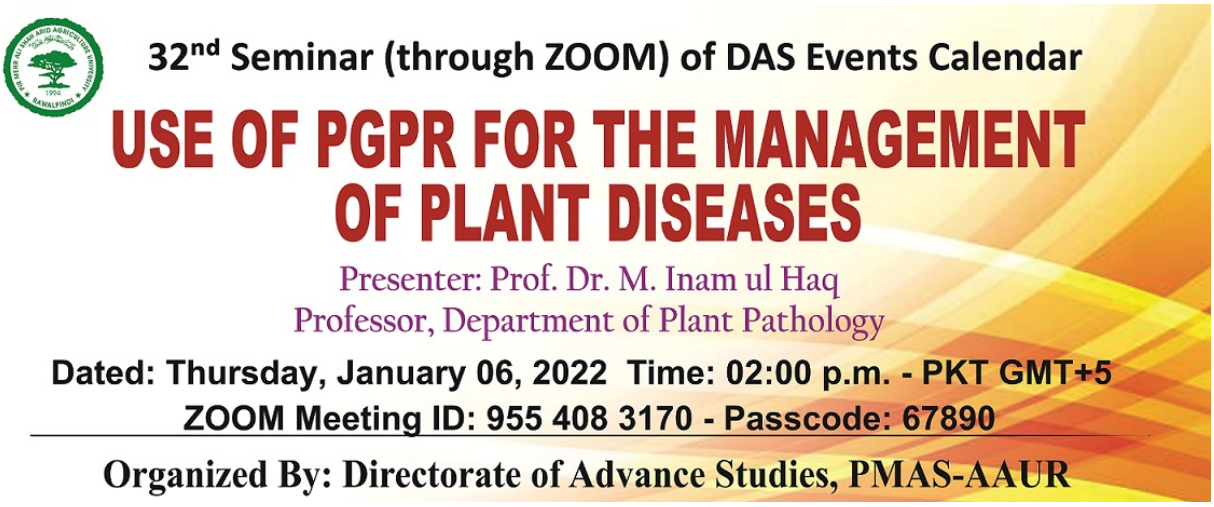
DIRECTORATE OF ADVANCED STUDIES
EVENT CATALOGUE
2021

32ND SEMINAR OF DAS EVENTS CALENDAR – 2021

USE OF PGPR FOR THE MANAGEMENT OF PLANT DISEASES



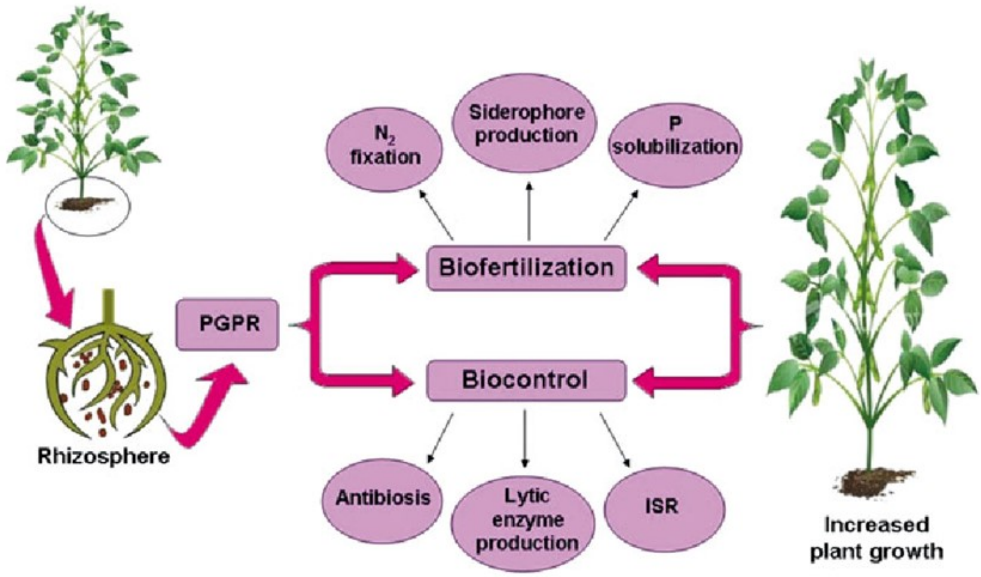
ACTIVITIES

PLANT GROWTH PROMOTING RHIZOBACTERIA

- Rhizobacteria colonizing the root surfaces and closely adhering soil interface, the rhizosphere (Kloepper, 1980).

Mechanisms of PGPR

- Increased mineral nutrient solubilization and nitrogen fixation (Glick et al., 1995)
- Repression of soil-borne pathogens (Glick et al., 1995)
- Improving plant stress tolerance to drought, salinity, and metal toxicity
- Production of phytohormones (Hu et al., 2005)
- Induction of systemic resistance (Huang et al., 2009)



OVERVIEW OF DISEASES AND PGPR

- *Pseudomonas fluorescens* controls downy mildew caused by *Sclerospora graminicola*
- *Bacillus* spp. can control bacterial leaf blight of rice caused by *Xanthomonas oryzae*
- *Pseudomonas* and *Bacillus* species are used as biological control agents against pests and plant diseases of potato and sugarbeet

COMMERCIAL PGPR PRODUCTS

Product	PGPR species	Target Crops
Nodulator	*Bradyrhizobium japonicum*	legumes
Diegall/Galltroll	*Agrobacterium radiobacter*	Fruits, nut, ornamentals,
Pixplus	*Bacillus cereus*	Cotton
Ballard	Bacillus pumilus	Oat trees, nurseries and landscapes
Epic, Kodiak, Subtilex	*Bacillus subtilis*	Peanut, beans, rice, pea, soyabean, barley
Blue circle, Intercept	*Burkholderia cepacia*	Alfalfa, barley, beans, cotton, maize, pea, sorghum
Bioject, Spotless	*Pseudomonas auerofaciens*	Turf and other crops
AtEze	*Pseudomonas chloroaphis*	Ornamentals and vegetable
Blight Ban, Conquer	*Pseudomonas fluorescens*	Almond, Cherry, potato

RESEARCH CONDUCTED SO FAR IN OUR LAB

RESEARCH STUDENTS

- Number of students have completed doctoral studies under my supervision

Sr. No.	Name	Title	Year
1	Dr. M. Ibrahim Tahir	Induction of resistance against bacterial wilt in potato	2016
2	Dr. Muhammad Raees	Surveillance and characterization of bacterial canker of Stone Fruits Using Biochemical and Molecular Methods	2018
3	Dr. Sajjad Hyder	Bio-pesticide development for the control of *Pythium* and *Phytophthora* spp. causing damping off disease in tomato and chilli	2019
4	Dr. M. Shah Jahan	Evaluation of biochar as a carrier material of antagonistic rhizobacteria for the management of root pathogenic fungi of chickpea	2019
5	Dr. Shazia Shahzaman	Chickpea root disease management using antagonistic rhizobacteria	2016
6	Dr. Shagufta Bibi	Screening and characterization of Rhizobacteria antagonistic to *Pseudomonas syringae* affecting stone fruits in Punjab and KPK	2019
7	Dr. Javaid A Tariq	Utilization of Plant Growth Promoting Rhizobacteria for the management of Rot Knot Nematode in Tomato	2008
8	Mphil Students	70 Students worked in different aspects of PGPR	

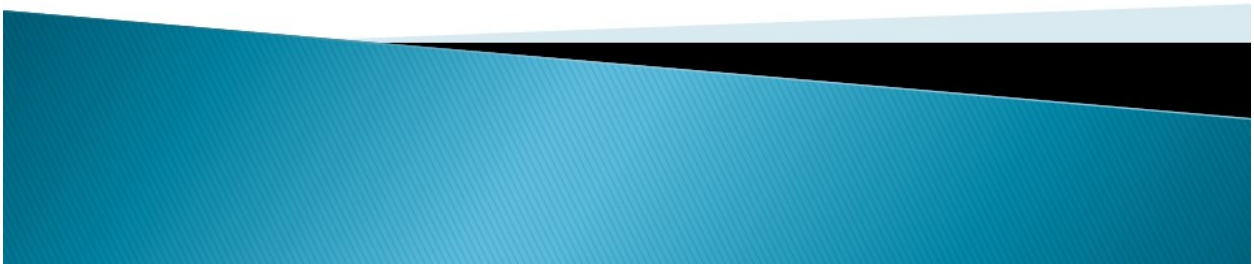
OUTCOMES OF THE STUDIES

- Number of antagonists are available that have proven efficiency against several important pathogens

- *Ralstonia solanacearum* (bacterial wilt of potato and tomato)
- *Pseudomonas syringae*
- Chickpea root fungal pathogens i.e. Fusarium, Verticillium
- *Pythium* spp. in chilli
- *Phytophthora* spp. in chilli
- Viral Pathogens
- Nematode Pathogens

INDUCTION OF RESISTANCE AGAINST BACTERIAL WILT IN POTATO (*Solanum tuberosum* L.)

Muhammad Ibrahim Tahir Ph D. Plant Pathology



OBJECTIVES

- Screening of antagonistic rhizobacteria against *R. solanacearum* and their evaluation in *In vitro* and in greenhouse
- Quantification of defense related enzymes at different intervals
- Analysis of salicylic and jasmonic acid responsive genes expression by RT-PCR for monitoring induction of systemic resistance in PGPR treated plants

RESULTS

SURVEY

District	Disease Incidence (%)	Disease Prevalence (%)	Severity Scale
Okara	21.4 ± 4.5	100	2–4
Sahiwal	19.66 ± 5.7	100	2–3
Sialkot	16.8 ± 3.9	100	2–3
Jhang	17.4 ± 2.2	100	1–3
Kasur	16.5 ± 5.8	100	1–3
Pakpattan	17.8 ± 4.6	100	2–4

PATHOGENICITY TEST OF *R. solanacearum*

Isolates	Virulence	Isolates	Virulence
Rs1	+	Rs14	–
Rs2	–	Rs15	+
Rs3	++	Rs16	–
Rs4	–	Rs17	+++
Rs5	–	Rs18	++
Rs6	++	Rs19	–
Rs7	++	Rs20	+
Rs8	++	Rs21	–
Rs9	+++	Rs22	–
Rs10	++	Rs23	–
Rs11	–	Rs24	++
Rs12	–	Rs25	++
Rs13	–	Rs26	–

PATHOGENICITY TEST OF *R. solanacearum*

Isolates	Virulence
Rs27	+
Rs28	++
Rs29	-
Rs30	-
Rs31	-
Rs32	-
Rs33	-
Rs34	+
Rs35	+

Isolates	Virulence
Rs36	+
Rs37	++
Rs38	-
Rs39	-
Rs40	-
Rs41	++
Rs42	-
Rs43	+++

ISOLATION OF RHIZOBACTERIA

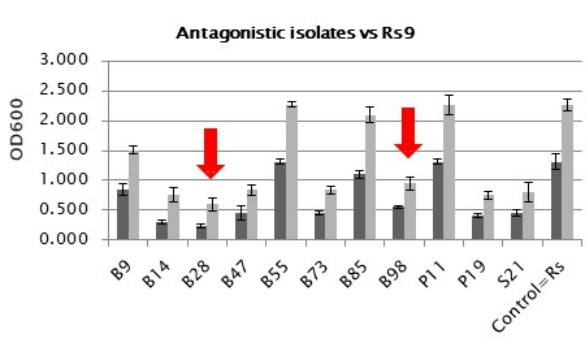
Areas	Bacillus sp. isolates	Pseudomonas isolates	Serratia isolates	Total
Okara	28	11	4	**43**
Sahiwal	15	21	6	**42**
Sialkot	11	13	4	**28**
Jhang	19	8	12	**39**
Kasur	21	9	8	**38**
Pakpattan	7	14	10	**31**
Total	**101**	**76**	**44**	**221**

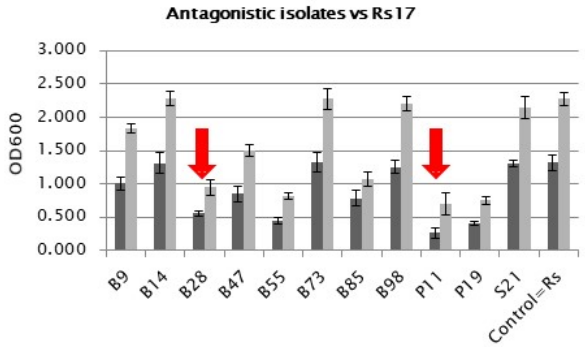
Screening of Rhizobacteria Against *R. solanacearum*

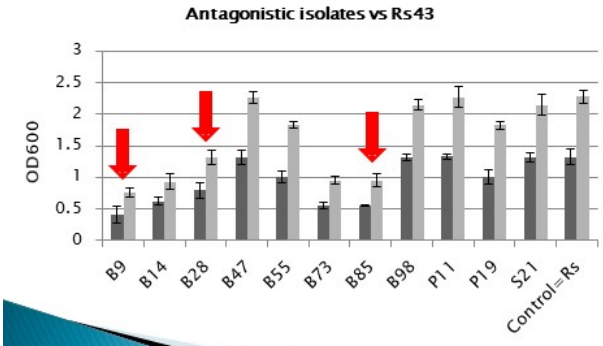
- Rhizobacterial isolates having zone of inhibition greater than 8 mm against either of *R. solanacearum* isolates

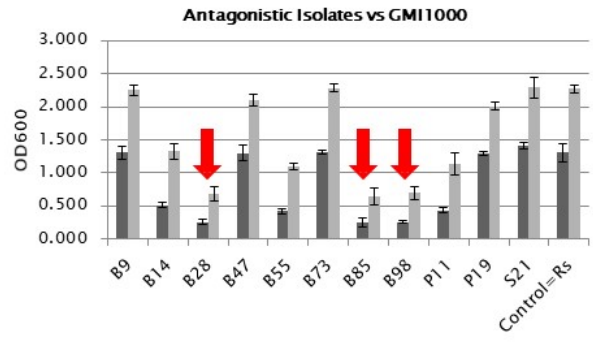
Isolates	Zone of inhibition (mm)			
	Rs9	Rs17	Rs43	GMI 1000
B9	3.4 ± 2.4 e	2.5 ± 1 e	**9.9 ± 3.2 a**	0 e
B14	10.6 ± 1.5 b	0 f	8.5 ± 3.0 ab	4.8 ± 1.5 cd
B28	**12.3 ± 2.3 a**	7.3 ± 2.2 c	4.6 ± 2.4 c	**10.6 ± 2.2 ab**
B47	8.5 ± 1.2 c	3.7 ± 2.7 de	0 e	0 e
B55	0 e	9.1 ± 2.3 ab	2.8 ± 1.2 d	6.4 ± 2.2 c
B73	9.6 ± 3.0 bc	0 f	7.8 ± 1.9 b	0 e
B85	0 e	4.5 ± 2.2 d	8.3 ± 1.9 b	**11.7 ± 3.3 a**
B98	7.9 ± 2.8 d	0 f	0 e	**10.3 ± 4.1 ab**
P11	0 e	**10.2** ± 2.8 a	0 e	5.6 ± 3.4 c
P19	9.8 ± 1.9 bc	9.5 ± 1.8 ab	2.5 ± 1.0 d	0 e
S21	8.8 ± 2.8 c	0 f	0 e	0 e
Control	0 e	0 f	0 e	0 e

Comparison of culture filtrate on *R. solanacearum* isolates growth









PLANT GROWTH PROMOTING TRAITS OF ANTAGONISTS

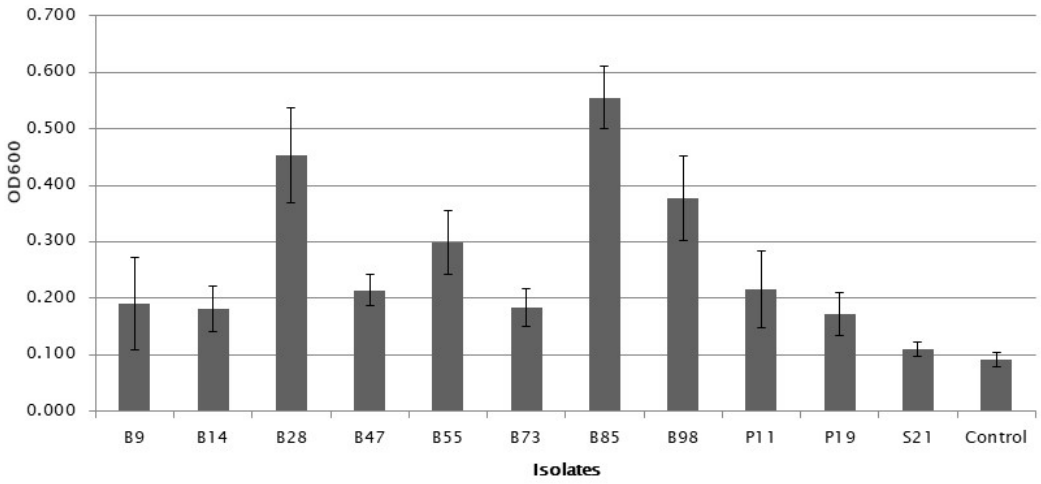
Isolates	*Siderophore production	P– solubilization	IAA production (µg/ml)	**Ammonia production	HCN Production	Chitinase production
B9	++	–	4 ± 0.41	+	-	-
B14	–	–	4.50 ± 0.45	+	-	-
B28	+++	+	**50 ± 3.5**	+	-	+
B47	+	–	4 ± 0.29	+	-	-
B55	–	–	12 ± 1.0	–	-	+
B73	–	–	3 .3 ± 1.1	+	-	+
B85	++	+	20 ± 0.24	+	-	+
B98	++	–	5.5 ± 0.21	+	-	+
P11	+	–	3.8 ± 1.5	–	-	-
P19	+	–	8.5 ± 2.8	+	-	-
S21	+	–	0	–	-	+
Control	–	–	0	–	-	-

* Strong (≥ 5mm halozone represented as '+++'), weak (2-5 mm represented as '++'), slight (1-2mm represented as '+') and non-siderophore producers as '-'

** Ammonia production present '+', absent '-'

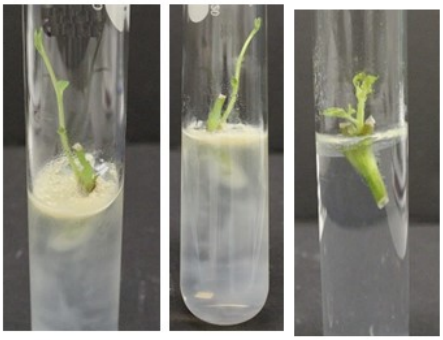
PLANT GROWTH PROMOTING TRAITS OF ANTAGONISTS

- Biofilm formation ability



PLANT GROWTH PROMOTING TRAITS OF ANTAGONISTS

- Root colonizing ability



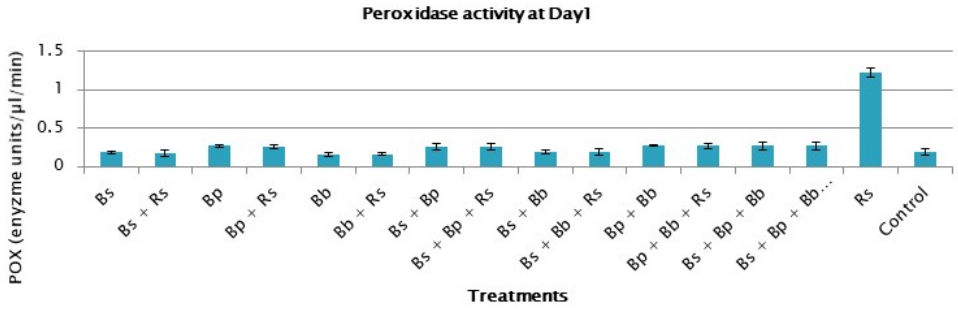
B85 B28 B98

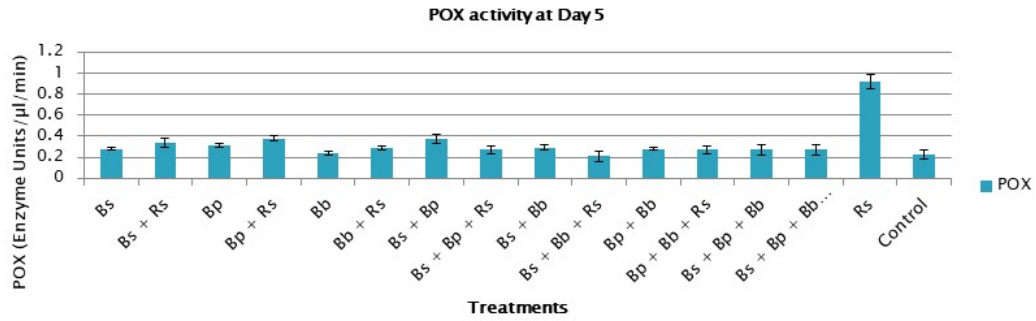
Isolates	Root Colonizing ability	Cfu/ml
B9	–	4
B14	–	6
B28	++	23
B47	–	4
B55	+	11
B73	–	5
B85	+++	31
B98	+	17
P11	+	8
P19	–	6
S21	–	3
Control	–	0

GREENHOUSE EVALUATION OF PGPR

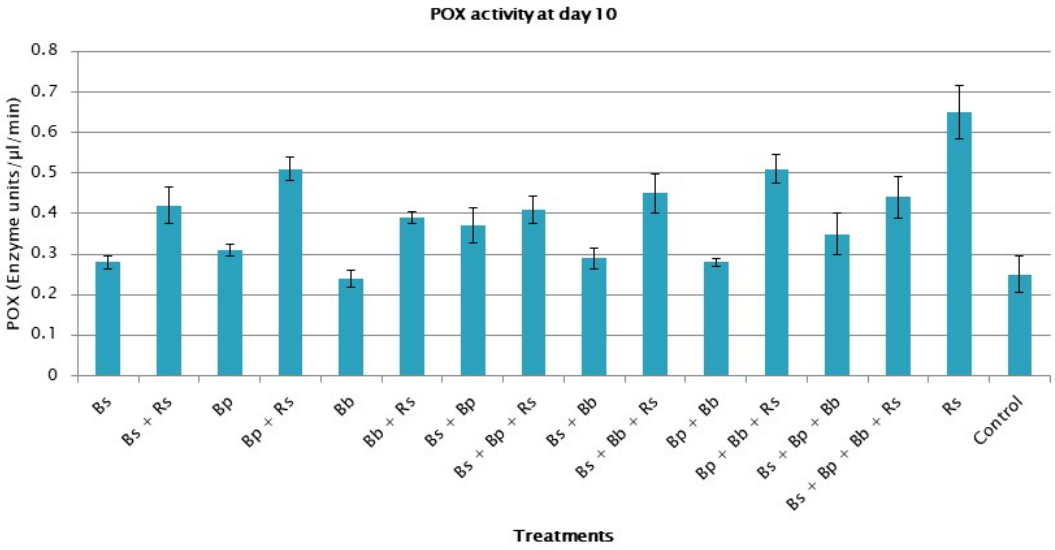
Treatments	Disease Incidence (%)	Shoot height (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
Bs	0 f	27.33 ab	16.52 ab	2.54 ab
Bs + Rs	16.4 c	26.10 bcd	15.65 bc	2.45 b
Bp	0 f	25.45 cde	15.39 bc	2.25 d
Bp + Rs	14.80 d	25.41 cde	15.48 bc	2.21 d
Bb	0 f	25.13 bcd	15.61 bc	2.23 d
Bb + Rs	18 b	24.85 bcd	15.60 bc	2.24 d
Bs + Bp	0 f	28.12 abc	15.61 bc	2.43 b
Bs + Bp + Rs	11.40 e	27.45 bcd	15.35 bc	2.41 b
Bs + Bb	0 f	27.33 bcd	15.21 bc	2.46 b
Bs + Bb + Rs	14.40 d	26.25 ab	15.61 bc	2.47 b
Bp + Bb	0 f	27.66 bcd	15.62 bc	2.20 d
Bp + Bb + Rs	13.80 d	26.60 bcd	15.64 bc	2.24 cd
Bs + Bp + Bb	0 f	**29.11 a**	**17.38 a**	**2.67 a**
Bs + Bp + Bb + Rs	9.60 e	27.20 ab	16.50 ab	2.54 ab
Rs	51 a	17.33 f	7.98 d	1.14 e
Control	0 f	23.33 e	15.26 c	2.18 d

QUANTIFICATION OF DEFENSE ENZYMES



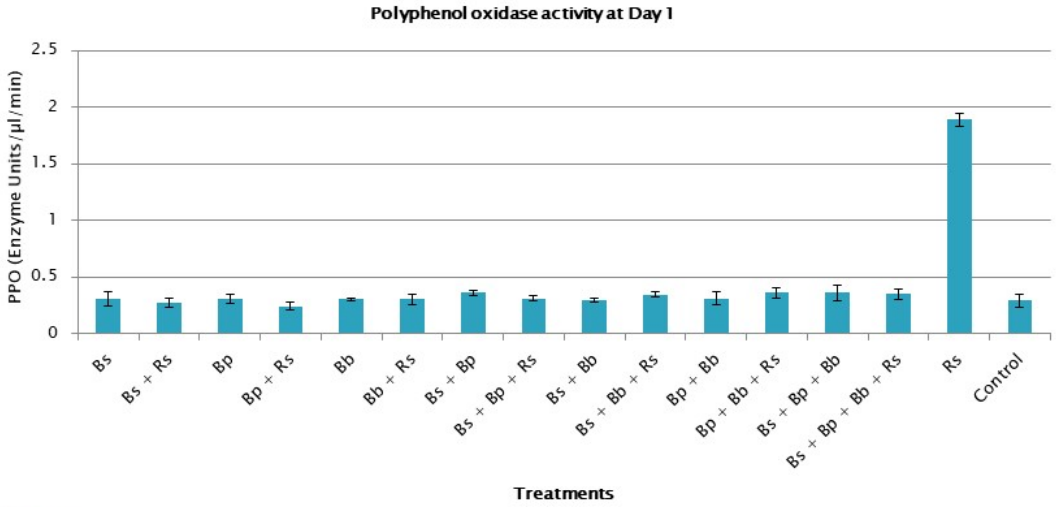


QUANTIFICATION OF DEFENSE ENZYMES



QUANTIFICATION OF DEFENSE ENZYMES

- Polyphenol oxidase (PPO)



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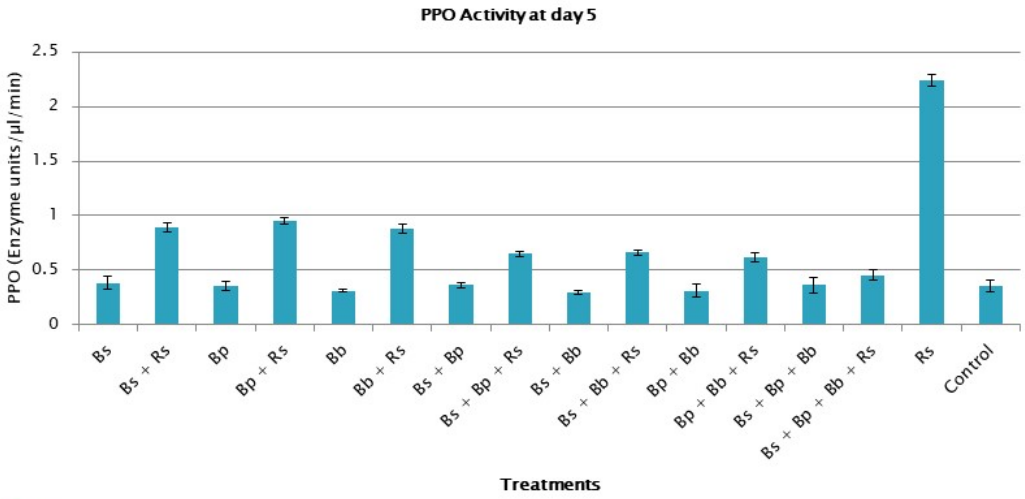
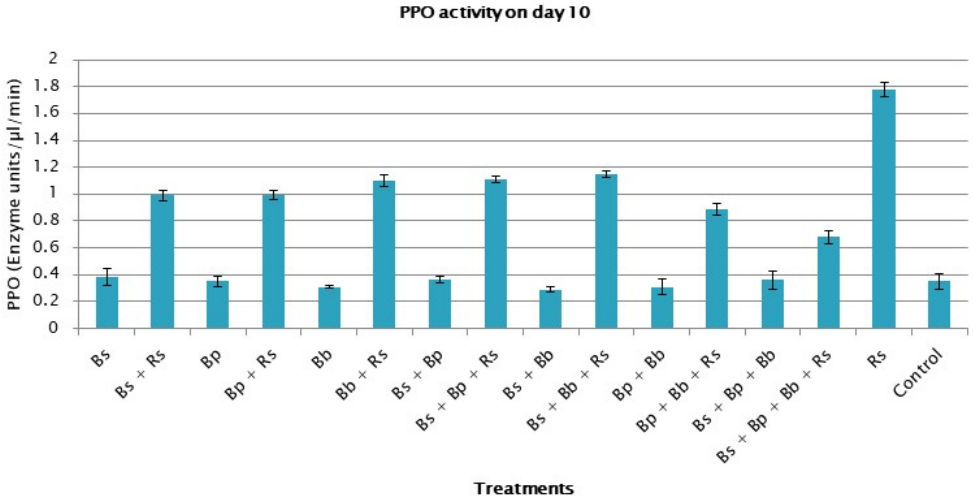
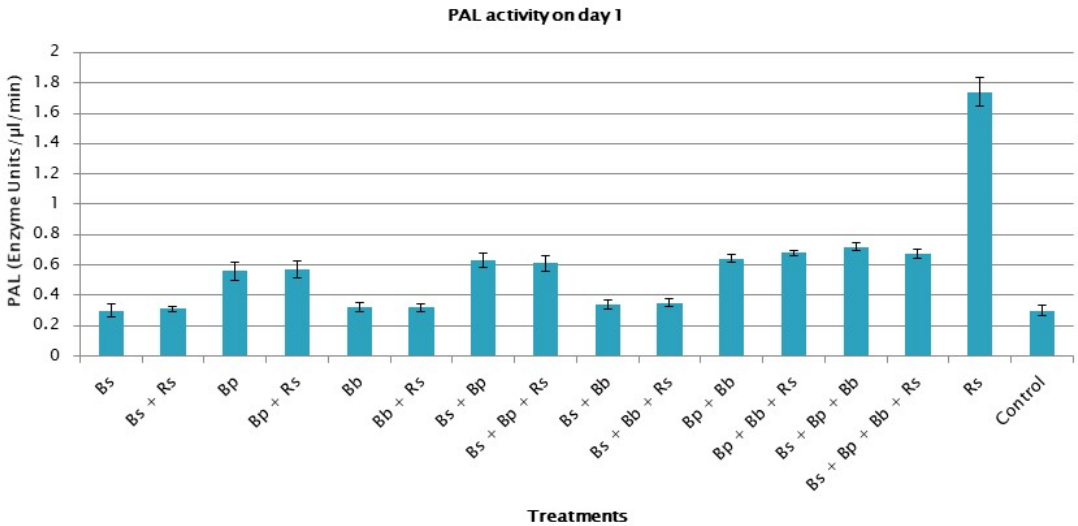


QUANTIFICATION OF DEFENSE ENZYMES



QUANTIFICATION OF DEFENSE ENZYMES

- Phenylalanine ammonia lyase (PAL)



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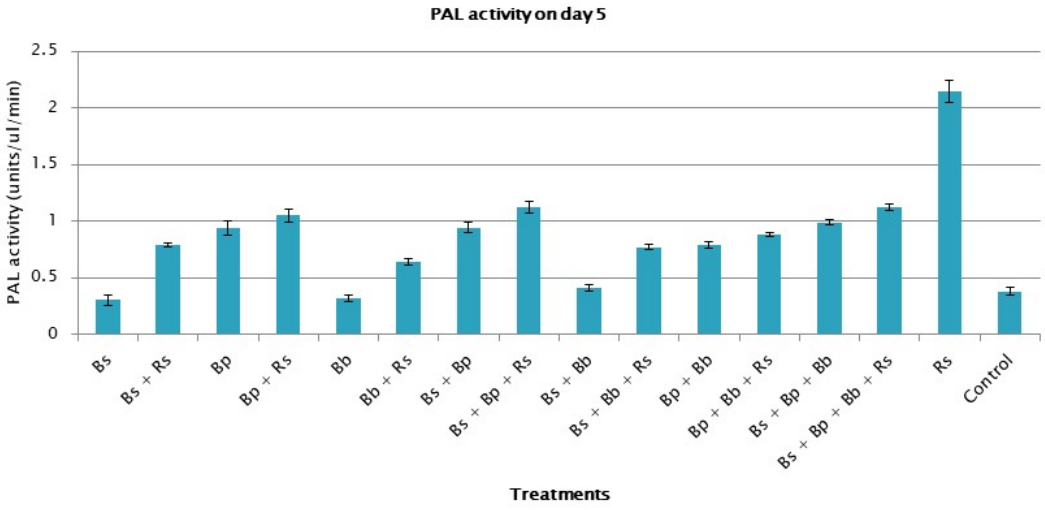

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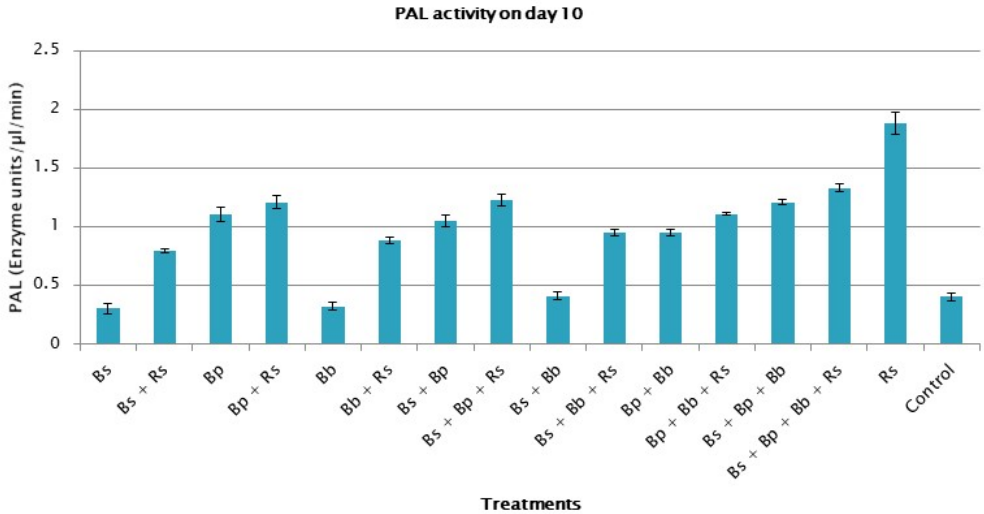

SUMMARY

- Highest disease incidence was found at Okara and Sahiwal (21 and 20% respectively) with severity scale ranging from 2-4
- From 221 rhizobacterial isolates, 11 isolates were found antagonistic against *R. solanacearum* among which Bacillus isolates were more effective
- Induced resistance by *B. subtilis* subsp. *inaquosorum* B28 and *B. pumilus* B85 have activated plant defense mechanism and it has reduced the disease incidence to as low as 9% as compared to control (51%).
- In combined application (Bs + Bp + Bb), PGPR are more effective as individual characters of each isolate have contributed towards better plant growth environment.

Evaluation of Biochar as a Carrier Material of Antagonistic Rhizobacteria for the Management of Root Pathogenic Fungi of Chickpea

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PhD Scholar- Plant Pathology
12-Arid-480

What is Biochar?

Biochar is the porous carbonaceous solid produced by thermochemical conversion of organic materials in an oxygen depleted atmosphere (Shackley & Sohi, 2010).

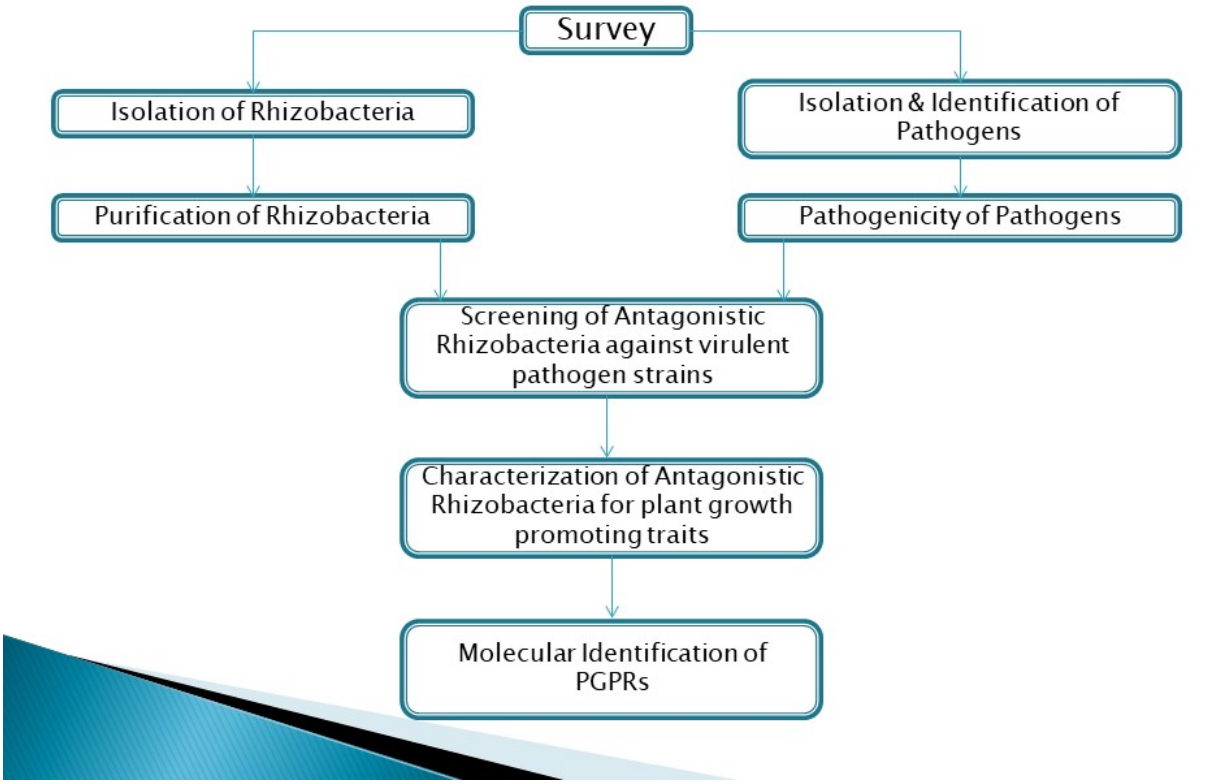
- High stability (structure changes)
- Highly absorbent (retain nutrients, chemicals)

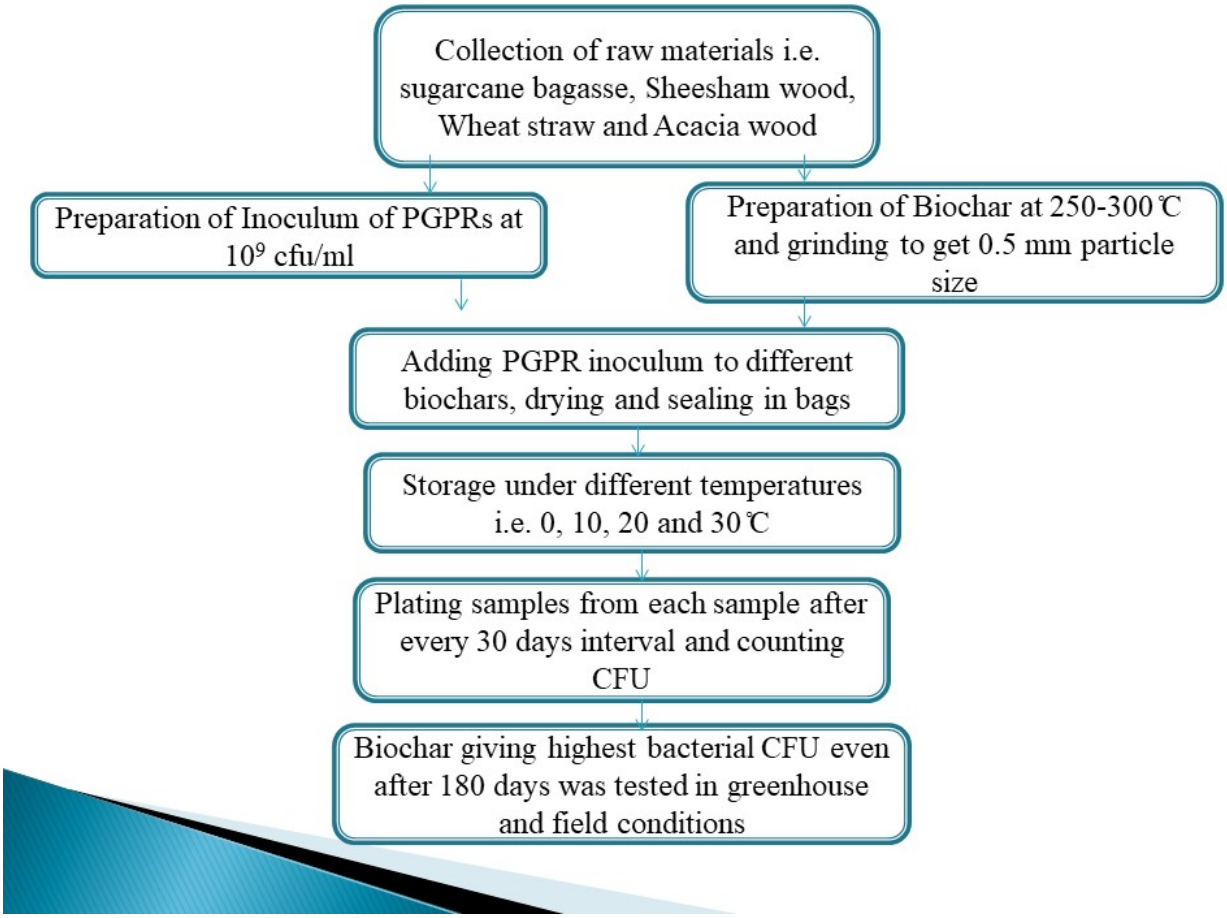
(Liang *et al*., 2006)

OBJECTIVES

- Determine the current status of fungal root diseases of chickpea in Layyah and Bhakkar districts of Punjab.
- Isolate and screen the antagonistic rhizobacteria inhibiting chickpea root fungal pathogens
- Evaluation of different biochar as a carrier of antagonistic rhizobacteria for its shelf life
- Assessing the efficacy of biochar with and without antagonistic rhizobacteria against root pathogenic fungi of chickpea under greenhouse conditions.

Layout of Study 1 and study 2



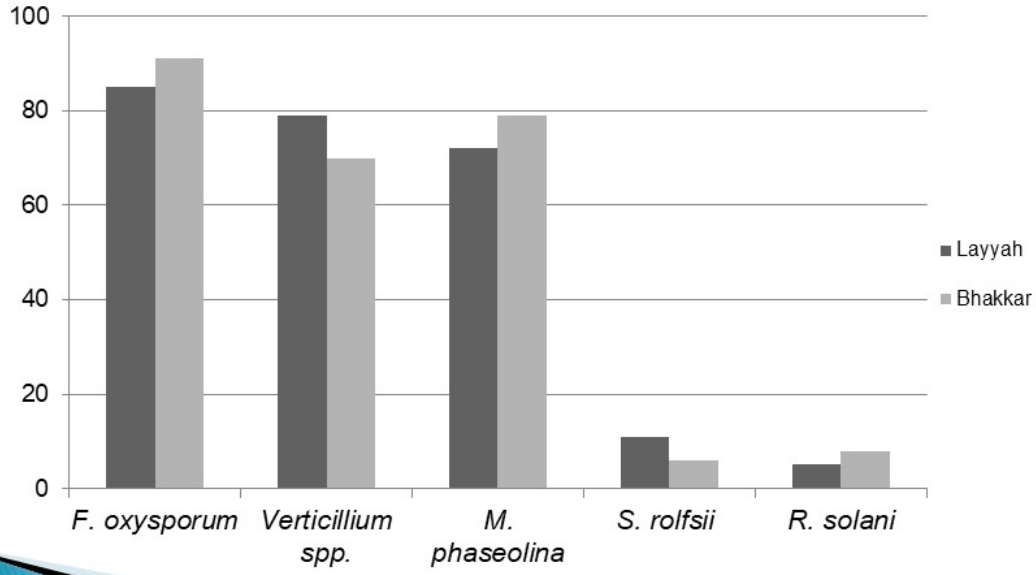


Survey Results

District	Areas	2013-14			2014-15			Mean DI (%)
		DI (%)	DP (%)	DS range	DI (%)	DP (%)	DS range	
Layyah	Sahoo wala	56	100	1-4	48	100	1-4	60.05±6.74
	Shah wala	58	100	2-4	54	100	2-4	
	Turkey wala	70	100	2-4	62	100	2-4	
	Sobay wal	66	100	2-4	70	100	2-3	
	Asar wala	72	100	2-4	76	100	2-4	
	Bhular wal	58	100	1-3	52	100	1-4	
	Bhalia wala	56	100	2-4	58	100	2-4	
	Sheiro wal	62	100	2-4	66	100	2-4	
	Yasin wal	70	100	2-4	68	100	2-4	
	Janay wal	54	100	1-4	62	100	1-4	
	Mochi wala	60	100	1-4	56	100	1-4	
	Shumar wala	62	100	2-4	54	100	2-4	
	Asar gillani wala	54	100	2-3	52	100	2-4	
	Okmay wala	56	100	2-3	54	100	2-3	
	Mochi moor	52	100	2-4	56	100	2-4	
	Lunger wala	56	100	2-4	62	100	2-3	
	Dera nawab sewag	70	100	1-4	60	100	1-3	
	Shumar moor	68	100	2-4	56	100	2-4	
	Darbar mai wleet	68	100	2-4	52	100	2-4	
	Manjhi wala	60	100	1-4	56	100	1-4	
	Mean	**61.4±6.43**	**100**		**58.7±7.06**	**100**		

District	Areas	2013-14			2014-15			Mean DI (%)
		DI (%)	DP (%)	DS range	DI (%)	DP (%)	DS range	
Bhakkar	Tehsil Mankeraa	48	100	1-4	54	100	1-4	57.5±6.78
	Mani	56	100	1-4	52	100	1-4	
	Kuriya	62	100	1-4	56	100	1-4	
	Lungha wala	56	100	2-4	62	100	2-4	
	Kiri wala	56	100	2-3	50	100	2-3	
	Pati blinda	68	100	1-4	64	100	1-4	
	Dagar shada	76	100	2-4	70	100	2-4	
	Wig sadar	50	100	2-4	48	100	2-4	
	Khanser	52	100	2-3	50	100	2-4	
	Dullewala	56	100	1-3	62	100	1-3	
	Jundan wala	58	100	1-4	60	100	1-4	
	Magasi wala	66	100	2-4	62	100	2-4	
	Thal Hayder abad	62	100	2-3	64	100	2-4	
	Tehsil deria khan	54	100	2-4	52	100	2-4	
	Maibal sharif	56	100	2-4	50	100	2-4	
	Tesil kaloor koot	64	100	2-4	56	100	2-4	
	Chandni chowk	66	100	1-3	54	100	1-3	
	Bhatti wala	56	100	2-4	50	100	2-3	
	Panj Girain	48	100	2-3	50	100	2-4	
	Mian Punja	60	100	1-4	64	100	1-4	
	Mean	**58.5±7.10**	**100**		**56.5±6.45**	**100**		

Frequency of chickpea root fungal pathogens from samples collected from Layyah and Bhakkar



Pathogenicity Tests Results

Fungal pathogen	Total isolates	Highly pathogenic	Slightly pathogenic	Non-pathogenic
F. oxysporum	72	12	45	15
Verticillium spp.	34	7	21	6
M. phaseolina	33	5	18	10
S. rolfsii	10	0	8	2
R. solani	6	0	6	0
Total	155	24	98	33

Evaluation of Rhizobacteria for Antagonism

Isolate/Pathogen	Zone of inhibition (mm)				
	F. oxysporum	*F. solani*	*S. rolfsii*	*Verticillium spp.*	*M. phaseolina*
Rh-3	5±1e	0±0d	8±2.5bc	3±1.1c	5±1.5c
Rh-7	10±1.5cd	0±0d	10±2.5b	9±3.3b	0±0e
Rh-8	19±1.7a	11±4.5a	6±1.8c	8±2.5b	9±2.5a
Rh-24	0±0f	0±0d	9±3.3b	0±0d	8±3.7a
Rh-30	0±0f	9±3.3ab	0±0e	5±1.5c	0±0e
Rh-31	8±2d	7±2.7bc	10±2.5	8±2.2b	0±0e
Rh-32	4±1.2e	10±1.8a	7±1.2c	0±0d	0±0e
Rh-33	12±3.5c	6±2.2c	13±5.0a	17±2.5a	6±1.7bc
Rh-43	9±2.3d	4±2.0c	0±0e	4±1.5c	0±0e
Rh-49	8±2.5d	9±3.5ab	0±0e	0±0d	7±3.3b
Rh-55	16±3.3b	8±2.3b	8±1.5bc	15±4.8a	6±2.2bc
Rh-56	0±0f	8±3.5b	11±2.8ab	3±0.8c	5±1.3c
Rh-77	8±1.9d	6±2.2c	4±1.2d	0±0d	4±1.2cd
Control	0±0f	0±0d	0±0e	0±0d	0±0e

PGP Traits of Rhizobacteria

Isolates	IAA production	P-solubilization	Siderophore production	Chitinase production	Biofilm formation
Rh-3	+	-	-	+	-
Rh-7	+	-	+	+	+
Rh-8	+	-	++	++	+
Rh-24	+	-	+	-	-
Rh-30	+	+	+	-	-
Rh-31	-	+	-	+	-
Rh-32	-	-	+	-	+
Rh-33	+	-	+	++	-
Rh-43	+	+	+	-	+
Rh-49	+	-	++	-	-
Rh-55	+	+	+	++	+
Rh-56	+	-	+	+	+
Rh-77	+	-	-	+	+
Control	-	-	-	-	-

Preparation of Biochar and its evaluation

- Wheat straw, sheesham wood, Sugar cane bagasse and Acacia wood were used for biochar production
- Heating in conventional pyrolysis tank at 250-350℃ (Pan *et al*., 2011)
- After preparation, the pyrolyzed product was ground to fine form (0.5 mm)

- **Evaluation**

- Antagonistic PGPRs suspension was prepared at 10^9 cfu/ml

- 30 ml inoculum was shaken at 25 ºC for 24 h with 10 g of carrier materials in flasks followed by aseptically drying of carrier materials and storage under different temperatures i.e. 0, 10, 20 and 30 C.

- Samples were taken every 30 day interval i.e 0, 30, 60, 90, 120, 150 and 180 days and cfu/g was calculated by plating.

Shelf life of *P. illinoisensis* Rh-8 on different carrier materials

Temperature	Carrier material	Bacterial population (lg CFU/g carrier material)							
		Days after inoculation (DAI)							Mean
		0	30	60	90	120	150	180	
0 ºC	Bagasse biochar	9.70	9.17	9.03	8.36	8.11	7.56	7.31	8.46i
	WS biochar	9.69	9.04	8.92	8.15	7.03	6.12	5.55	7.78k
	Sheesham biochar	9.70	9.18	8.95	8.47	7.78	7.00	6.53	8.23j
	AW biochar	9.69	9.09	8.77	8.26	7.84	7.05	6.68	8.19jk
	Peat	9.70	9.19	9.04	8.49	7.49	7.03	6.41	8.23j
	Lignite	9.68	9.07	8.85	8.23	7.23	6.91	6.46	8.13jk
10 ºC	Bagasse biochar	9.70	9.83	10.14	10.31	10.73	11.19	10.92	10.40a
	WS biochar	9.68	9.75	9.92	9.68	9.08	8.49	8.05	9.24g
	Sheesham biochar	9.70	9.85	10.07	10.26	10.31	9.95	9.81	9.99cd
	AW biochar	9.69	9.77	9.94	10.13	9.94	9.83	9.61	9.84d
	Peat	9.71	9.80	10.14	10.32	10.57	10.03	9.95	10.07c
	Lignite	9.70	9.66	9.94	10.17	9.78	9.23	8.75	9.60fg
20 ºC	Bagasse biochar	9.71	10.03	11.26	11.34	10.57	9.74	9.14	10.26b
	WS biochar	9.68	9.94	10.15	10.65	10.08	9.28	8.64	9.77e
	Sheesham biochar	9.70	9.95	10.27	10.86	10.41	9.65	9.01	9.98cd
	AW biochar	9.69	9.77	9.94	10.13	9.94	9.83	8.89	9.74e
	Peat	9.70	10.13	11.35	10.87	9.61	9.03	8.53	9.89d
	Lignite	9.70	9.91	10.70	10.07	9.47	8.99	8.27	9.59fg
30 ºC	Bagasse biochar	9.70	11.52	11.07	9.77	9.02	8.44	7.99	9.64fg
	WS biochar	9.68	10.83	10.64	9.32	8.87	8.19	7.35	9.27g
	Sheesham biochar	9.70	9.85	10.07	10.26	10.31	9.95	9.81	9.99cd
	AW biochar	9.69	10.25	9.94	10.13	9.94	9.83	9.61	9.91d
	Peat	9.68	11.36	11.07	8.30	7.96	7.35	7.06	8.97h
	Lignite								

Shelf life of B. *subtilis* Rh-33 on different carrier materials

Temperature	Carrier material	Bacterial population (lg CFU/g carrier material)							Mean
		Days after inoculation (DAI)							
		0	30	60	90	120	150	180	
0 °C	Bagasse biochar	9.70	9.00	8.63	8.21	7.88	7.34	7.01	8.25f
	WS biochar	9.69	8.95	8.32	7.89	7.03	6.12	5.35	7.62h
	Sheesham biochar	9.70	9.00	8.70	8.22	7.78	7.15	6.75	8.19f
	AW biochar	9.69	9.09	8.77	8.26	7.84	7.05	6.48	8.17f
	Peat	9.70	9.10	8.75	8.23	7.81	7.18	6.75	8.22f
	Lignite	9.68	9.07	8.85	8.23	7.23	6.91	6.46	8.06g
10 °C	Bagasse biochar	9.70	9.23	9.45	9.94	10.19	10.53	10.01	9.86a
	WS biochar	9.68	8.75	8.62	8.12	7.58	7.11	6.25	8.02
	Sheesham biochar	9.70	9.11	9.35	9.81	10.08	10.29	9.61	9.71b
	AW biochar	9.70	8.99	9.14	8.63	8.12	8.00	7.61	8.60e
	Peat	9.68	9.25	9.48	9.99	10.08	10.31	9.83	9.80ab
	Lignite	9.68	9.25	9.48	9.99	10.08	10.31	9.83	9.80ab
20 °C	Bagasse biochar	9.71	9.89	10.25	10.61	10.08	9.75	9.33	9.95a
	WS biochar	9.66	9.85	10.05	10.21	10.02	9.11	8.47	9.62bc
	Sheesham biochar	9.70	9.85	10.27	10.59	10.01	9.58	9.13	9.88a
	AW biochar	9.69	9.77	9.94	10.13	9.94	9.83	8.89	9.74b
	Peat	9.70	9.87	10.32	10.63	10.00	9.55	9.10	9.88a
	Lignite	9.70	9.72	10.27	10.54	9.95	9.47	9.04	9.81ab
30 °C	Bagasse biochar	9.71	10.21	10.25	9.61	8.98	8.52	8.21	9.36c
	WS biochar	9.72	10.15	10.23	9.52	8.78	8.35	8.00	9.25cd
	Sheesham biochar	9.70	10.15	10.27	9.59	9.01	8.44	8.08	9.32c
	AW biochar	9.69	10.21	10.08	9.44	8.64	8.28	7.93	9.18d
	Peat	9.70	10.17	10.27	9.59	9.01	8.47	8.08	9.33c
	Lignite	9.69	10.21	10.08	9.44	8.64	8.28	7.93	9.18d

Shelf life of *P. psychrotolerans* Rh-55 on different carrier materials

Temperature	Carrier material	Bacterial population (lg CFU/g carrier material)							Mean
		Days after inoculation (DAI)							
		0	30	60	90	120	150	180	
0 °C	Bagasse biochar	9.70	9.15	9.08	8.39	8.21	7.61	7.29	8.46i
	WS biochar	9.69	9.04	8.92	8.15	7.03	6.12	5.55	7.78k
	Sheesham biochar	9.70	9.18	8.95	8.47	7.78	7.00	6.53	8.23j
	AW biochar	9.69	9.13	8.81	8.26	7.94	7.05	6.78	8.19jk
	Peat	9.70	9.19	9.04	8.49	7.49	7.03	6.41	8.23j
	Lignite	9.72	9.09	8.89	8.23	7.23	6.95	6.46	8.13jk
10 °C	Bagasse biochar	9.70	9.63	10.10	10.27	10.65	11.11	10.93	10.50a
	WS biochar	9.68	9.78	9.92	9.64	9.18	8.41	8.05	9.24g
	Sheesham biochar	9.70	9.85	10.07	10.26	10.31	9.95	9.81	9.99cd
	AW biochar	9.69	9.79	9.94	10.14	9.94	9.85	9.61	9.84d
	Peat	9.71	9.81	10.14	10.25	10.57	10.05	9.95	10.07c
	Lignite	9.70	9.64	9.94	10.17	9.73	9.23	8.78	9.60fg
20 °C	Bagasse biochar	9.71	10.03	11.26	11.34	10.55	9.74	9.14	10.13b
	WS biochar	9.68	9.94	10.15	10.65	10.08	9.28	8.64	9.71e
	Sheesham biochar	9.70	9.98	10.29	10.86	10.41	9.61	9.01	9.88cd
	AW biochar	9.69	9.77	9.94	10.18	9.94	9.83	8.89	9.69e
	Peat	9.73	10.13	11.31	10.87	9.63	9.03	8.53	9.89d
	Lignite	9.70	9.91	10.70	10.07	9.44	8.94	8.27	9.62fg
30 °C	Bagasse biochar	9.70	11.50	11.07	9.73	9.02	8.41	7.99	9.60fg
	WS biochar	9.68	10.84	10.64	9.32	8.87	8.19	7.35	9.17g
	Sheesham biochar	9.70	9.84	10.07	10.26	10.33	9.95	9.81	9.28cd
	AW biochar	9.69	10.25	9.93	10.13	9.94	9.87	9.61	9.51d
	Peat	9.68	11.36	11.07	8.30	7.96	7.35	7.06	8.94h
	Lignite	9.67	11.04	10.80	8.02	7.89	7.27	6.89	8.76hi

Greenhouse Evaluation of biochar

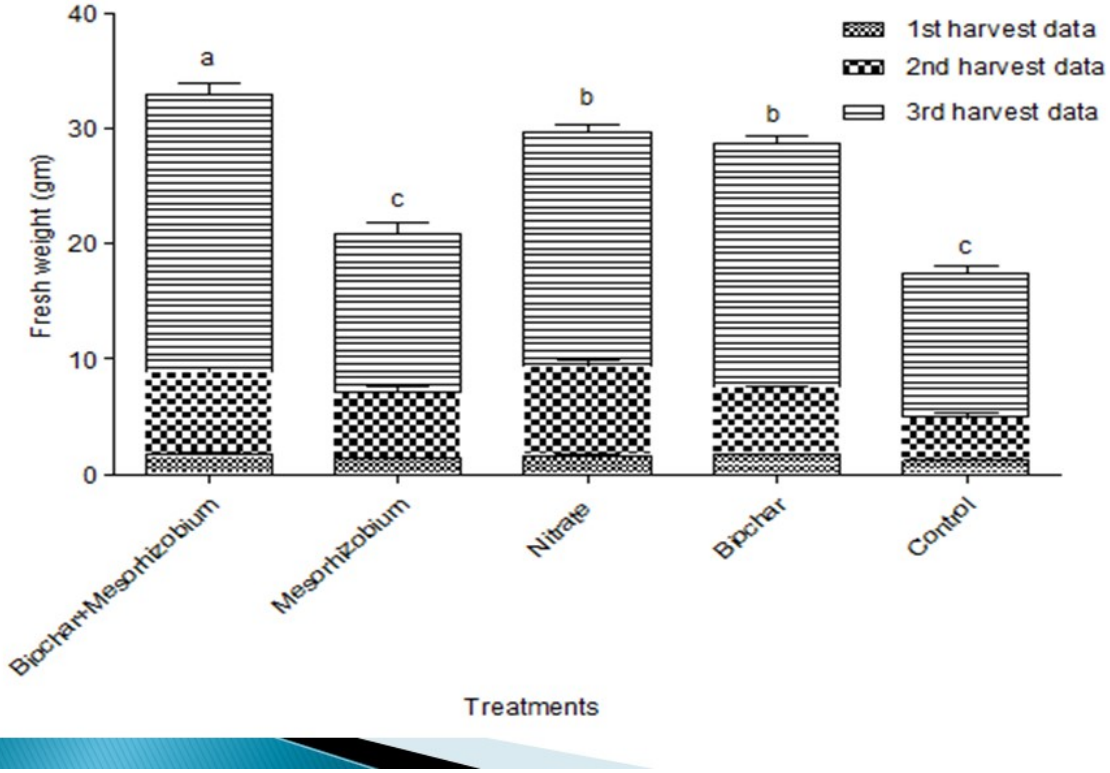
Treatment	DI (%)	RL (cm)	RFW (g)	RDW (g)
Bagasse biochar	0i	33.25bc	8.42a	1.28a
Bagasse biochar + Rh-8	0i	38.6a	8.6a	1.30a
Bagasse biochar + Rh-8 + F. oxysporum	25f	29.50cd	7.78ab	1.22abc
Bagasse biochar + Rh-8 + Verticillium	10h	31.30c	7.81ab	1.25ab
Bagasse biochar + Rh-8 + M. phaseolina	20g	35.3b	7.4bc	1.13bc
Bagasse biochar + F. oxysporum	67b	25.65d	6.4cd	1.00cd
Bagasse biochar + Verticillium	37d	25.00d	6.9c	1.04cd
Bagasse biochar + M. phaseolina	50c	24.70de	6.1d	1.00cd
Rh-8	0i	35.2b	7.9ab	1.21abc
Rh-8 + F. oxysporum	40d	32.5bc	7.2c	1.05cd
Rh-8 + Verticillium	22fg	33bc	7.2c	1.10bcd
Rh-8 + M. phaseolina	35e	33bc	6.7cd	1.03cd
F. oxysporum	75a	19.5f	3.75d	0.67de
Verticillium	40d	22.45e	3.66d	0.75de
M. phaseolina	63b	18.7f	3.5de	0.52e
Control	0i	30.5c	7.5b	1.15bc

Plants after 20 days

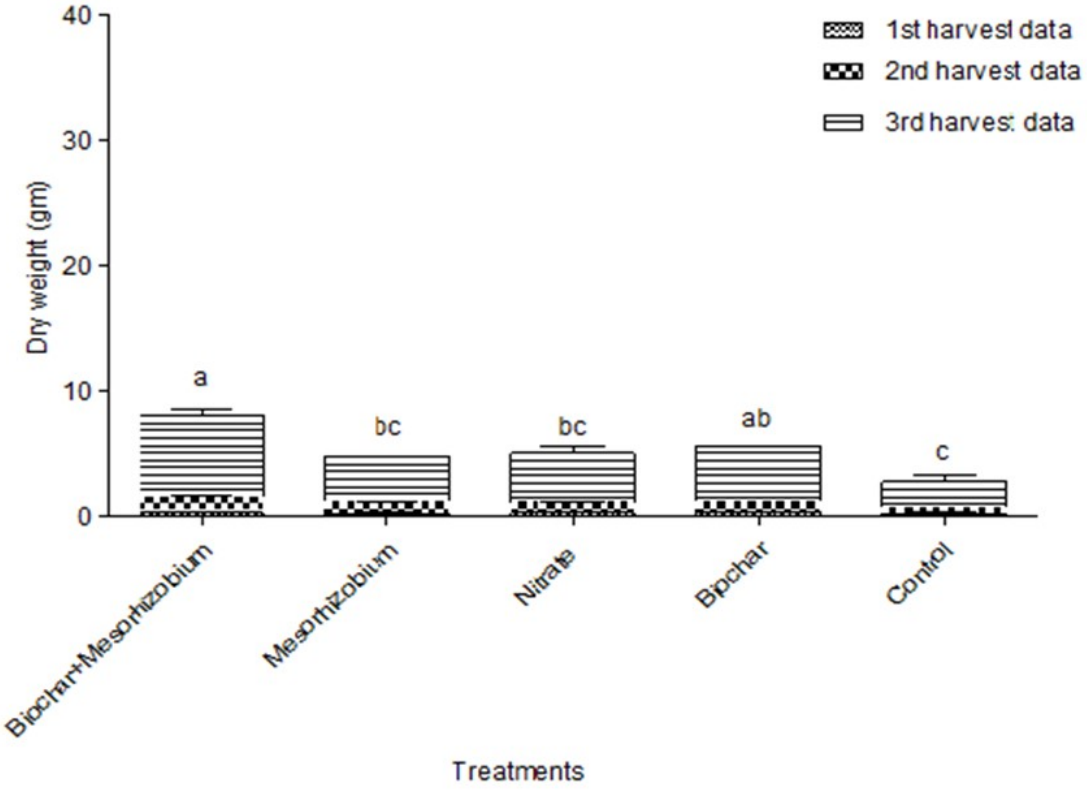
Plants after 40 days

Results

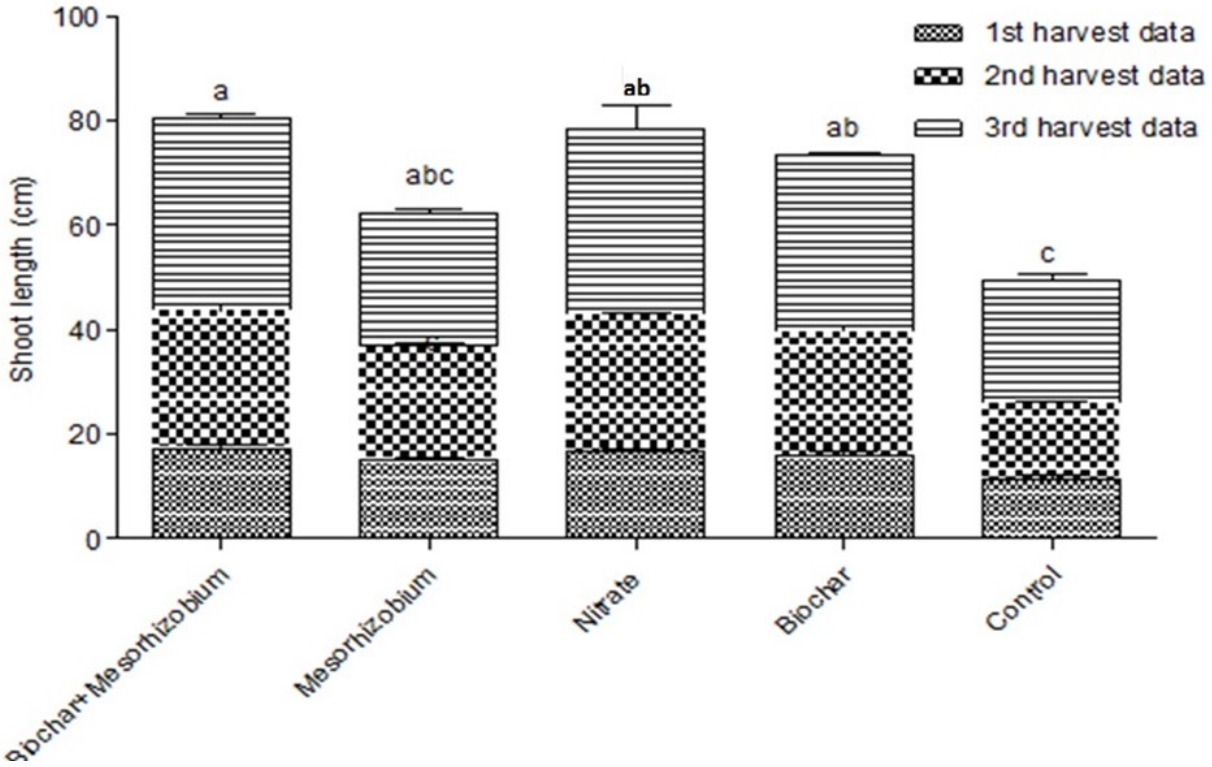
Fresh weight



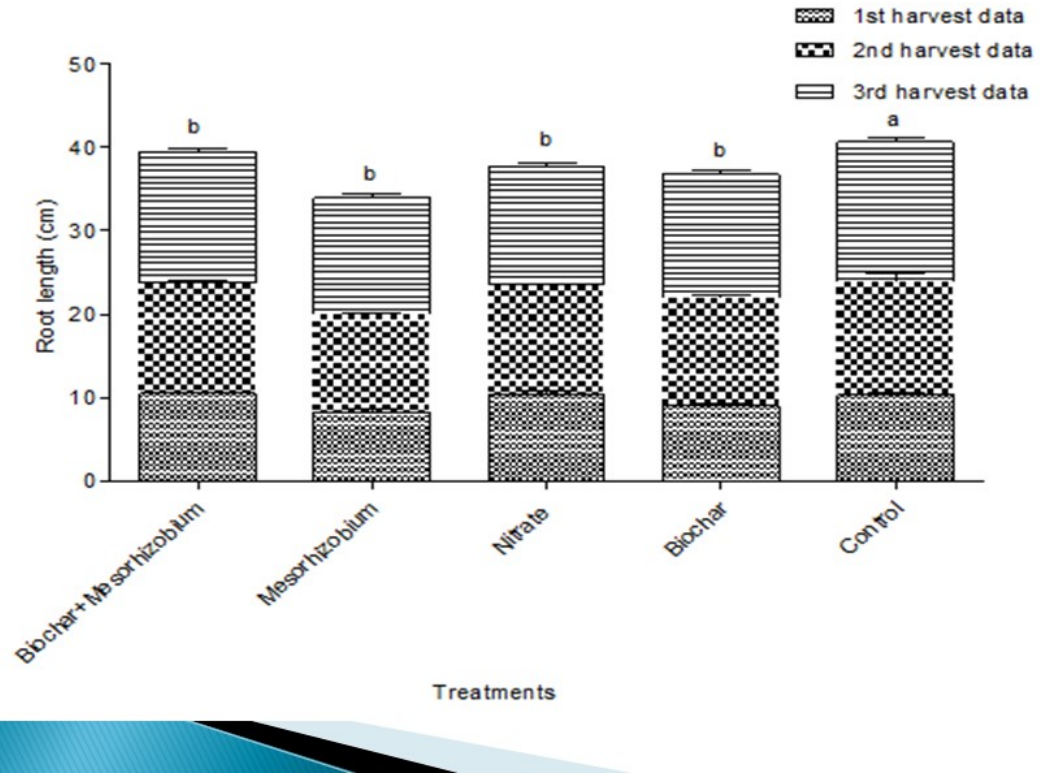
Dry weight

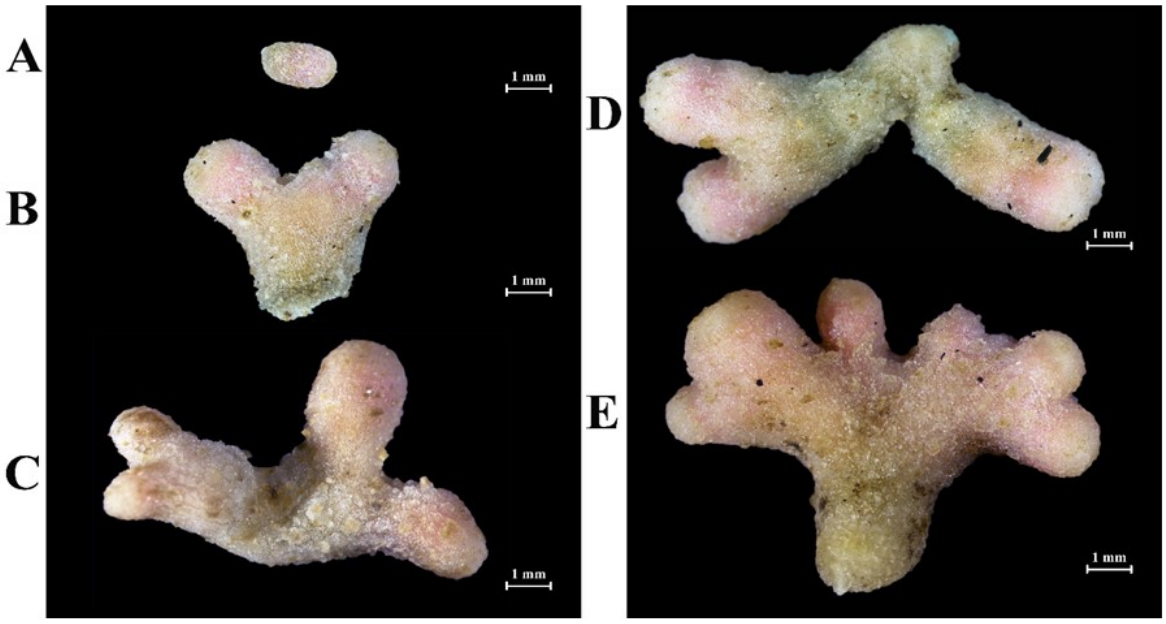


Shoot length



Root length





a) Nodule of control (Water) treated chickpea plants b) Nitrate treated nodule c) M. ciceri treated nodule d) biochar treated nodule and in e) Biochar + *Mesorhizobium ciceri* treated nodule after 60 days

Antagonistic effect of Treatments

- **Treatments**
  i. *Mesorhizobium ciceri*
  ii. Sugarcane Bagasse (SB) biochar (5g/each pot)
  iii. *M. ciceri* + SB Biochar
  iv. Control
- Poison Food Technique
- Diameter of the colony was measured till one week

Colony Diameter of Fungal Pathogens after 7 days

Treatment	Colony diameter (cm)		
	F. oxysporum	F. solani	Phytophthora spp.
Biochar	1.43 b	1.20 b	1.13 b
Mesorhizobium	1.21 c	1.18 b	1.14 b
Biochar + Mesorhizobium	0.99 d	0.86 c	0.64 c
Control	4.45 a	3.98 a	2.80 a

Future research and developmental strategies

- Future research in rhizosphere biology will rely on the development of molecular and biotechnological approaches to increase our knowledge of rhizosphere biology.
- The proven antagonists can be tested in other crops/varieties and pathogens under different agro-ecological zones of Pakistan.
- Higher yield and cost-effective PGPR and their products should be used by agriculture farmers.
- Research on nitrogen fixation and phosphate solubilization by PGPR is progress on but research done on potassium solubilization is little all over the world.
- Fresh alternatives should be continuously explored for the use of bio inoculants.

Continue..

- The application of multi-strain bacterial consortium over single inoculation could be an effective approach for reducing the harmful effects of stress on plant growth
- An efficient delivery method and carrier be investigated for longer benefits.
- Molecular screening of effective isolates should be done and available antagonists can be genetically improved for better outputs.
- The synthetic metabolites / products released by antagonists should also be tested
- An integrated approach to be worked out for disease control as biological control alone may not be effective when applied in the field.
- PGPR can also be used as bioremediation. So, will be helpful in cleaning the environment.

Conclusion

- PGPR are economically and environmentally beneficial for plant growth promotion.
- PGPR may function as bio-fertilizers, bio-inoculant and bio-control other growth-promoting activities.
- In the future, they might replace chemical fertilizers and pesticides which have many hazardous effects on agriculture.

Research Project

- Research Project entitled "Utilization of plant growth promoting and nodule forming rhizobacteria in the integrated control of root infecting fungi of Sunflower and Soybean" Funded by HEC, Islamabad (2003-06).
- HEC project: "Surveillance and pathogen characterization of bacterial canker of stone fruits using biochemical and molecular methods and its biomanagement".
- PGTF-INT/15/KO7-project: "Surveillance and pathogen characterization of bacterial canker of stone fruits using biochemical and molecular methods and its biomanagement".
- PARB funded Project: "Development of Bio-pesticide for the Control of Soil-borne Diseases of Tomatoes and Chilies caused by Pythium and Phytophthora spp."

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