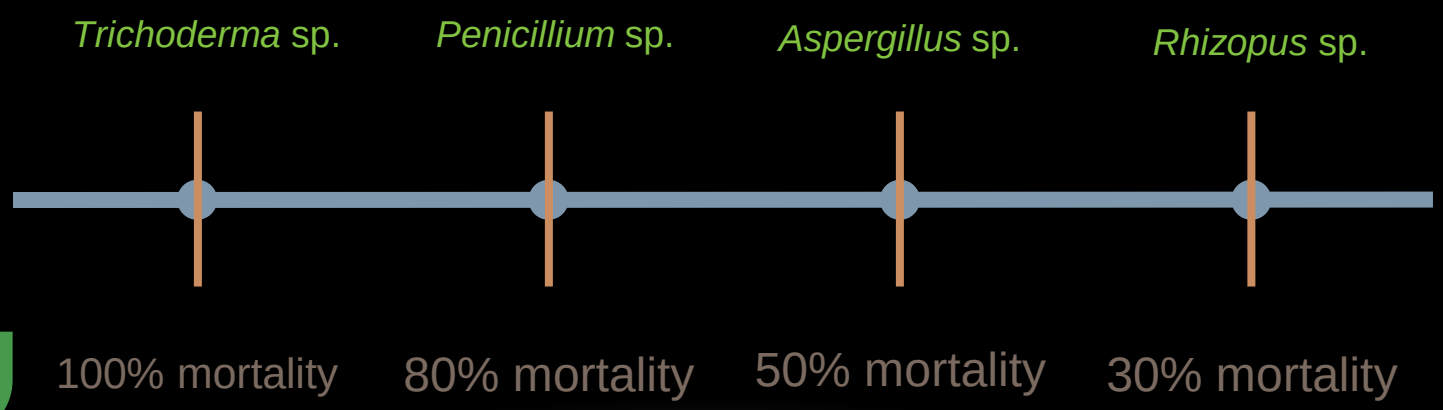
Ability to Suppress the Growth of *Fusarium* sp.

Treatment	Repetition					Mean
	1	2	3	4	5	
Trichoderma	71,42	67,85	63,63	71,42	81,48	$71,16_{a}$
Aspergillus	33,33	60,97	42,85	53,48	65,11	$51,148_{b}$
Rhizopus	42,85	33,33	33,33	65,11	67,85	$48,494_{b}$
Penicillium	42,85	33,33	55,81	33,33	52,5	$43,564_{b}$
Mean	47,6125	48,87	48,905	55,835	66,735	53,5915

***Cercospora* sp.**				
Colletotrichum capsici				
***Fusarium* sp.,**				
	***Trichoderma* sp.**	***A. niger***	***Rhizopus* sp.**	***Penicilium* sp.**

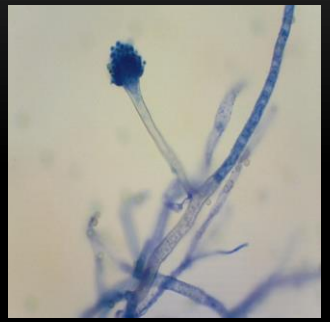

without pre-immunisation
pre-immunisation by *Trichoderma*
Botrytis cinerea
Increased activiation of messengers
Improved resistance against pathogens
SA salicylic acid
ABA abscisic acid
JA jasmonic acid
Trichoderma

Endophytic Fungi as Entomopatogen (*Tenebrio molitor*) (Sopialena et all., 2018)

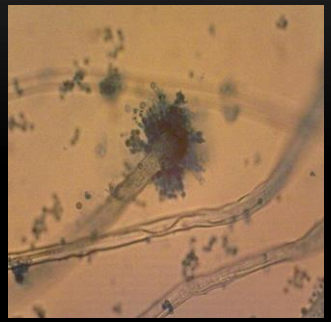


Jamur Endofit sebagai Pengendali Penyakit pada Tomat

CENDAWAN ENDOFIT PADA TOMAT

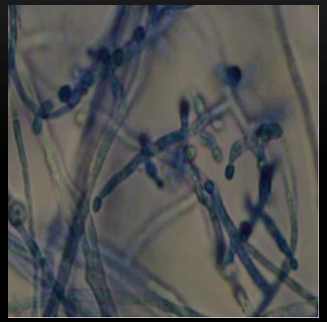


a



b

c



d

(a)*Aspergilus niger*
(b)*Aspergilus flavus*
(c)*Rhizopus* sp.
(d)*Trichoderma* sp.

Antagonism of Endophytic Fungi to *Colletotrichum coccodes* (Wilt Disease) on Chilli's Plant

Treatment	Repetition					Mean
	1	2	3	4	5	
A. flavus	39,46	27,87	30,40	37,53	30,58	33,17 [a]
A. niger	28,18	43,67	31,16	37,67	41,49	36,43 [a]
***Rhizopus* sp.**	43,97	32,43	38,36	37,44	39,64	38,37 [a]
***Trichoderma* sp.**	44,73	43,26	40,94	45,37	42,38	43,34 [b]

Endophytic Fungi on Pepper

No.	Endophytic Fungi	Jumlah Jamur Endofit			
		Hilly Area		Flat Area	
		Root	Leaf	Root	Leaf
1	***Fusarium* sp.**	7	5	5	2
2	*Nigrospora* sp.	1	0	1	1
3	*Trichoderma* sp.	6	1	4	1
4	***Aspergillus* sp.**	11	4	5	1
	∑Individu	25	10	15	5
		35		20	
	∑Genus	4		4	
	Indeks of Diversity	0.240	0.358	0.216	0.347
	Indeks of Homogenicity	0.173	0.090	0.156	0.250
	Indeks of Dominancy	0.958	0.889	0.929	0.750

Exploration of Endophytic Microbia to Improve Sugar Plant Health and Production of Sugar (Titiek Yulianti, Balai Penelitian Tanaman Pemanis Dan Serat Indonesian Research Institute For Sweetener And Fiber Crops)

- Endophyt of *Acetobacter diazotrophicus* (sinonim of *Gluconacetobacter diazotrophicus*) provide 60-80% Nitrogen through its ability fo fixate N_2 from the air and this endophyt suitable function as biofertilizer.
- Also found other endophytic microbia to stimulate root growth and plants, also helps to protect sugar plant from pathogens.

Diversity of Endophytic Microbia in Sugar Plant and it's function

No	Jenis Endofit	Fungsi	Referensi
1.	Azospirillum	Pemfiksasi N_2	Gangwar dan Kaur (2009)
2.	Burkholderia spp.	Agensia hayati (anti jamur dan anti bakteri, salah satunya pirol-nitrin)	van Antwerpen et al. (2002); Omarjee et al. (2004); Mendez et al. (2007)
3.	Epicoccum nigrum	Perangsang pertumbuhan akar dan agensia hayati untuk patogen F. verticillioides, Colletotri- chum falcatum, Ceratocystis paradoxa, dan Xanthomonas albilineans.	Fávaro et al. (2012)
4.	Eschericia coli	Penambang P, penghasil Siderophore, dan penghasil hormon IAA	Gangwar dan Kaur (2009)
5.	Gluconacetobacter diazotrophicus	Pemfiksasi N_2	Asis Jr. et al. (2004)
6.	Gluconacetobacter diazotrophicus	Meningkatkan ketahanan tanaman melawan X. albisineans, patogen penyebab penyakit leaf scald	Arencibia et al. (2006); Blanco et al. (2010).
7.	Herbaspirillum rubrisubalbicans	Pemfiksasi N_2	Asis Jr. et al. (2004);

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Distribution of the Endophytic Fungi in Apple Leaves

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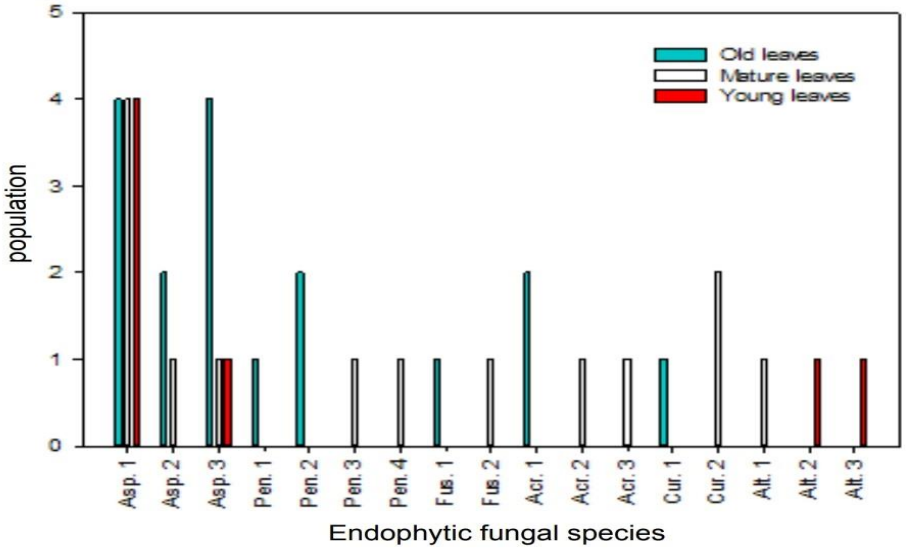
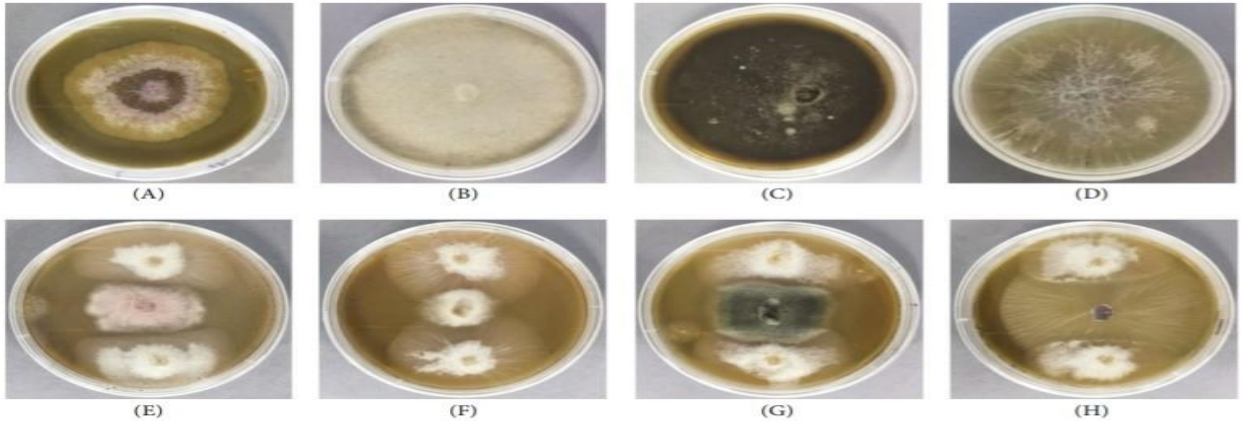
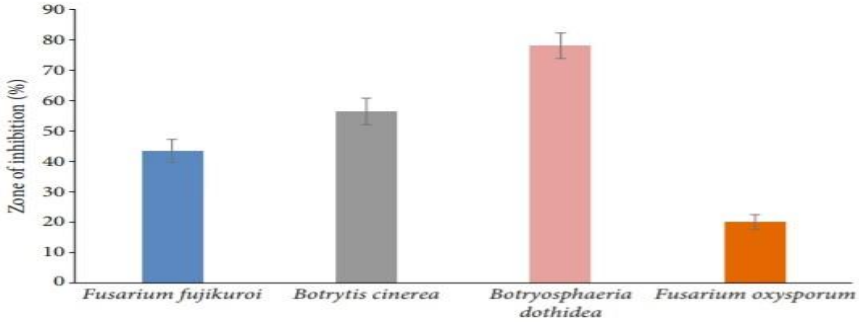


Fig. 1. Population of colonized endophytic fungi in each age of leaf; *Aspergillus* sp1 (Asp.1), *Aspergillus* sp2 (Asp.2), *Aspergillus* sp3 (Asp.3), *Penicillium* sp1 (Pen.1), *Penicillium* sp2 (Pen.2), *Penicillium* sp3 (Pen.3), *Penicillium* sp4 (Pen.4), *Fusarium* sp1 (Fus.1), *Fusarium* sp2 (Fus.2), *Acremonium* sp1 (Acr.1), *Acremonium* sp2 (Acr.2), *Acremonium* sp3 (Acr.3), *Curvularia* sp1 (Cur.1), *Curvularia* sp2 (Cur.2), *Alternaria* sp1 (Alt.1), *Alternaria* sp2 (Alt.2), and *Alternaria* sp3 (Alt.3).



(a)



(b)

FIGURE 3: Antifungal effects of the endophytic *Acremonium* sp. against four pathogenic strains using dual culture assay. (a) A 5 mm plug of pathogenic fungi was cultured in the middle of the PDA plate surrounded by two plugs of the endophytic fungi. Plates (A), (B), (C), and (D) are controls of *F. fujikuroi*, *B. cinerea*, *B. dothidea*, and *F. oxysporum*, respectively. PDA plates (E), (F), (G), and (H) contain dual cultures. (b) Antifungal activities were measured as the size of the zones of inhibition (ZI) of the pathogenic fungi. Zones of inhibitions were measured after two weeks of fungal growth. Means were averages ± standard deviation. The experiment was repeated thrice with $n = 5$.

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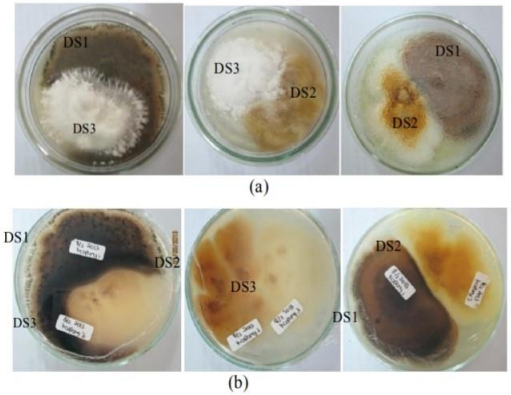


Figure 1. Antagonism test of the fungal isolates for 11 days (a) top view, (b) bottom side view.

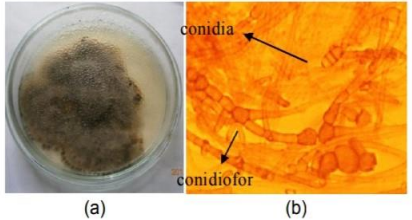


Figure 2. DS1 Isolate (a) 11 days old, (b) hyphae (400x).

Table 1. Fungal isolates from *Rhizophora mucronata*

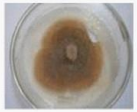
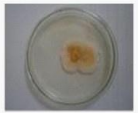
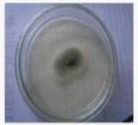
Isolate	on PDA medium	Characteristic
DS1		Colour of the colony is cream to dark brown, wavy edges, form concentric ring
DS2		Inner part of the colony is light yellow, outer part is pink. The colony is undulate (wavy)
DS3		White colony, thick mycelia, the margin is entire (smooth, with no irregularities)
DS4		Colour of the colony is green to grayish, wavy edges of the colony
DS5		Colour of the colony is light yellow, wavy edges and form concentric ring

Table 1. Fungal endophytes from three *Rhodiola* species and their frequency of colonization (FC%).

Genus (stated in GenBank)	Phylum; Class; Order	Fungal isolate (Representative strains)	Isolate number				FC%			
			Rct	Rac	Rsc	Total	Rct	Rac	Rsc	total
Ilyonectria	Ascomycota; Sordariomycetes; Hypocreales	Rct18, Rct20, Rct21, Rct22, Rct29, Rct31, Rct32, and Rsc7	7	0	1	8	9.86	0	1.92	4.44
Hypocrea	Ascomycota; Sordariomycetes; Hypocreales	Rct8, Rct10, and Rac32	2	1	0	3	2.82	1.75	0	1.67
Fusarium	Ascomycota; Sordariomycetes; Hypocreales	Rct6, Rct7, Rct12, Rct13, Rct14, Rct26; Rac41, and Rsc8	6	1	1	8	8.45	1.75	1.92	4.44
Neonectria	Ascomycota; Sordariomycetes; Hypocreales	Rct23, Rct25, Rct27, Rct30, Rct71, and Rac36	5	1	0	6	7.04	1.75	0	3.33
Thelonectria	Ascomycota; Sordariomycetes; Hypocreales	Rct24	1	0	0	1	1.41	0	0	0.56
Beauveria	Ascomycota; Sordariomycetes; Hypocreales	Rct28	1	0	0	1	1.41	0	0	0.56
Bionectria	Ascomycota; Sordariomycetes; Hypocreales	Rct38	1	0	0	1	1.41	0	0	0.56
Cordyceps	Ascomycota; Sordariomycetes; Hypocreales	Rct61	1	0	0	1	1.41	0	0	0.56
Trichoderma	Ascomycota; Sordariomycetes; Hypocreales	Rac43 and Rsc4	0	1	1	2	0	1.75	1.92	1.11
Gibberella	Ascomycota; Sordariomycetes; Hypocreales	Rac15, Rac27;Rsc58, and Rsc71	0	2	2	4	0	3.51	3.85	2.22
Lecythophora	Ascomycota; Sordariomycetes; Coniochaetales	Rct47	1	0	0	1	1.41	0	0	0.56
Coniochaeta	Ascomycota; Sordariomycetes; Coniochaetales	Rct69	1	0	0	1	1.41	0	0	0.56
Phomopsis	Ascomycota; Sordariomycetes; Diaporthales	Rac6, Rac9, Rac44, Rac59, Rac60, and Rac61	0	6	0	6	0	10.53	0	3.33
Cytospora	Ascomycota; Sordariomycetes; Diaporthales	Rsc29	0	0	1	1	0	0	1.92	0.56
Biscogniauxia	Ascomycota; Sordariomycetes; Xylariales	Rsc5	0	0	1	1	0	0	1.92	0.56
Pestalotiopsis	Ascomycota; Sordariomycetes; Xylariales	Rsc44	0	0	1	1	0	0	1.92	0.56
Chaetomium	Ascomycota; Sordariomycetes; Sordariales	Rac51 and Rsc3	0	1	1	2	0	1.75	1.92	1.11
Sordariomycetes	Ascomycota; Sordariomycetes	Rac37	0	1	0	1	0	1.75	0	0.56

(Continued)

CONCLUTION

Endophytic Fungi has a lot of very functions to plants, and it has coevolve to the plant well. Needs to develop the technology well on endophytic fungi.

THANK YOU