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Antibacterial Activities Of Ethanol And Aqueous Extracts Of Vetiver (Khus-Grass), PGPR And Bavistin Against Rhizobacteria Pathogenic To Plants

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Abstract:
Rhizosphere is regarded as the narrow zone of the soil that is directly influenced by the root secretions. It is considered as a "hot spot" of microbial colonization and activity. In the soil the microbial interaction with the plant-roots leads to destructive, associative or symbiotic associations. Beneficial association of microbes with roots is caused by bacteria, Actinomycetes (filamentous bacteria), cyanobacteria and fungi (fungal symbiosis).
In the present investigation, an effort has been made to control the plant pathogenic bacteria using ethanol and aqueous extract of Vetiver (Khas Grass) alone and in combination with consortium of some plant growth promoting bacteria (PGPR) for the integrated management of bacterial diseases and to improve the yield of crop plants.
A consortium of 19 rhizobacteria isolated from Paddy fields of Pandaul was prepared by mixing 1 mL of pure culture of each of the rhizobacterial isolate. In this consortium the rhizobacterial isolates included Bacillus polymyxa, B. subtilis, B. megaterium, B. pumilus, Azotobacter chroococcum, A. beijerinckii, Pseudomonas fluorescence, P. putida, P. aeruginosa, Arthrobacter chroococcum, Citrobacter sp., Erwinia amylovora, Stenotrophomonas maltophila, Agrobacterium fabrum, Flavobacterium anhuiense, Acinetobacter soli, Azospirillum sp., Burkholderia sp. and Rhizobium sp. These isolates were proved to produce Indole acetic acid (IAA), substances for solubilization of inorganic phosphates and siderophores, and hence called plant growth promoting rhizobacteria (PGPR). The antibacterial activity of different concentrations of aqueous and ethanol extracts alone and in consortium of PGPR against plant pathogenic bacteria viz., Xanthomonas compestris pv. oryzae, Clavibacter tritici/Corynebacterium tritici, Erwinia carotovora, Pseudomonas syringae pv. lapsa and Pseudomonas angulata has been performed. Similarly, the effect of five different concentrations of Bavistin, a systemic fungicide on growth inhibition of bacteria pathogenic to plants was evaluated.
The results revealed that the aqueous and ethanol extracts of Vetiver Grass showed concentration dependent growth inhibition of Xanthomonas compestris pv.oryzae, Clavibacter tritici, Erwinia carotovora, Pseudomonas syringae and Pseudomonas angulata. Bavistin did not cause significant growth inhibition of the present five plant pathogenic bacteria. The growth inhibition of plant pathogenic bacteria showed synergistic effect when treated with aqueous and ethanol extracts along with PGPR.
It can be concluded that the aqueous and ethanol extracts of Vetiver Grass singly as well as with consortium of nineteen rhizobacterial isolates can inhibit the growth of plant pathogenic bacteria.
Key Words: *Rhizobacteria, PGPR, Vetiver' extract, Bavistin, Paddy*

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I. INTRODUCTION

Rhizosphere is regarded as the narrow zone of the soil that is directly influenced by the root secretions. It is considered as a "hot spot" of microbial colonization and activity (Bolton *et al*., 1993) [1], and the largest ecosystem on earth with huge energy flux (Barriuso *et al*., 2008) [2]. Soil which is not a part of the rhizosphere is considered as bulk soil (Gobat *et al*., 2004) [3]. The rhizosphere zone of soil contains many bacteria, actinomycetes and fungi that feed on sloughed-off plant cells, proteins and sugars secreted by roots. These organic substances are termed rhizodiposition. Except for some pathogenic microorganisms, many rhizosphere microbes improve the growth and health of plants by various mechanisms viz. nitrogen fixation and production of plant growth hormones (Patten and Glick, 1996, 2002; Andrews and Harris, 2000) [4, 5, 6].

In the soil the microbial interaction with the plant-roots leads to destructive, associative or symbiotic associations. Beneficial association of microbes with roots is caused by bacteria, Actinomycetes (filamentous bacteria), cyanobacteria and fungi (fungal symbiosis). *Azospirillum*, *Azotobacter* and *Beijerinckia* cause associative symbiosis with the roots of several non-leguminous plants viz. Corn, Wheat, Sorghum etc. Species of

Pseudomonas, *Bacillus* and *Streptomyces* are non-symbiotic rhizobacteria which favorably affect the growth of plants (Kloepper, 1980) [7]. These non-symbiotic bacteria increase the growth of plants due to change in balance of rhozosphere microflora producing an indirect effect on the crops, control of pathogens and other harmful microorganisms in the rhizosphere, production of growth hormones like Indole acetic acid (IAA) and gibberellins, release of nutrients produced due to nitrogen fixation by rhizobacteria.

In the rhizosphere region the plant-microbe interaction may be beneficial, neutral, variable, or deleterious for plant growth. The rhizobacteria that exhibit beneficial effect on plant growth and development are termed Plant growth Promoting Rhizobacteria (PGPR) (Kloepper and Scroth, 1978; Subba Rao, 1999) [8, 9]. The different strains of PGPR-bacteria are responsible for growth promotion activities of plants (Cattelan *et al*., 1999) [10]. This includes the ability to produce or change the concentration of plant growth hormones viz. Indole acetic acid (IAA), Gibberellic acid (GA), Cytokinin, and Ethylene; fix atmospheric dinitrogen; suppress or inhibit the growth of deleterious microorganisms by production of siderophore, β-1,3-glucanase, chitinases, antibiotics and cyanide; and dissolve phosphates and other nutrients. *Azotobacter* and *Azospirillum* are known to plant growth due to their ability to fix atmospheric dinitrogen, and plant growth stimulating hormone Indole acetic acid (IAA) is also involved in this process (Kennedy, 1998) [11]. The use of phosphate solubilizing bacteria is also known to increase plant growth (De Freitas *et al*., 1997) [12].

Rice is the staple food. It is widely cultivated in India, China, Japan, Pakistan, SriLanka and countries of South eastern Asia. Rice is a crop of wide physiological adaptability and grown in both tropical and temperate countries at an altitude of over 2000 m. above sea-level. The crop grows best on clayey loams but can also be cultivated on various types of soils viz. damp alluvial soil, high sandy soil, gravelly or stony soil, boggy soil, or terraces, hill slopes, high lands or low lands subject to flooding. High temperature and high humidity are most favorable for the growth of crop.

Rice plant is susceptible to various fungal diseases viz. Blast disease, Brown-spot, and Bakanae disease caused by *Piricularia oryzae*, *Helminthosporium oryzae* and *Gibberella fujikuroi* respectively. These can be treated by spraying fungicides e.g., Bordeaux mixture and growing resistant varieties. Inoculation of rhizobacteria showing fungicidal and antibacterial activities to rice field can also be as biocontrol agents to treat bacterial and fungal diseases of rice. The rice crop is also severely affected by a number of bacterial pathogens residing in the rhizosphere of paddy. *Xanthomonas compestris* pv. oryzae and *Xanthomonas compestris* pv. oryzicola are two most popular bacterial pathogens causing bacterial blight and bacterial leaf streak diseases in paddy.

In the present investigation, an effort has been made to control the plant pathogenic bacteria using ethanol and aqueous extract of Vetiver (Khas Grass) alone and in combination with consortium of some plant growth promoting bacteria (PGPR) for the integrated management of bacterial diseases and to improve the yield of crop plants.

II. MATERIALS AND METHODS

The pure cultures of bacterial pathogens viz., *Xanthomonas compestris* pv. oryzae causing blight disease of Paddy, *Clavibacter tritici*/*Corynebacterium tritici* causing Yellow Ear Rot or Tundu disease of Wheat, *Erwinia carotovora* causing Black leg wilt and Soft rot of Potato, *Pseudomonas syringae* pv. lapsa causing Stalk Rot of Maize and *Pseudomonas angulata* causing Angular leaf spot and wilt fire disease of Tobacco were procured from Plant Pathology Laboratory, IARI, New Delhi. A consortium of 19 rhizobacteria isolated from Paddy fields of Pandaul was prepared by mixing 1 mL of pure culture of each of the rhizobacterial isolate. In this consortium the rhizobacterial isolates included *Bacillus polymyxa, B. subtilis, B. megaterium, B. pumilus, Azotobacter chroococcum, A. beijerinckii, Pseudomonas fluorescence, P. putida, P. aeruginosa, Arthrobacter chroococcum, Citrobacter sp., Erwinia amylovora, Stenotrophomonas maltophila, Agrobacterium fabrum, Flavobacterium anhuiense, Acinetobacter soli, Azospirillum* sp., *Burkholderia* sp. and *Rhizobium* sp. In our previous investigation, these isolates were proved to produce Indole acetic acid (IAA), substances for solubilization of inorganic phosphates and siderophores, and hence called plant growth promoting rhizobacteria (PGPR).

The leaves of Vetiver grass were collected from local gardens of Darbhanga. One kilogram of leaves was pieced, air dried for three days and mashed using a blender. The dried leaves were macerated using absolute ethanol and warm water separately. The mixture was then filtered using Whatman filter paper No. 1. The filtrate was evaporated at 40^0C and this crude ethanol and aqueous extracts was used for further study as suggested by Suriani *et al*., (2019) [13]. Five different concentrations of ethanol and aqueous extracts viz. 0.5 %, 1.0 %, 1.5 %, 2.0 % and 2.5% were prepared from the crude ethanol extract.

The antibacterial activity of different concentrations of aqueous and ethanol extracts alone and in consortium of PGPR against plant pathogenic bacteria viz., Xanthomonas compestris pv. oryzae, *Clavibacter tritici*/*Corynebacterium tritici, Erwinia carotovora, Pseudomonas syringae* pv. lapsa and *Pseudomonas angulata* has been performed with following treatments:

Aqueous extract (Va) of Vetiver (Khas Grass):

1. Va 0 Control
2. Va 1 0.5%
3. Va 2 1.0 %
4. Va 3 1.5 %
5. Va 4 2.0 %
6. Va 5 2.5 %
7. Va 6 0.5 % + PGPR
8. Va 7 1.0 % + PGPR
9. Va 8 1.5 % + PGPR
10. Va 9 2.0 % + PGPR
11. Va 10 2.5 % + PGPR
12. **PGPR alone**

Ethanol extract (Ve) of Vetiver (Khas Grass)

1. Ve 0 Control
2. Ve 1 0.5 %
3. Ve 2 1.0%
4. Ve 3 1.5 %
5. Ve 4 2.0 %
6. Ve 5 2.5 %
7. Ve 6 0.5 % + PGPR
8. Ve 7 1.0 % + PGPR
9. Ve 8 1.5 % + PGPR
10. Ve 9 2.0 % + PGPR
11. Ve 10 2.5 % + PGPR
12. PGPR alone

Similarly, the effect of five different concentrations of a systemic fungicide Bavistin viz. 0.1 %-0.5% on growth inhibition of bacteria pathogenic to plants was evaluated. The results obtained have been presented in Table-1, 2 and 3; Figure-1, 2 and 3.

Table-1: Antibacterial activity of different concentrations of aqueous extracts of Vetiver (Khas Grass) alone and in consortium of PGPR against five plant pathogenic bacteria

Treatment (%)	Plant pathogenic bacteria									
	Xanthomonas compestris pv.*oryzae*		*Clavibacter tritici*		*Erwinia carotovora*		*Pseudomonas syringae*		*Pseudomonas angulata*	
	Dia. of colony (mm)	% inhibition	Dia. of colony (mm)	% inhibition	Dia. of colony (mm)	% inhibition	Dia. of colony (mm)	% inhibition	Dia. of colony (mm)	% inhibition
Va 0	60.5	0	68.5	0	70.5	0	70.0	0	67.5	0
Va 0.5	58.5	3.30	65.6	4.23	68.5	2.83	68.0	2.85	65.5	2.96
Va 1.0	55.5	8.26	58.5	14.59	57.0	19.14	56.5	19.28	53.5	20.74
Va 1.5	47.5	21.48	41.5	39.41	40.6	42.41	38.7	44.71	35.6	47.25
Va 2.0	30.7	49.25	28.7	58.10	27.9	60.42	25.7	63.28	21.5	68.14
Va 2.5	25.5	57.85	23.5	65.69	22.7	67.80	19.8	71.71	18.7	72.29
Va 0.5 + PGPR	52.5	13.22	50.5	26.27	48.6	31.06	45.5	35.0	41.5	38.51
Va 1.0 + PGPR	42.5	29.75	40.7	40.58	36.5	48.22	34.5	50.71	31.5	53.33
Va 1.5 + PGPR	28.7	52.56	24.6	64.08	19.5	72.34	17.6	74.85	15.7	76.74
Va 2.0 + PGPR	18.5	69.42	15.5	77.37	12.6	82.12	11.8	83.14	10.5	84.44
Va 2.5 + PGPR	6.5	89.25	4.5	93.43	3.5	95.03	2.5	96.42	3.0	99.55
PGPR alone	10.7	82.31	9.7	85.83	10.5	85.10	10.7	84.71	11.0	83.70

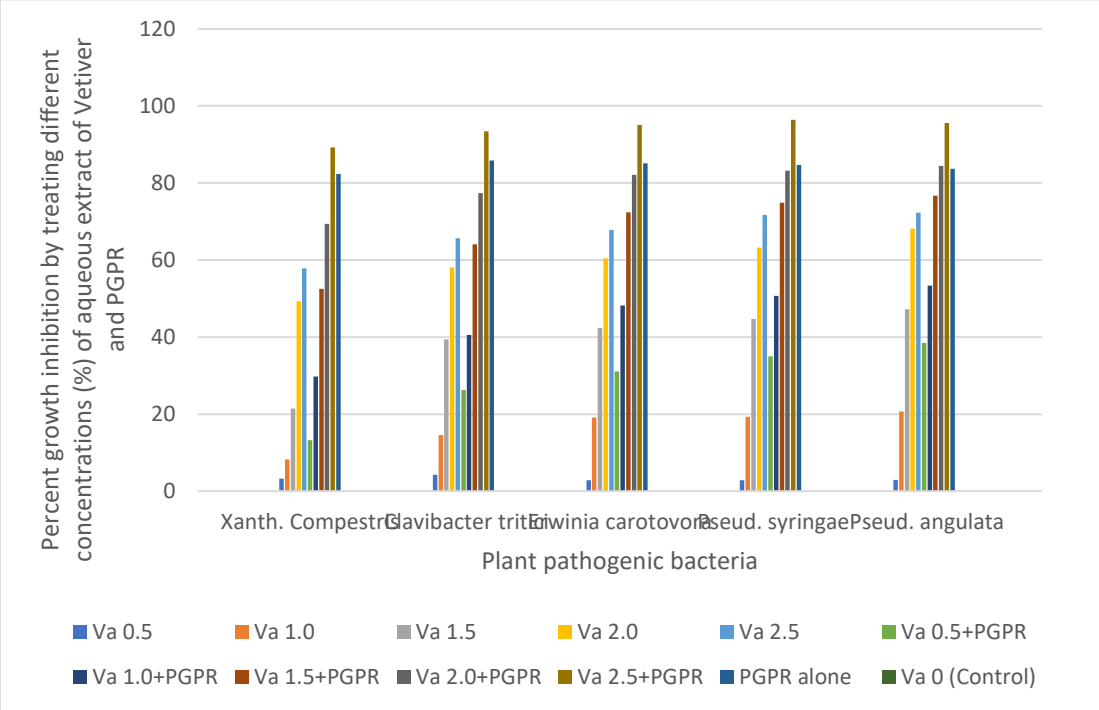

Figure-1: Effect of different concentrations (0.5 % to 2.5 %) of aqueous extracts alone and in consortium with PGPR on growth inhibition of five plant pathogenic bacteria.

Table-2: Antibacterial activity of different concentrations of ethanol extract of Vetiver (Khas Grass) alone and in consortium with PGPR against plant pathogenic bacteria.

Treatment (%)	Plant pathogenic bacteria									
	Xanthomonas compestris pv. *oryzae*		*Clavibacter tritici*		*Erwinia carotovora*		*Pseudomonas syringae*		*Pseudomonas angulata*	
	Dia. of colony (mm)	% inhibition	Dia. of colony (mm)	% inhibition	Dia. of colony (mm)	% inhibition	Dia. of colony (mm)	% inhibition	Dia. of colony (mm)	% inhibition
Ve 0 (Control)	60.5	0	68.5	0	70.5	0	70.5	0	67.5	0
Ve 0.5	59.5	1.65	64.5	5.83	65.5	7.09	65.0	7.14	62.5	7.40
Ve 1.0	55.5	8.26	51.4	24.96	51.7	26.66	51.0	27.14	52.5	22.22
Ve 1.5	51.7	14.54	46.5	32.11	40.6	42.41	39.5	43.57	47.5	29.62
Ve 2.0	46.5	23.14	41.5	39.41	32.5	53.90	30.6	56.28	39.8	41.03
Ve 2.5	42.7	29.42	35.7	47.88	17.5	75.17	15.7	77.57	30.7	54.51
Ve 0.5 + PGPR	52.5	13.22	47.5	30.65	60.5	14.18	58.5	16.42	45.5	32.59
Ve 1.0 + PGPR	42.6	29.58	35.5	48.17	40.5	42.55	37.5	46.42	31.5	53.33
Ve 1.5 + PGPR	27.6	54.38	21.6	68.47	29.5	58.15	24.6	64.85	20.6	69.48
Ve 2.0 + PGPR	15.5	74.38	13.7	80.00	7.0	90.07	6.5	90.71	12.5	81.48
Ve 2.5 + PGPR	7.5	87.60	5.0	92.70	3.0	95.74	2.5	96.42	6.5	90.37
PGPR alone	12.5	79.33	9.0	86.86	11.0	84.39	12.0	82.85	12.0	82.22

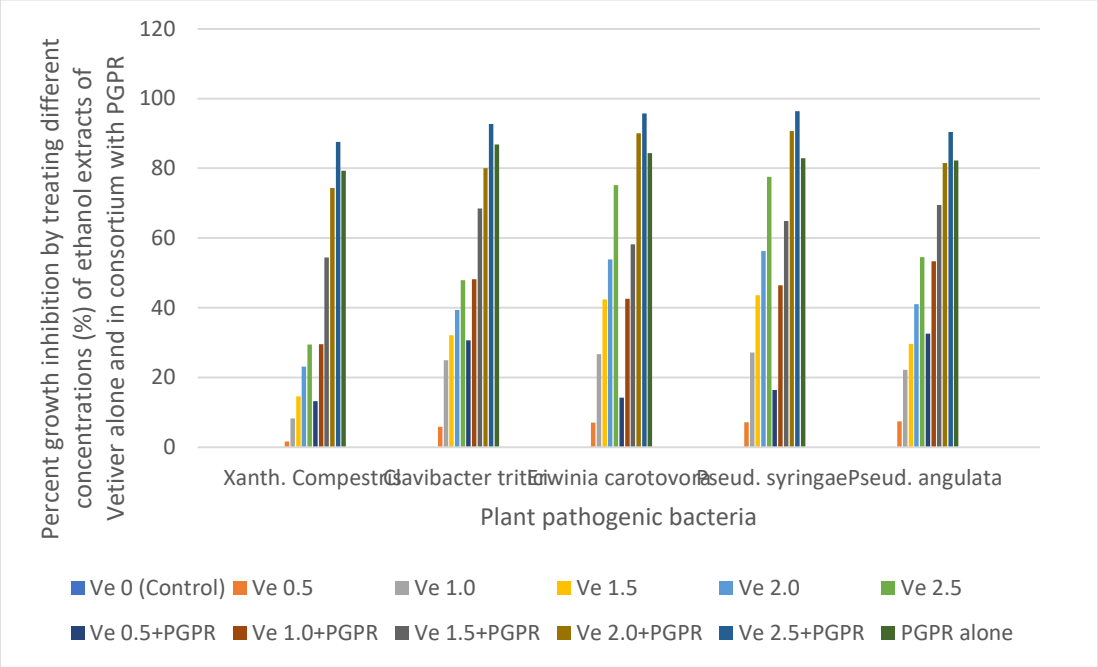

Figure-2: Effect of different concentrations (0 % to 2.5 %) of ethanol extracts of Vetiver alone and in consortium with PGPR on growth inhibition of five plant pathogenic bacteria

Table-3: Antibacterial activity of different concentrations of Bavistin against plant pathogenic bacteria

Treatment (%)	Plant pathogenic bacteria									
	Xanthomonas compestris pv. oryzae		*Clavobacter tritici*		*Erwinia carotovora*		*Pseudomonas syringae*		*Pseudomonas angulata*	
	Dia. of colony (mm)	% inhibition	Dia. of colony (mm)	% inhibition	Dia. of colony (mm)	% inhibition	Dia. of colony (mm)	% inhibition	Dia. of colony (mm)	% inhibition
B 0% (Control)	60.5	0	68.5	0	70.5	0	70.0	0	67.5	0
B 0.1	60.4	0.16	68.4	0.14	70.3	0.28	70.0	0	67.3	0.29
B 0.2	59.8	1.15	67.8	1.02	69.8	0.99	69.8	0.28	67.0	0.74
B 0.3	59.5	1.65	67.5	1.45	68.5	2.83	68.5	2.14	67.0	0.74
B 0.4	59.0	2.47	66.8	2.48	67.9	3.68	67.8	3.14	66.8	1.03
B 0.5	59.0	2.47	66.5	2.91	67.9	3.68	67.8	3.14	66.5	1.48

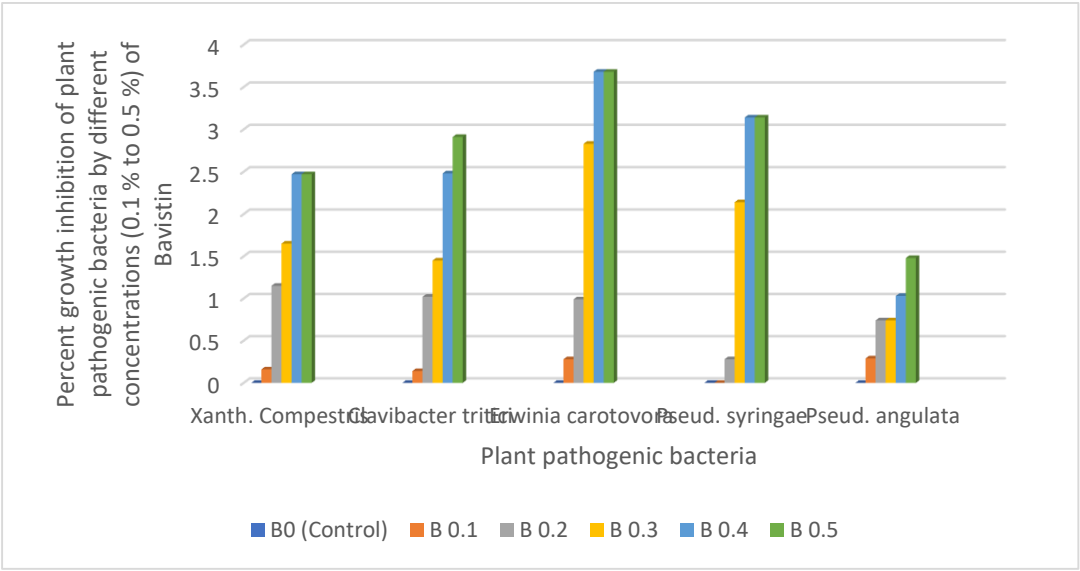

Figure-3: Effect of different concentrations of Bavistin (0.1 % to 0.5 %) on growth inhibition of five plant pathogenic bacteria

III. RESULTS AND DISCUSSION

The antibacterial activity of different concentrations aqueous and ethanol extracts of Vetiver (Khas Grass) alone and in consortium with 1 mL of nineteen plant growth promoting rhizobacterial isolates against five plant pathogenic bacteria was studied. The aqueous extracts of Vetiver Grass showed concentration dependent growth inhibition of *Xanthomonas compestris* pv.*oryzae*, *Clavibacter tritici*, *Erwinia carotovora*, *Pseudomonas syringae* and *Pseudomonas angulata*. At 0.5 % of aqueous extract of Vetiver, the growth inhibition of these plant pathogenic bacteria was 3.30 %, 4.23 %, 2.83 %, 2.85 % and 2.96 % respectively. The growth inhibition of these plant pathogenic bacteria increased on increasing the concentration of aqueous extracts. At 2.5 % concentration of aqueous extracts, the growth inhibition was maximum, about 57.85 %, 65.69 %, 67.80 %, 71.71 % and 72.29 % respectively (Table-1; Figure-1). It was found that the growth inhibition of phytopathogenic bacteria viz., *Xanthomonas compestris* pv. *oryzae*, *Clavibacter tritici*, *Erwinia carotovora*, *Pseudomonas syringae and Pseudomonas angulata* was highest when treated with aqueous extracts along with consortium of nineteen rhizobacterial isolates. The growth inhibition of these plant pathogenic bacteria was maximum about 89.25 %, 93.43 %, 95.03 %, 96.42 % and 95.55 % respectively when treated with 2.5 % of aqueous extract of Vetiver along with a consortium of 19 PGPR (Table-1; Figure-1). The growth inhibition of plant pathogenic bacteria thus showed synergistic effect when treated with aqueous extracts along with PGPR.

The ethanol extracts of Vetiver Grass also exhibited concentration dependent growth inhibition of five plant pathogenic bacteria viz., *Xanthomonas compestris* pv. *oryzae*, *Clavibacter tritici*, *Erwinia carotovora*, *Pseudomonas syringae* and *Pseudomonas angulata* (Table-2; Figure-2). At 0.5 % of ethanol extract of Vetiver Grass (Ve 0.5) the growth inhibition of these pathogenic bacteria was minimum, about 1.65 %, 5.83 %, 7.09 %, 7.14 % and 7.40 % respectively. The growth inhibition of these five pathogenic bacteria was highest, about 29.42 %, 47.88 %, 75.17 %, 77.57 % and 54.51 % respectively when treated with 2.5 % of ethanol extract of Vetiver Grass (Ve 2.5). The ethanol extracts of Vetiver Grass also showed synergistic effect on growth inhibition of plant pathogenic bacteria. The growth inhibition of these plant pathogenic bacteria was maximum, about 87.60 %, 92.70 %, 95.74 %, 96.42 % and 90.37 % respectively when treated with 2.5 % of ethanol extract of Vetiver Grass along with a consortium of 19 PGPR (Table-2; Figure-2).

The results related to antibacterial activity of five different concentrations of Bavistin viz., 0.1 %, 0.2 %, 0.3 %, 0.4 % and 0.5 % on five plant pathogenic bacteria revealed that Bavistin did not cause significant growth inhibition of five plant pathogenic bacteria viz., *Xanthomonas compestris* pv. *oryzae*, *Clavibacter tritici*, *Erwinia carotovora*, *Pseudomonas syringae* and *Pseudomonas angulata*. The growth inhibition of these bacteria was only 2.47 %, 2.91 %, 3.68 % 3.14 % and 1.48 % when treated with 0.5 % of Bavistin (Table-3; Figure-3). This fungicide was proved to be bacteriostatic rather than bactericidal. Bavistin is a systemic fungicide and is effective in controlling plant pathogenic fungi and is ineffective against Oomycetous pathogens and bacteria.

Higher the concentration of aqueous and ethanol extracts, the higher the growth inhibition of plant pathogenic bacteria viz., *Xanthomonas compestris* pv. *oryzae*, *Clavibacter tritici*, *Erwinia carotovora*, *Pseudomonas syringae* and *Pseudomonas angulata*. The highest growth inhibition of plant pathogenic bacteria was observed when treated with a mixture of extracts and PGPR. PGPR is very influential in inhibiting growth of pathogenic bacteria and thereby increasing the growth of crops. PGPR function to increase plant growth because they produce growth hormones such as IAA (Sudirga *et al*., 2014; Collas *et al*., 2019) [14, 15]. Plant growth promoting rhizobacteria such as species of Bacillus, Pseudomonas and Enterobacter are known to improve plant growth by suppressing the growth of plant pathogens (Kumar *et al*., 2010; Sukanova *et al*., 2017) [16, 17]. PGPR along with plant extracts can be used as biocontrol in plants and can function as a growth enhancer and to increase yield status of crops. The plant extracts along with PGPR can also be used for sustainable agriculture (Shaikh *et al*., 2018) [18]. PGPR is an alternative source of biofertilizer to improve soil fertility and thus, reduce the demand of chemical fertilizers and pesticides (Shaikh *et al*., 2018) [18]. PGPR isolates from plants are known to be antifungal against several pathogenic fungi and bacteria because these rhizobacterial isolates produce siderophores, chitinases, cellulases, IAA, substances for phosphate solubilization and ammonia (Basu *et al*., 2021) [19].

IV. CONCLUSIONS:

It can be concluded that the aqueous and ethanol extracts of Vetiver Grass singly as well as with consortium of nineteen rhizobacterial isolates can inhibit the growth of plant pathogenic bacteria. The extracts along with PGPR showed synergistic effect in growth inhibition of pathogenic bacteria. A preparation of extracts of Vetiver Grass along with PGPR in appropriate amount has the potential to improve the growth parameters of crops and exert potential antibacterial against the phytopathogenic bacteria. The preparation has the potential to be developed as the best organic or biofertilizer cum-biopesticide for sustainable agriculture development and effective control of phytopathogenic bacteria.

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CONFLICT OF INTEREST: Authors declare no conflict of interest directly or indirectly.

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