The 8th National Fungus Day of Egypt Online | اليوم الوطنى الثامن لفطريات مصر أونلاين

The 8th National Fungus Day of Egypt Online

Date: February 24, 2023

Theme: "Mycology by Amateur and Young Mycologists"

Strategic Crops and Endophytic Fungi (The New Hope Towards Sustainability)

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2023

Problems

- Several regions in the world have experienced a decrease in water availability and an increase in soil salinisation and desertification.

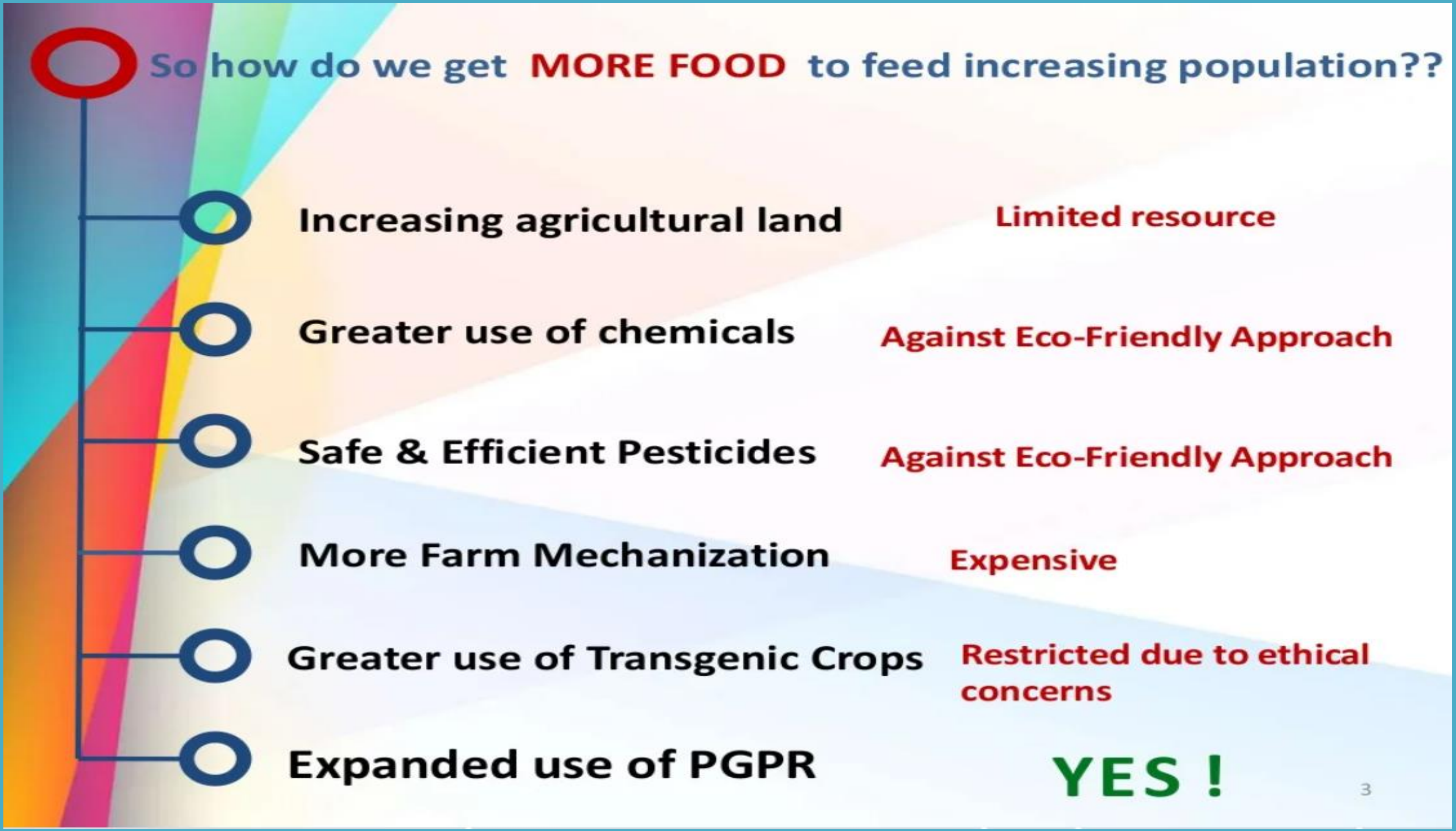
- Other problems related to the excessive soil use, deforestation and inappropriate irrigation practices (Chadha et al. 2015; Lugtenberg et al. 2016).

Current Situations

Crops	Abiotic stresses	Yield reduction (%)	References
Rice	Drought	23.2–24.0%	Yang et al. (2019)
	Heat	13.8–28.5%	Aghamolki et al. (2014)
	Drought and heat	20–80%	Lawas et al. (2018)
Maize	Drought	17.91–20.62%	Hussain et al. (2019)
	Heat	14.56–16.61%	Hussain et al. (2019)
	Drought and Heat	73%	Chukwudi et al. (2021)
Wheat	Drought	44.66 %	Qaseem et al. (2019)
	Heat	53.05 %	Qaseem et al. (2019)
	Drought and Heat	56.47 %	Qaseem et al. (2019)

Current studies about global food and agriculture revealed that world production may need to be increased by 60%– 110% before 2050 to avoid food shortage (Schmidhuber and Tubiello 2007; Edgerton 2009).

Current Solutions and Difficulties



Future Hope ????

The Use of Endophytic Fungi as Plant Growth Promoting Agent is the Future

Endophytes

- **These microorganisms are defined as endophytes that can not cause any symptomatic disease for their host plant (Manon et al., 2015).**
- **Concludes that endophytes are microbes which inhabit internal plant tissues for at least part of their life cycles and cause no harm to the host plant under any circumstance Cocq et al., 2017).**

Classification of Endophytic Fungi

Claviciptaceae

Class 1 :Colonizing both cool and warm-season grasses systemically (Bischoff and White, 2005).

Non-Claviciptacea

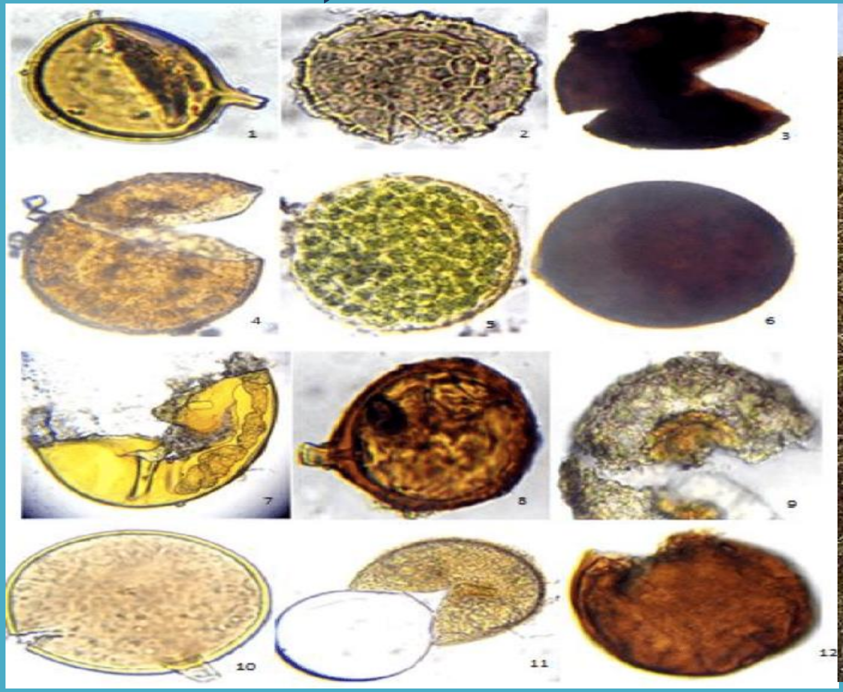
Class 2 : Fungi colonizing host tissues of plant systemically.

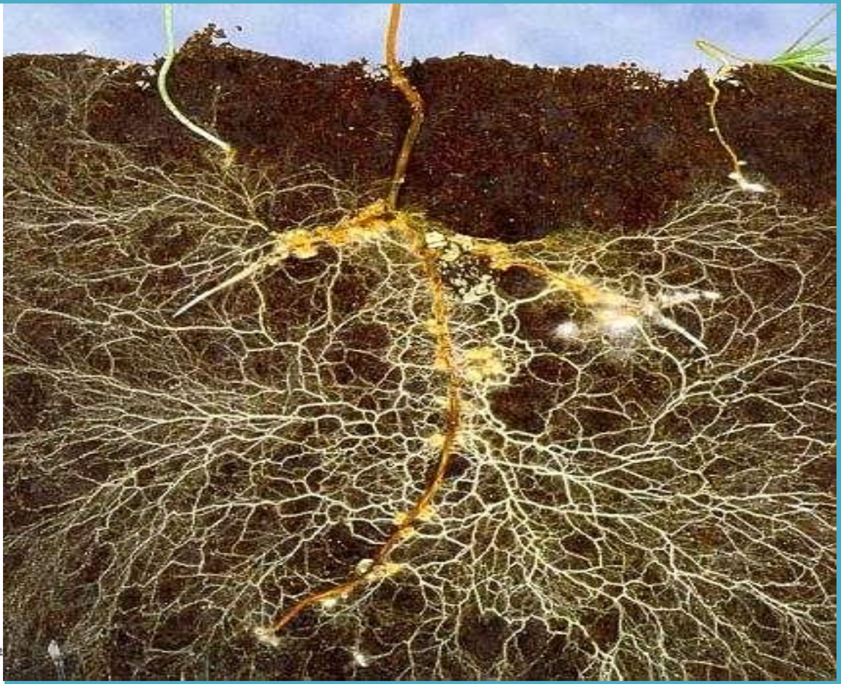
Class 3: Fungi colonizing the above-ground plant tissues.

Class 4: fungi with dark septate endophytes that colonize plant roots are belonging to Ascomycota, and non-mycorrhizal members of Sebacinales, Basidiomycota (Rodriguez et al., 2009 and Andrade-Linares and Franken, 2013).

Endomycorrhiza

- Fungi can penetrate root cell walls and establish inside the cell membrane which made these fungi more invasive in a symbiotic relationship with their plant host (Bonfante and Genre, 2010).
- Classified into five subgroups arbuscular, ericoid, arbutoid, monotropoid, and orchid mycorrhiza (Petrosyan *et al.*, 2003).



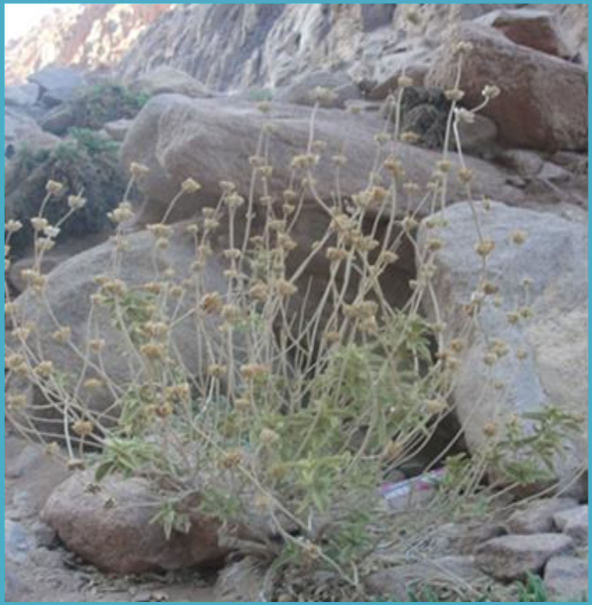


Where Endophytes?

- The diversity of endophytic fungi associated with plants can greatly vary according to environmental conditions, artic environments, hot deserts, and mangrove, temperate and tropical forests (Vega et al., 2010).

Why wild plants?

- Microbial communities colonizing wild medicinal plants under extremely harsh conditions play an important role in mitigating many biotic and a biotic stresses within arid regions such as salinity, heat, drought and low input of chemical fertilizers (Alsharif et al., 2020).
- Plant growth-promoting microorganisms improve plant health and productivity under many extreme conditions through forming symbiotic interactions with their plant host (Abdelaal and Sahar, 2015;Torre-Ruiz et al., 2016).



Benefits of endophytic colonization by fungi

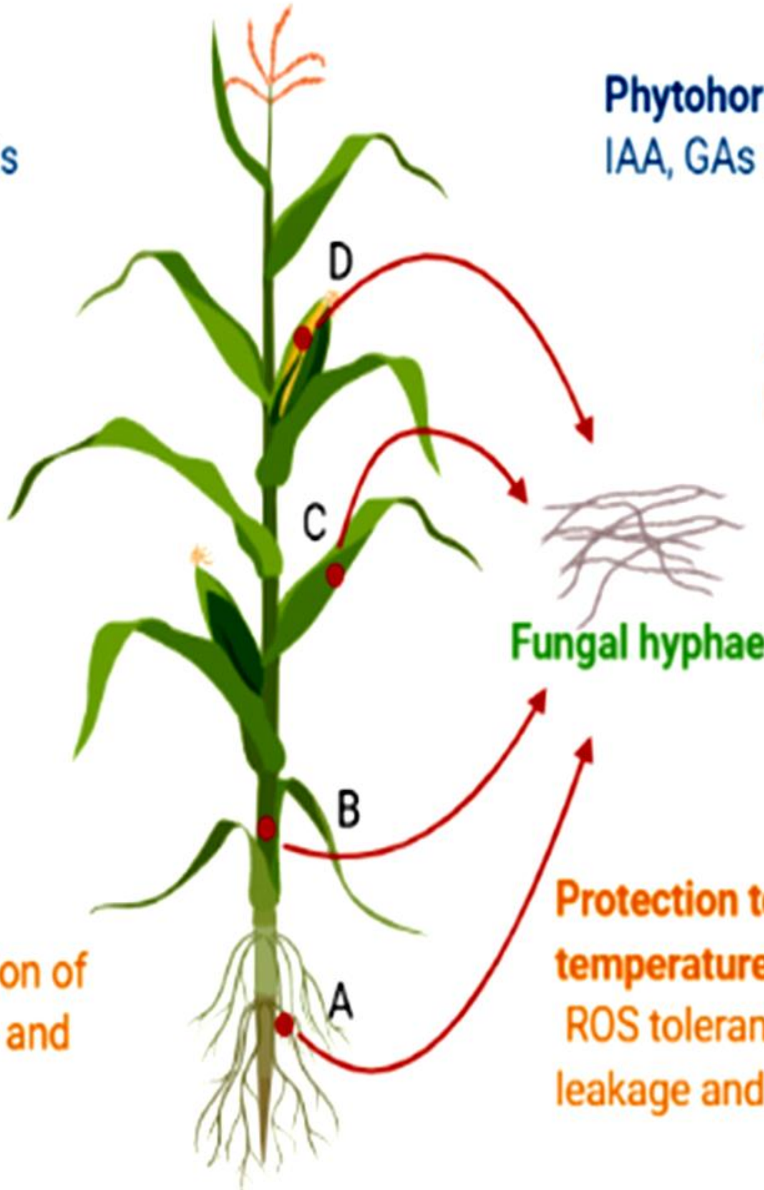
Direct benefits and Indirect benefits

Nutrient acquisition
N, P, K, Mg, other macronutrients and also micronutrients

Phytohormones Production
IAA, GAs and cytokinins

Activation of sistemic resistance
ISR and ASR

Secondary metabolites
Alkaloids, steroids, terpenoids and others including VOCs



Siderophores
Iron acquisition and ISR (?)

Protection to biotic stress (pathogens, herbivores and nematodes)
secondary metabolites, activation of sistemic resistance, parasitism and competition

Protection to abiotic stress (drought, salinity, temperature and heavy metals)
ROS tolerance, ↓ lipid peroxidation, ↓ electrolyte leakage and regulation of phytohormones

Cereal crops and their Endophytic Fungi

Wheat

- *Aspergillus flavus*
- *Cladosporium cladosporioides*
- *Trichoderma harzianum*
- *Fusarium proliferatum*

Plant growth promoting traits

- IAA production
- Siderophores
- Resist saline conditions
- Alleviate heavy metal pollution (Ripa et al., 2019)

Oryza Sativa (Rice)

- *Penicillium chrysogenum*
- *Fusarium oxysporum*
- *Cladosporium cladosporioides*

- **Antagonistic activity against phytopathogens**
- **Production of bioactive compounds**
- **Production of organic acids (Naik et al., 2009)**

Zea mays (Maize)

Leaves

- *Acremonium*
- *Fusarium*
- *Penicillium*

Roots

- *Fusarium*
- *Trichoderma*
- *Aspergillus*
- *Alternaria alternata*
- *Botryodiplodia*

Activity

Productionn of secondary bioactive compounds which promote growth of plants

(Potshangbam et al. ;2017)

Native Egyptian Endophytes could Alleviate low Nitrogen input in Wheat Crop

- **Wild medicinal plants are a reservoir of many bioactive compounds that are safe for humans and the environment compared with chemical and synthetic compounds used to treat many diseases .**
- **Many reports suggest that wild plants growing in harsh conditions may harbor plant growth-promoting (PGP) rhizobacteria. However, very little is known about the microbiota that colonizes the roots of desert plants.**
- **Wild medicinal plants in Sinai attract the attention of many ecologists, taxonomists, and phytochemists owing to their importance as large reservoirs for a diverse microbial community that has a crucial role in alleviating drought stress through increasing biomass production and enhancing plant growth.**

Wild Plants Collected

- ***Cheilanthes vellea* (rare fern)**
- ***Conyza stricta***
- ***Silene schimperiana***
- **Exploring their role in plant growth promoting activity.**
- **This study represents first report about endophytic fungi of rare fern *Cheilanthes vellea* and their role in wheat plant growth promotion.**

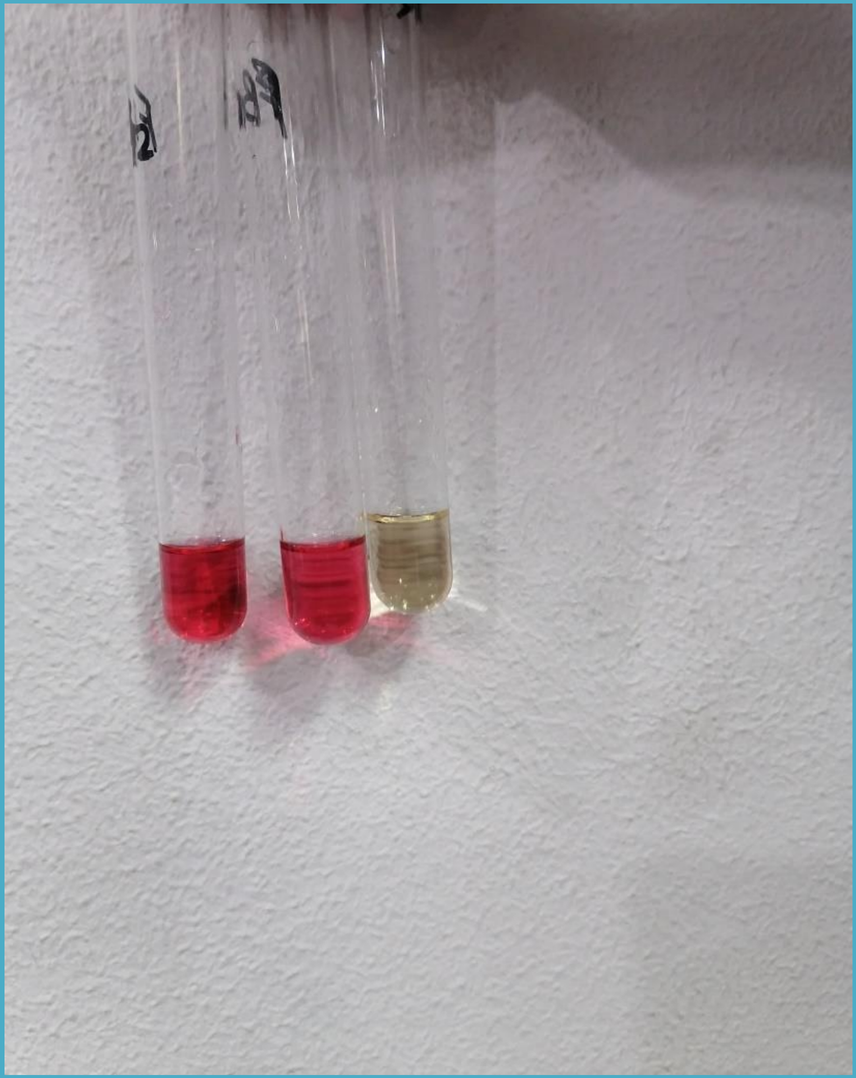


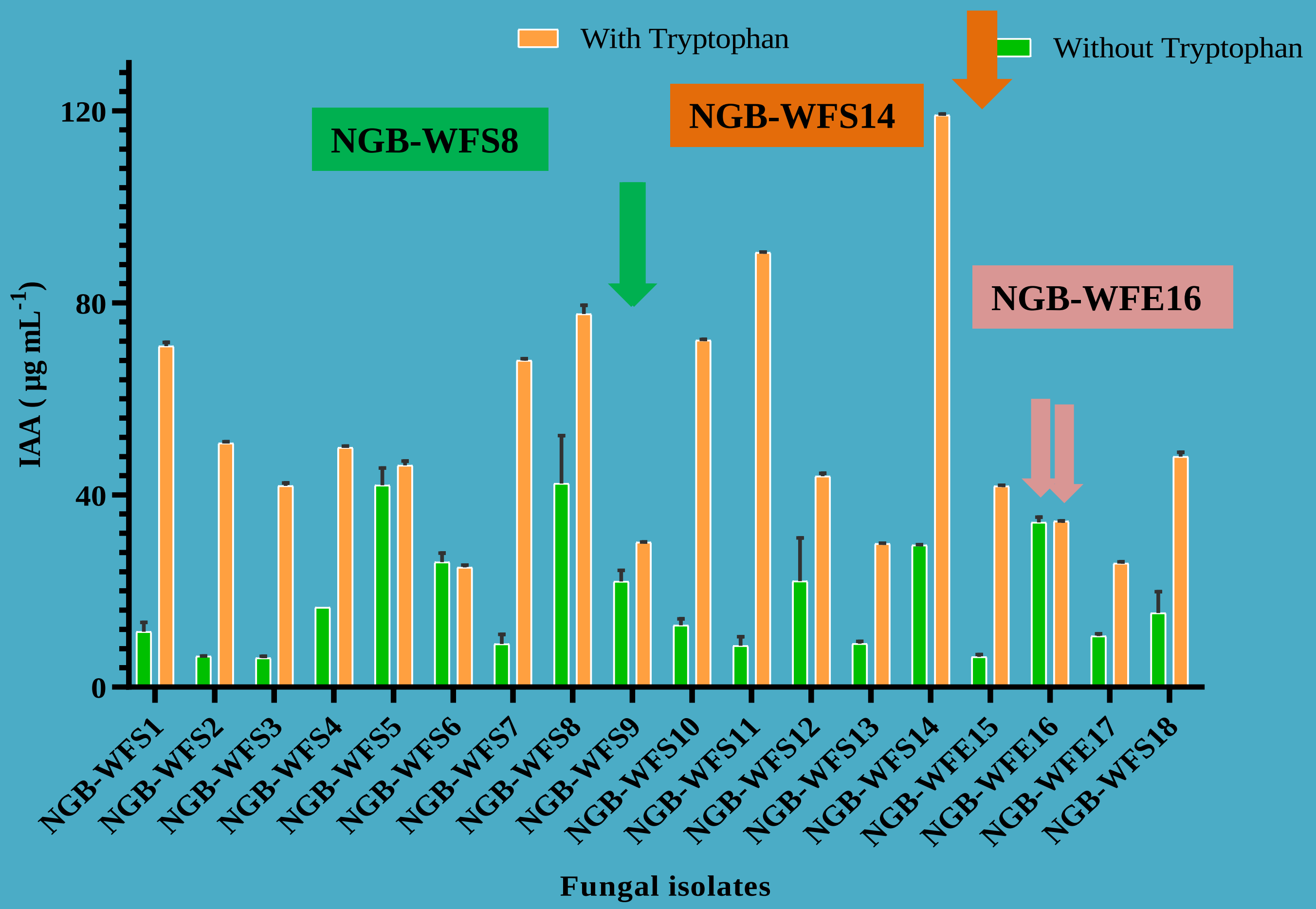



IAA Determination in Endophytic Culture Filterate

- IAA determination using Salkovisky reagent in presence or absence of Tryptophan as percursor of IAA production

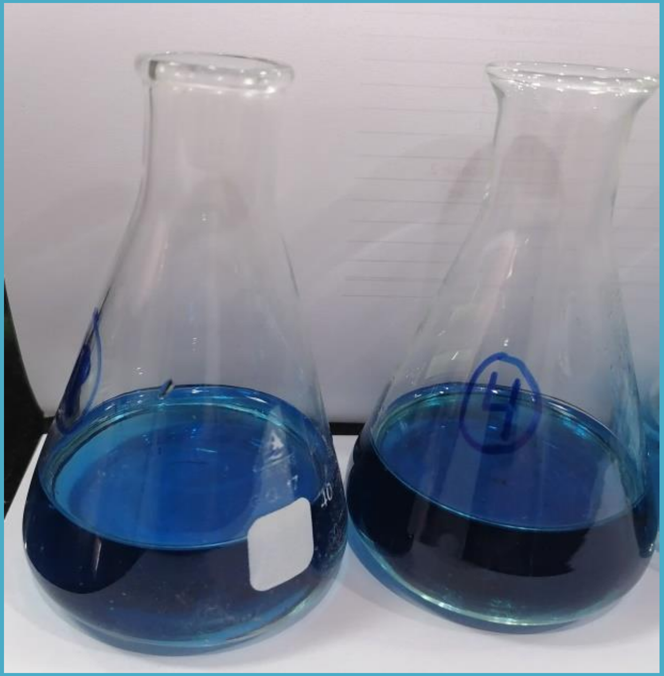
- Presence of pink or red color revealed presence of IAA





With Tryptophan
Without Tryptophan
NGB-WFS8
NGB-WFS14
NGB-WFE16
IAA (µg mL⁻¹)
120
80
40
0
NGB-WFS1
NGB-WFS2
NGB-WFS3
NGB-WFS4
NGB-WFS5
NGB-WFS6
NGB-WFS7
NGB-WFS8
NGB-WFS9
NGB-WFS10
NGB-WFS11
NGB-WFS12
NGB-WFS13
NGB-WFS14
NGB-WFE15
NGB-WFE16
NGB-WFE17
NGB-WFS18
Fungal isolates

Phosphate Soulbilization Efficiency



Blue color indicates the presence of organic acids and solubilized phosphorus in the Pikoviskays liquid broth using chloromolbydic acid

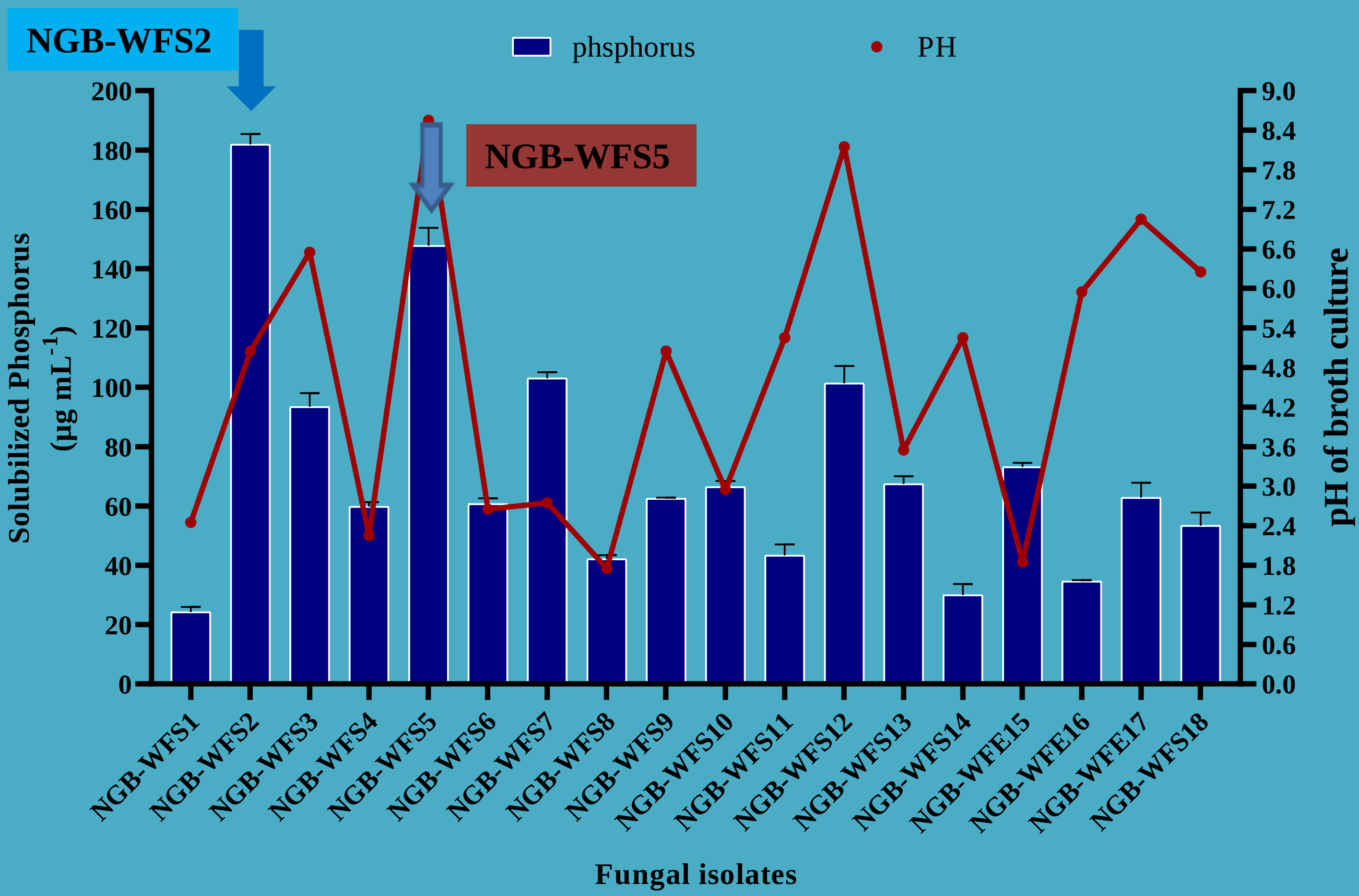


Fig. (9): Solubilized phosphorus concentrations and corresponding pH of PVK broth inoculated with the tested fungal isolates after 10 days of incubation.

Siderophore and HCN production, and inhibition percentages of fungal pathogen by fungal isolates

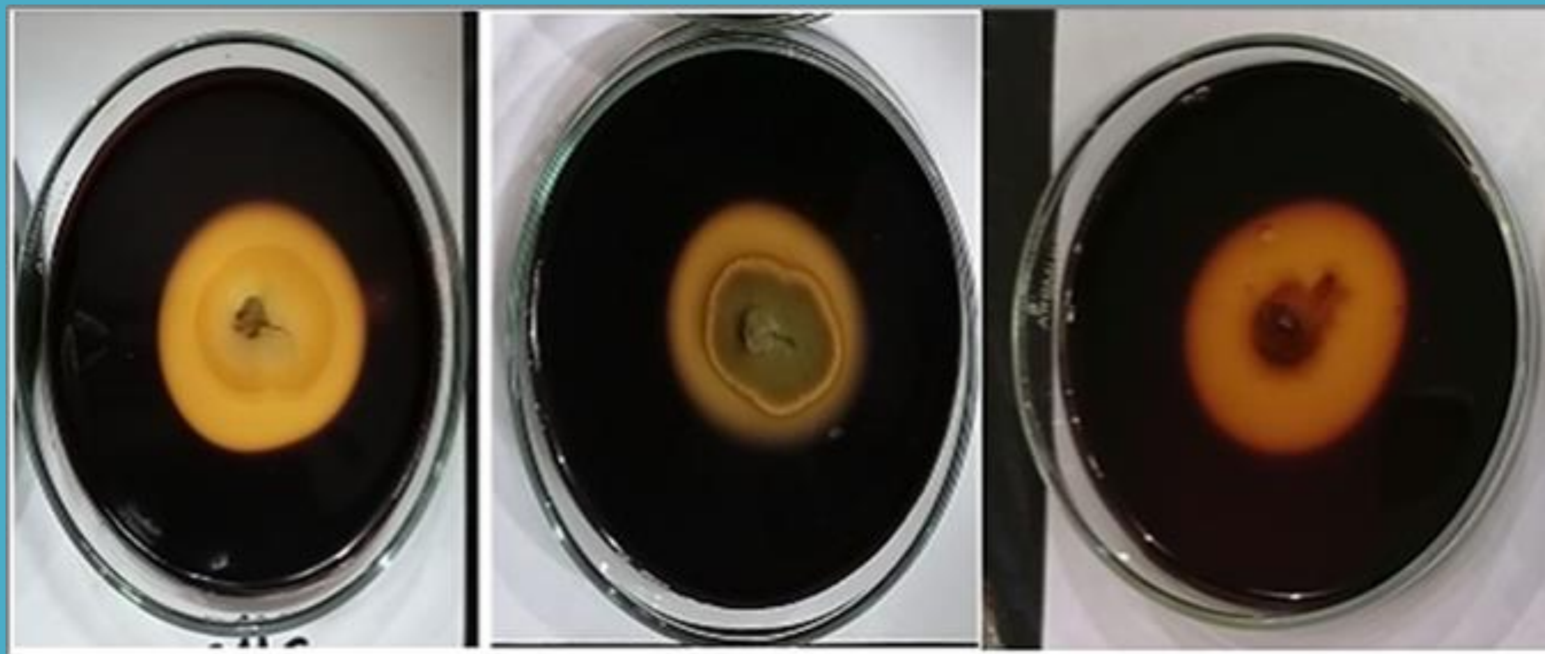
Isolates	Siderophore production (%SU)			HCN production	Inhibition % of fungal pathogen (*A. alternata*)		
NGB-WFS1	25.9	±	0.10	++	65.9	±	0.8
NGB-WFS2	3.1	±	0.05	++	59.5	±	2.4
NGB-WFS3	19.3	±	0.05	+	63.5	±	7.9
NGB-WFS4	33.7	±	0.05	++	69.1	±	0.8
NGB-WFS5	1.5	±	0.10	++	73.8	±	4.0
NGB-WFS6	33.6	±	0.55	++	64.3	±	0.8
NGB-WFS7	23.0	±	0.25	-	62.7	±	0.8
NGB-WFS8	0.0	±	0.00	++	77.0	±	0.8
NGB-WFS9	44.7	±	0.15	+	56.4	±	0.8
NGB-WFS10	61.7	±	0.05	-	59.6	±	5.6
NGB-WFS11	57.1	±	1.85	-	59.5	±	0.8
NGB-WFS12	16.8	±	0.10	++	58.0	±	4.0
NGB-WFS13	36.2	±	0.30	++	55.6	±	4.8
NGB-WFS14	17.3	±	0.30	-	52.4	±	4.8
NGB-WFE15	75.4	±	0.14	-	57.9	±	0.8
NGB-WFE16	**96.5**	±	0.43	-	76.2	±	9.5
NGB-WFE17	14.6	±	0.32	++	63.5	±	1.6
NGB-WFS18	44.4	±	0.09	+++	**87.3**	±	1.6

Control (*A. alternata*) **NGB-WFS 14** **NGB-WFE16**

Antagonistic effect of tested fungal isolates against phytopathogen Alternaria alternata in dual culture assay. (1) Control (*A. alternata*) (2) NGB-WFS 14, (3) NGB-WFE16.

Enzymatic index for hydrolytic enzymes produced by fungal isolates.

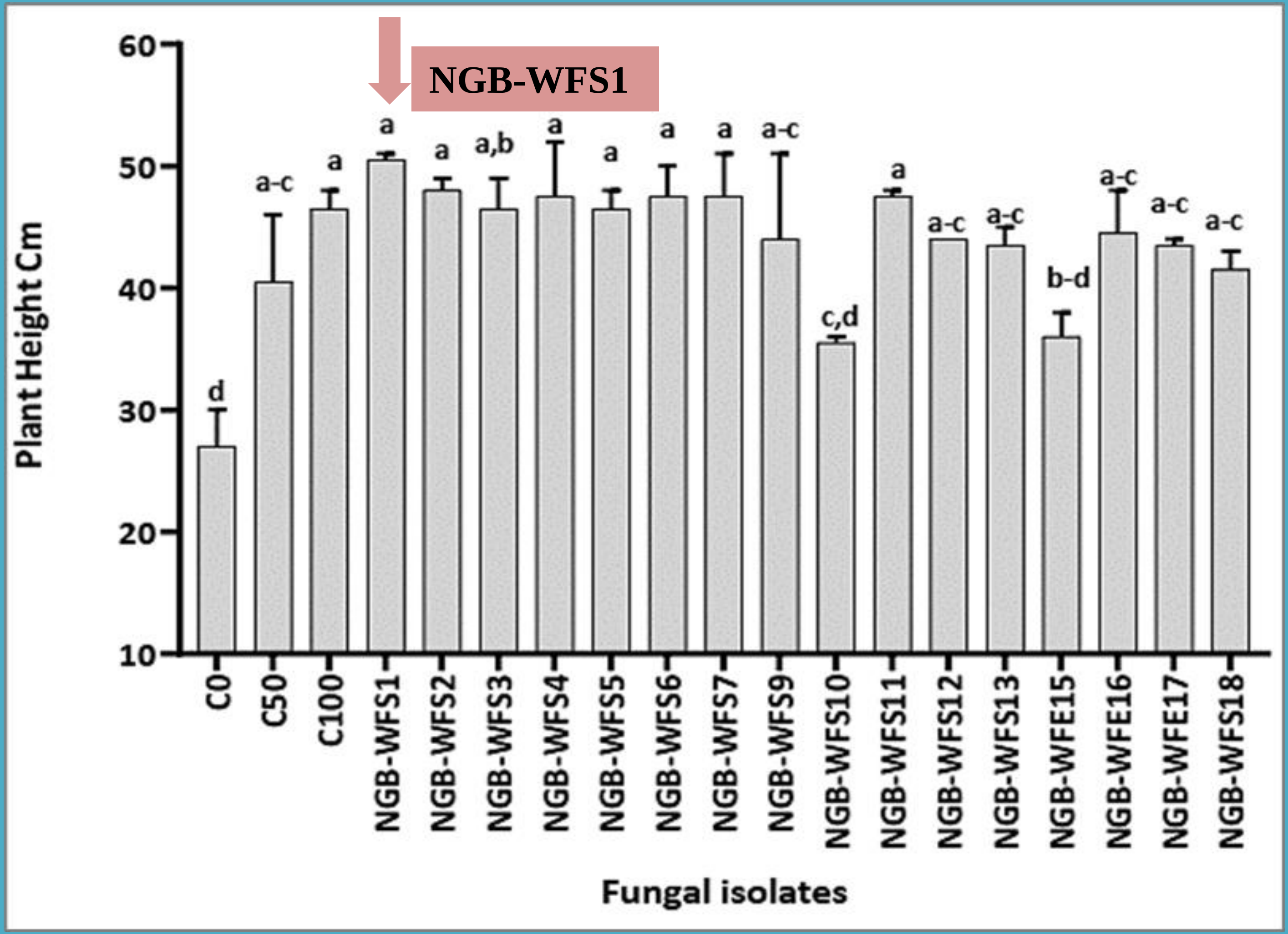
Isolate Code	Enzyme index (EI)											
	Cellulase			Xylanase			Pectinase			Chitinase		
NGB-WFS1	1.4	±	0.42	**1.8**	**±**	**0.00**	1.1	±	0.00	1.3	±	0.21
NGB-WFS2	**2.0**	**±**	**0.00**	1.5	±	0.14	2.0	±	0.00	1.2	±	0.00
NGB-WFS3	1.6	±	0.07	1.6	±	0.21	1.4	±	0.14	1.2	±	0.07
NGB-WFS4	1.0	±	0.00	1.0	±	0.00	0.0	±	0.00	1.0	±	0.00
NGB-WFS5	1.0	±	0.00	1.0	±	0.00	1.0	±	0.00	1.1	±	0.00
NGB-WFS6	1.1	±	0.00	0.0	±	0.00	0.0	±	0.00	1.0	±	0.00
NGB-WFS7	1.4	±	0.00	1.3	±	0.07	1.3	±	0.07	1.4	±	0.00
NGB-WFS8	1.1	±	0.07	1.0	±	0.00	0.0	±	0.00	1.0	±	0.00
NGB-WFS9	1.6	±	0.07	1.3	±	0.00	1.4	±	0.07	1.8	±	0.07
NGB-WFS10	1.0	±	0.00	1.1	±	0.07	0.5	±	0.71	**3.9**	**±**	**0.14**
NGB-WFS11	1.1	±	0.14	**1.8**	**±**	**0.71**	1.3	±	0.21	3.0	±	0.42
NGB-WFS12	1.6	±	0.00	1.4	±	0.21	1.5	±	0.00	1.3	±	0.00
NGB-WFS13	1.3	±	0.21	1.0	±	0.35	1.3	±	0.28	1.4	±	0.07
NGB-WFS14	1.5	±	0.07	1.3	±	0.00	1.4	±	0.14	1.0	±	0.00
NGB-WFE15	1.1	±	0.07	1.7	±	0.07	1.1	±	0.00	1.1	±	0.00
NGB-WFE16	1.3	±	0.07	0.7	±	0.92	**2.1**	**±**	**1.41**	1.0	±	0.00
NGB-WFE17	1.0	±	0.00	1.5	±	0.71	1.1	±	0.00	1.2	±	0.28
NGB-WFS18	1.0	±	0.00	1.0	±	0.00	1.9	±	0.14	1.0	±	0.00



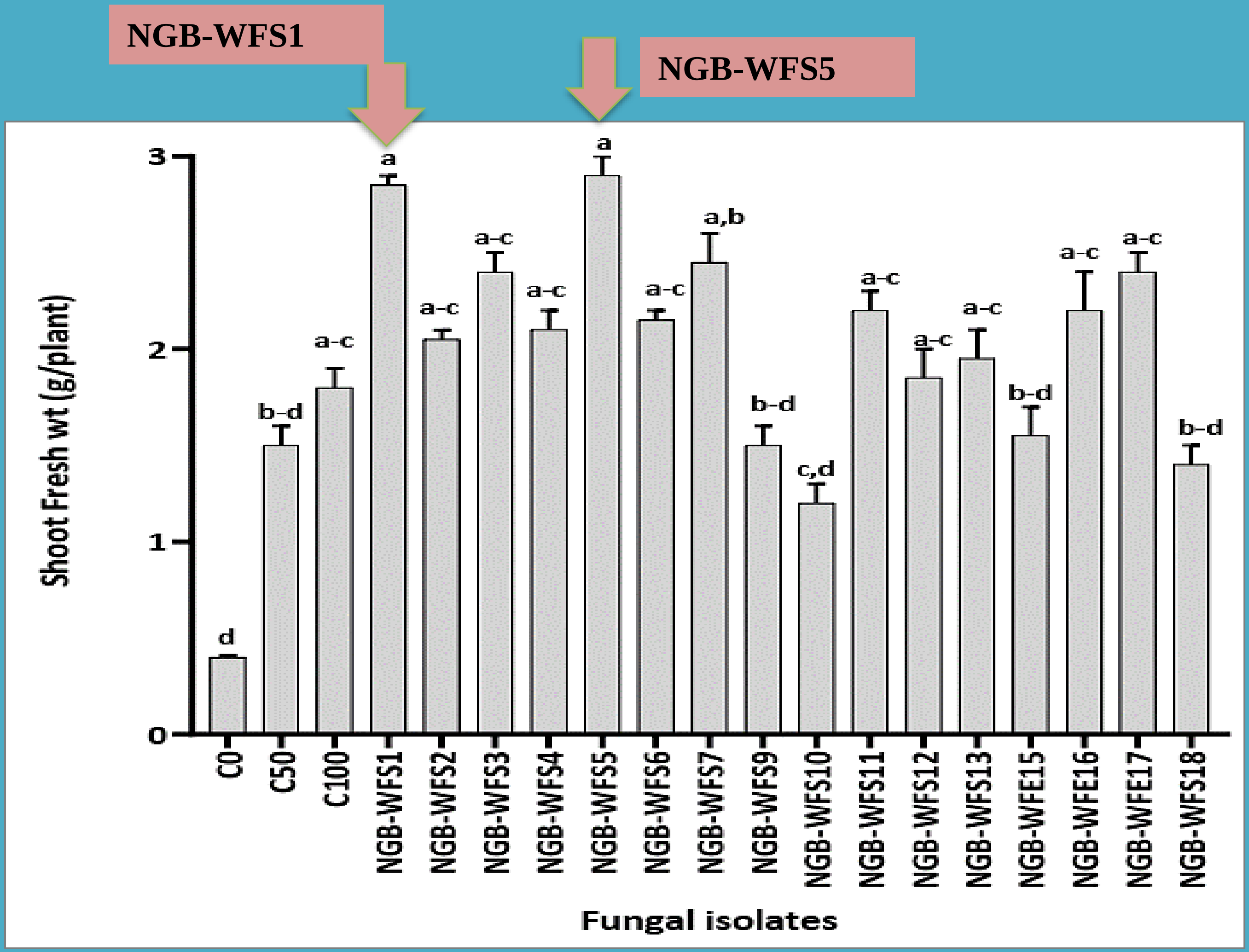
NGB-WFS2 NGB- WFS11 NGB-WFS10

Enzyme production by fungal isolates on agar plate showing a clear zone on specific medium after staining with iodine by isolate (1) NGB-WFS2 on CMC, (2) NGB- WFS11 on xylan, and (3) NGB-WFS10 on chitin amended medium.

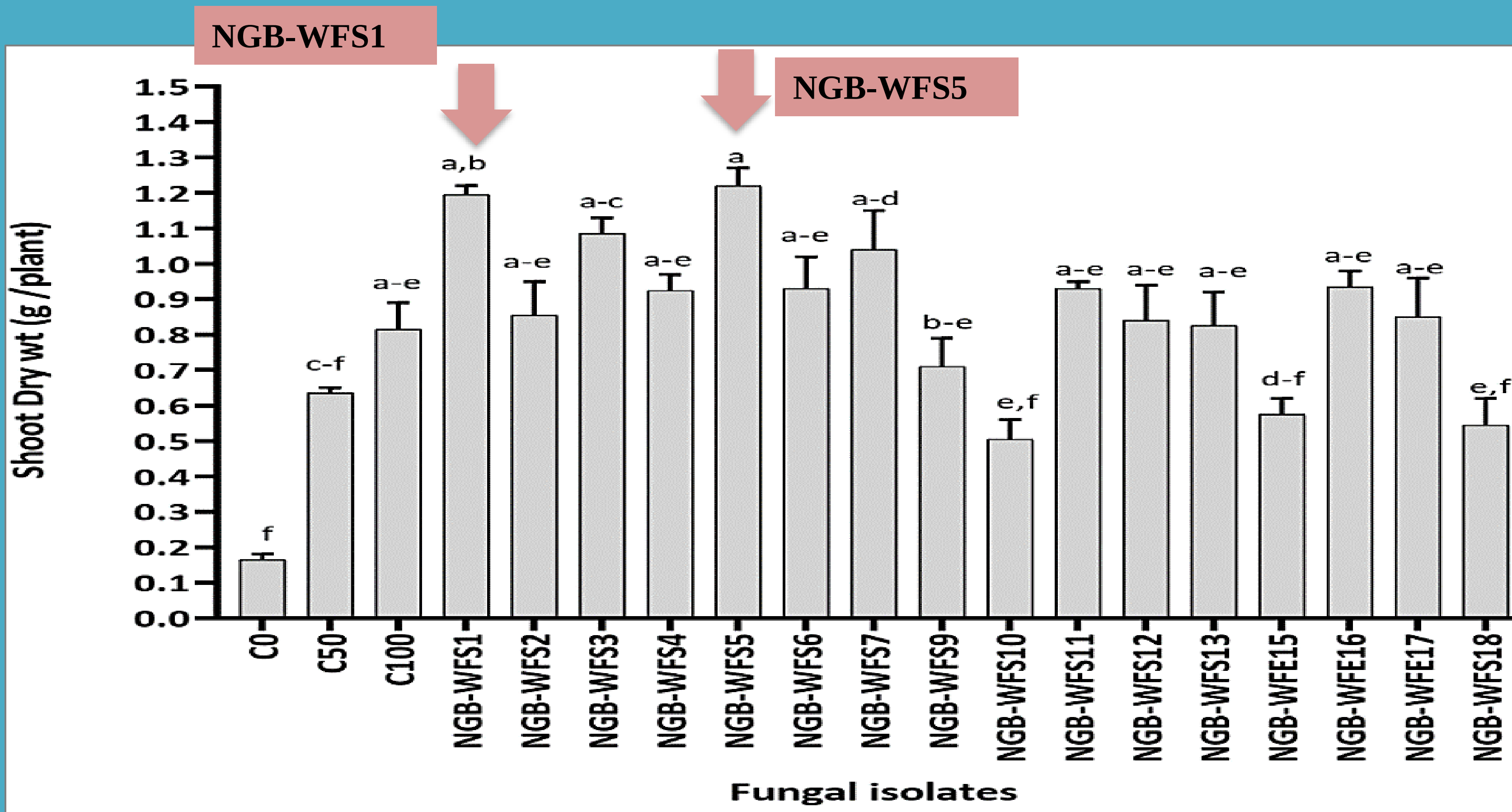
Evaluation of Active plant Growth-promoting (PGP) Isolates for Enhancement of Wheat Plants Growth in Pot Experiment under low Nitrogen (N) inputs



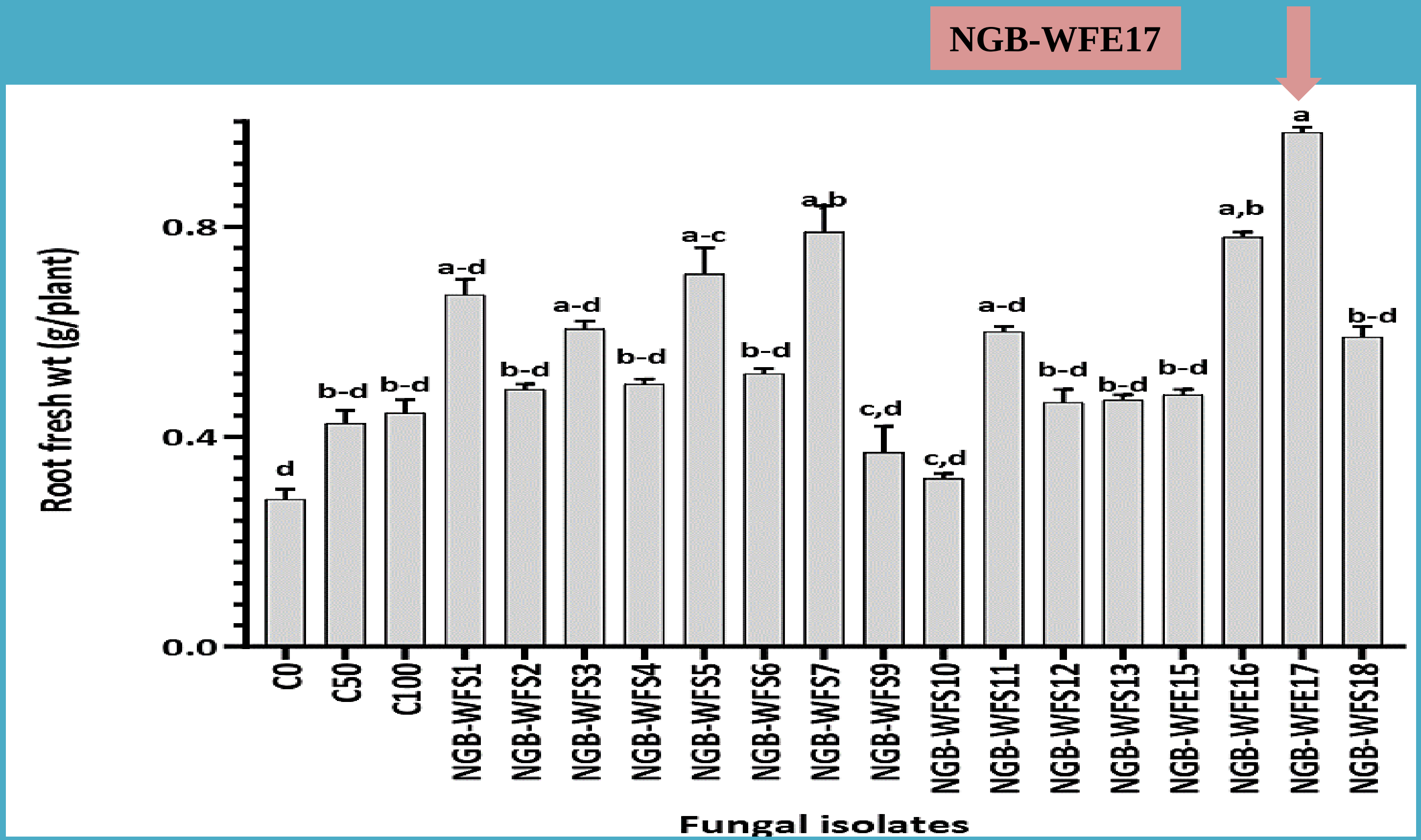
Effect of fungal inoculation on height of wheat plants. Data are presented as means of three replicates. Bars sharing different letter(s) are statistically different according to Duncan multiple range ($p \leq 0.05$).



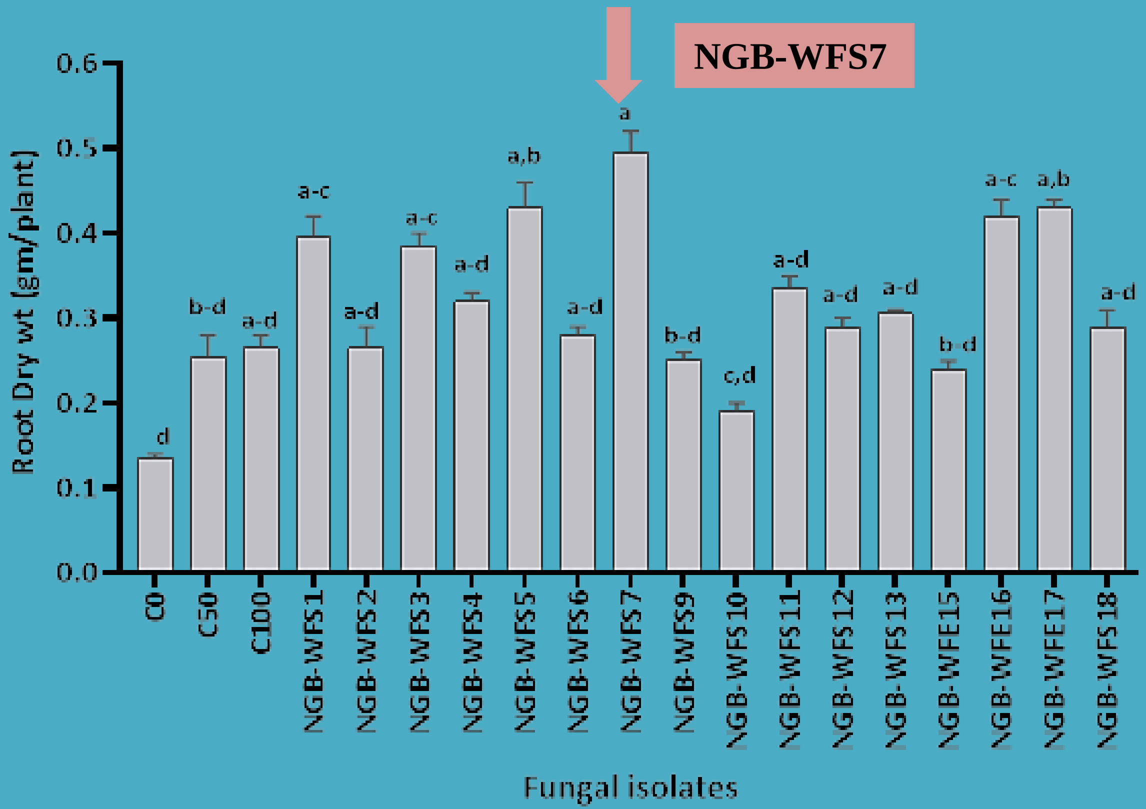
Effect of fungal inoculation on shoot fresh weight of wheat plants. Data are presented as means of three replicates. Bars sharing different letter(s) are statistically different according to Duncan multiple range ($p \leq 0.05$).



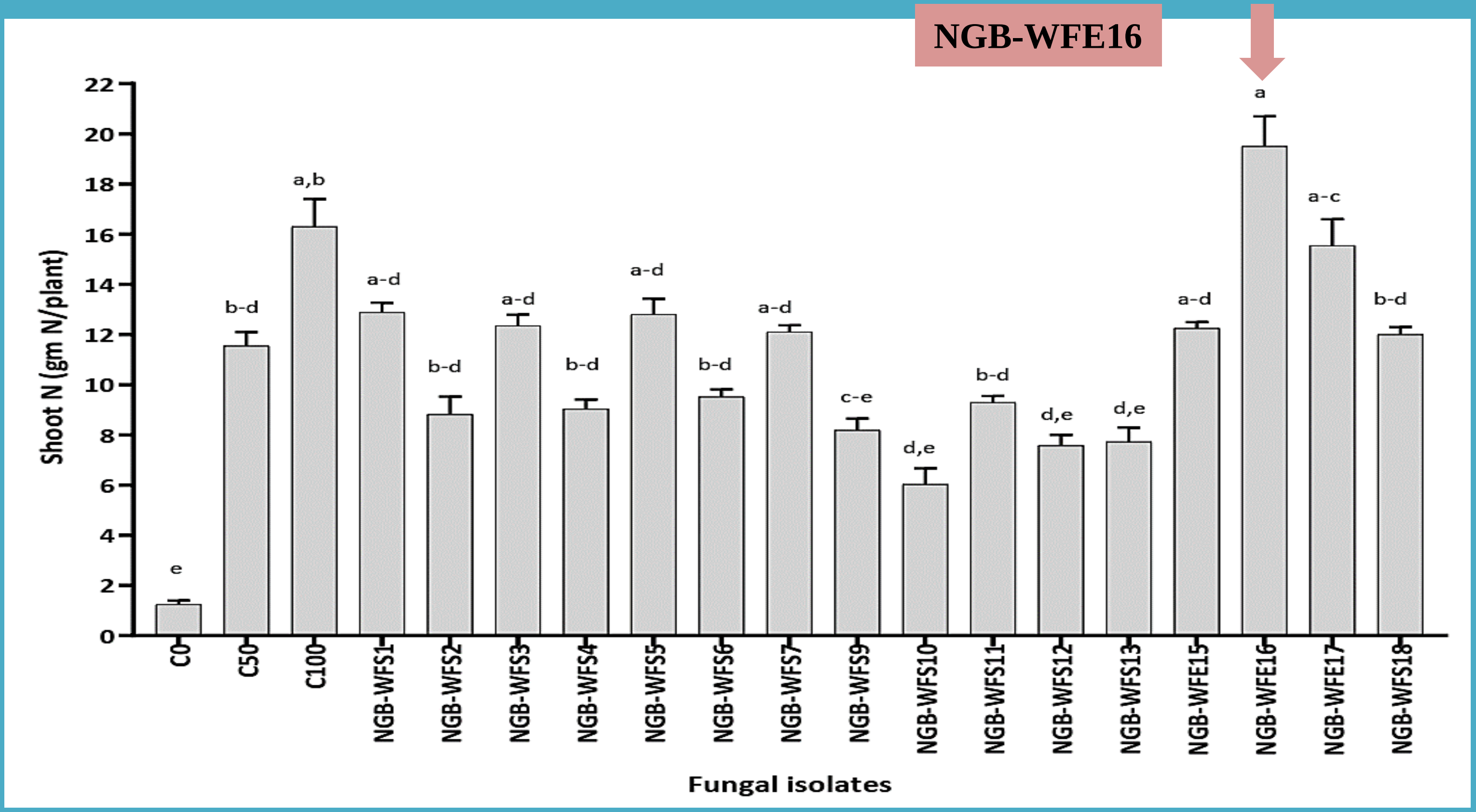
Effect of fungal inoculation on shoot dry weight of wheat plants.
Data are presented as means of three replicates. Bars sharing different letter (s) are statistically different according to Duncan multiple range ($p \leq 0.05$).



Effect of fungal inoculation on root fresh weight of wheat plants.
Data are presented as means of three replicates. Bars sharing different letter(s) are statistically different according to Duncan multiple range ($p \leq 0.05$).



Effect of fungal inoculation on root dry weight of wheat plants.
Data are presented as means of three replicates. Bars sharing different letter(s) are statistically different according to Duncan multiple range ($p \leq 0.05$).



Effect of fungal inoculation on shoot-N content of wheat plants.
Data are presented as a means of three replicates. Bars sharing different letter(s) are statistically different according to Duncan multiple range ($p \leq 0.05$).

Effect of fungal inoculation on photosynthetic pigments contents of wheat plants under greenhouse conditions

Isolate	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Total Chlorophyll (mg g^{-1})	Carotenoids (mg g^{-1})
NGB-WFS1	2.0 ^{a}	0.56 ^{ab}	2.6 $^{a-d}$	0.77 ^{ab}
NGB-WFS2	1.8 $^{a-d}$	0.43 $^{b-d}$	2.2 $^{c-g}$	0.63 $^{b-e}$
NGB-WFS3	1.7 $^{a-e}$	0.40 ^{cd}	2.1 $^{d-g}$	0.60 $^{c-e}$
NGB-WFS4	1.6 $^{a-e}$	0.38 ^{cd}	2.1 $^{d-g}$	0.58 $^{c-e}$
NGB-WFS5	1.7 $^{a-e}$	0.40 ^{cd}	2.1 $^{d-g}$	0.58 $^{c-e}$
NGB-WFS6	1.5 ^{de}	0.34 ^{de}	1.9 ^{fg}	0.54 ^{de}
NGB-WFS7	1.5 $^{c-e}$	0.37 ^{ce}	1.9 ^{fg}	0.56 ^{de}
NGB-WFS9	1.6 $^{b-e}$	0.38 ^{cd}	2.0 $^{e-g}$	0.59 $^{c-e}$
NGB-WFS10	1.7 $^{a-e}$	0.40 ^{cd}	2.1 $^{d-g}$	0.58 $^{c-e}$
NGB-WFS11	1.8 $^{a-d}$	0.45 $^{b-d}$	2.3 $^{c-f}$	0.66 $^{b-d}$
NGB-WFS12	1.6 $^{a-e}$	0.37 $^{c-e}$	2.0 $^{e-g}$	0.59 $^{c-e}$
NGB-WFS13	1.7 $^{a-e}$	0.40 ^{cd}	2.1 $^{d-g}$	0.62 $^{c-e}$
NGB-WFE15	1.9 $^{a-c}$	0.43 $^{b-d}$	**3.1 ^{a}**	0.65 $^{b-d}$
NGB-WFE16	1.8 $^{a-d}$	0.34 ^{de}	**3.0 ^{ab}**	0.59 $^{c-e}$
NGB-WFE17	1.9 $^{a-c}$	0.45 $^{b-d}$	2.7 $^{a-c}$	0.66 $^{b-d}$
NGB-WFS18	2.0 ^{ab}	0.66 ^{a}	**3.0 ^{ab}**	**0.81 ^{a}**
Control (0%N)	1.1 ^{f}	0.23 ^{e}	1.3 ^{h}	0.49 ^{e}
Control (50%N)	1.8 $^{a-e}$	0.42 $^{b-d}$	2.2 $^{c-g}$	0.63 $^{b-e}$
Control (100%N)	2.0 ^{ab}	0.52 $^{a-c}$	2.5 $^{b-e}$	0.72 $^{a-c}$

Control (0%N)

Control (50%N)

Control (100%N)

NGB-WFE16

Greenhouse experiment showed the effect of the most potent plant growth -promoting fungal isolate on the growth performance of wheat plants.

Future work and recommendation

- **Wild plants is a promising source for plant growth promoting fungi**

- **isolation sources affect significantly on the PGP traits of microorganisms.**

- **Wild plants associated microbes are efficient alternatives for achieving sustainable agriculture and reducing the reliance on chemical N-fertilizers.**

- **Make further studies to explore the effect of this inoculants as PGP microorganisms under field conditions.**

- **Formulate the effective PGP fungi of this study as bio-inoculants for wheat crop enhancement.**


Thank you