Potential of a marine *Pseudomonas aeruginosa* strain OG101 to combat *Fusarium oxysporum* associated wilt in legume crops

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Abstract

The continuous and overwhelming activity of humans releasing greenhouse gas (GHG) into the atmosphere causes additional warming and long term changes in climatic components that lead to a direct or indirect effect on the ecosystem. Changes in climate matters during crop season and it can decrease the mean value of crop production. The present study focuses on the use of bacterial fertilizer and biocontrol agent for the better growth of pulseslike chickpea (*Cicer arietinum*) and cowpea (*Vigna unguiculata*) plant which helps to improve physical, chemical and biological health of the soil by fixing the nitrogen and reducing the addition of chemical fertilizers. *Pseudomonas* strain OG101 controls mycelial growth of *Fusarium oxysporum f.sp. ciceris* and *F. oxysporum f.sp. pallidoroseum* up to 24.4% and 20.5%, respectively. In addition, OG101 showed a significant improvement in the germination index of 93.3% and 98.3% with disease index of 1.6% and 3.3% in chickpea plant and cowpea plant, respectively. Pot experiments of chickpea and cowpea plants refer to use it as a seed bacterization for the best effect for growth promotion and its biocontrol activity against *F. oxysporum f.sp. ciceris* and *F. oxysporum f.sp. pallidoroseum* respectively.

Keywords: Biocontrol; Biofertilizer; Pulses; wilt; disease index; germination index; Chickpea; Cowpea

Introduction

Leguminosae (Fabaceae) family is the third-largest family of angiospermsand comprises of 800 genera and more than 20,000 species(Stagnari et al. 2017). This family comprises of peas, beans, lentils, peanuts, and other podded plants which areused as a food by animals and humans. However in India, estimated loss of food legumes stands at nearly 10-15% because of various diseases caused by pathogens (Pande et al. 2009). Biotic stress is an important factor that limits the edible legumes, for example,chickpea, pigeon pea, pea, and lentils. Fusarium species majorly affect the crop plants in agriculture and are found in soils over the world. Wilt disease mainly caused by Fusarium species in legumes. Several management practices have been adopted such as (agronomic, chemical, biological and use of resistant varieties) to minimize disease. In which chemical is the best method adopted for the management of disease.

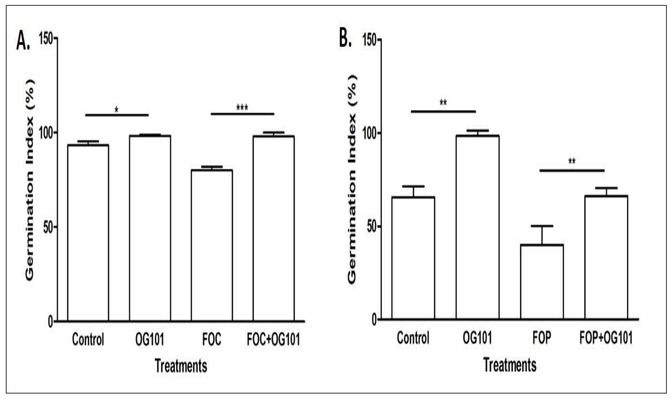
However, chemical management leads to emission of greenhouse gases. In addition, GHGs are produced by agricultural practices either directly during agricultural activities (cultivation and harvesting) or indirectly (herbicides, pesticides, and fertilizers) (Cowie et al. 2014). Fertilizer-induced GHG emissions represent the largest source of total agricultural emissions majority contributed during the production of chemical fertilizers.

Legumes can fix atmospheric nitrogen and serve as a source of proteins by themselves as well as inclusive cropping reduces the need to supply excess nitrogen fertilizers. Along with this, microorganisms play a crucial role via promotion of CO_2 sequestration to mitigate climate change directly via their own growth or indirectly via growth promotion of other plants by supplying nitrogen to soil (Kou-giesbrecht and Menge, 2019). The integrated application of biofertilizers and biocontrol agents instead of only chemical fertilizer and pesticides reduces the overall emission of GHGs such as CO_2 and N_2O.

The present study focuses on the investigation of the interaction of *Pseudomonas aeruginosa* strain OG101 as a sustainable crop production as a PGPB and biocontrol agent of leguminous crop by invading pathogenic wilt causing *Fusarium* i.e., *Fusarium oxysporum* ***f.sp***. *Ciceris* and *Fusarium oxysporum* ***f.sp***. *Pallidoroseum* for chickpea (*Cicer arietinum*) and cowpea (*Vigna unguiculata*) plant, respectively.

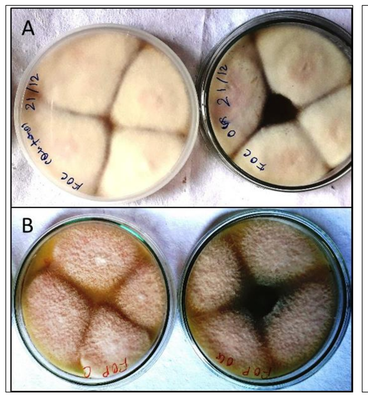
Results

Water Agar Assay



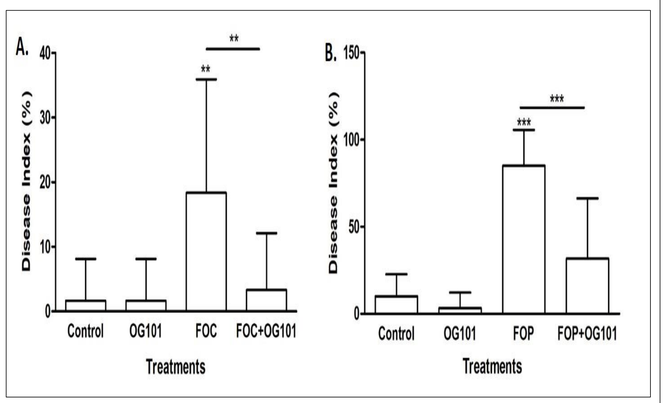
In-vitro % seed germination of chickpea plant (A) and cowpea plant (B).

Antifungal Activity



Antifungal activity of *Pseudomonas aeruginosa* OG101 against *Fusarium oxysporum* ***f. sp.*** *ciceris* (FOC) and *Fusarium oxysporum f. sp. pallidoroseum* (FOP).

Emergence Assay



In-vitro % disease emergence index of chickpea plant (A) and cowpea plant (B).

Pot trials (Vegetative parameters)

Seed Bacterization

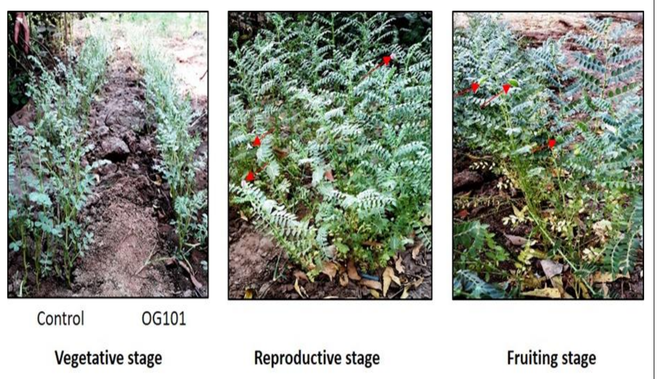
Vegetative parameter	Chickpea Legume plant				Cowpea Legume plant			
	Control	OG101 treatment	FOC	FOC+ OG101	Control	OG101 treatment	FOP	FOP+ OG101
No. of Leaves	27.3± 9.45	47.6± 1.52*	5±1	8.33±0.57*	5.6±0.57	7±1	5±1	8.33±0.57*
Length of Shoot (cm)	14.3±1.15	19.6±1.5***	19.5±0.43***	26.9±0.75***	24.7±1.07	27.7±1.41	19.5±0.4**	26.9±0.75
length of Roots (cm)	6.2±0.26	8.93±0.40***	4.93±0.37	7±0.1***	6.1±0.2	8.7±0.72**	4.9±0.37	7±0.1
Total length (cm)	20.5±0.98	28.6±1.35***	24.4±0.70***	33.96±0.85***	30.86±1.24	36.43±2.0	24.4±0.7	33.9±0.85
Fresh Root mass (gm)	0.42±0.01	0.54±0.03**	0.08±0.01***	0.15±0.07**	0.13±0.007	0.15±0.03	0.08±0.01	0.15±0.07
Fresh Leaf mass (gm)	0.24±0.02	0.27±0.01	0.27±0.007**	0.27±0.007	0.3±0.01	0.46±0.02**	0.27±0.007	0.71±0.01
Total Fresh mass (gm)	0.93±0.06	1.08±0.02**	0.71±0.01***	0.84±0.09*	0.94±0.007	1.17±0.07*	0.71±0.01**	0.84±0.09

Seed Bacterization with Soil application

Vegetative parameter	Chickpea Legume plant				Cowpea Legume plant			
	Control	OG101 treatment	FOC	FOC+ OG101	Control	OG101 treatment	FOP	FOP+ OG101
No. of Leaves	36±5.56	49±3.065	28±12.1	38±9.84	6 ± 1	8 ± 1	6.66±1.15	7.66±0.57
Length of Shoot (cm)	14.7±0.64	15.53±0.83	9.46±1.65**	14.3±0.6	27.3 ± 1.20	26.8 ± 2.55	21.46±1.55	26.4±4.50
length of Roots (cm)	4.2±1.49	21.16±1.35***	12.26±2.63**	6.03±0.5	5.66 ± 0.51	11.36 ± 0.51***	6.33±0.49	5.83±0.15
Total length (cm)	18.9±1.9	36.7±1.64***	21.73±4.28	20.3±0.8	33 ± 1.41	38.23 ± 2.36	27.8±1.55	32.2±4.35
Fresh Root mass (gm)	0.2 ±0.01	0.30±0.06	0.23±0.05	0.25±0.03	0.09 ± 0.10	0.08 ± 0.06	0.18±0.03*	0.13±0.01
Fresh Leaf mass (gm)	0.06±0.01	0.21±0.08*	0.17±0.015	0.16±0.01	0.30 ± 0.04	0.44 ± 0.08	0.48±0.02	0.57±0.06
Total Fresh mass (gm)	0.2±0.01	0.16±0.015*	0.19±0.03	0.18±0.02	0.98 ± 0.09	1.12 ± 0.11	1.19±0.08	1.52±0.02

P Value has been calculated using one way ANOVA and its interpretation is as follows:
Ns (P value greater than 0.05) Non-significant as compared to control
*** (P value between 0.05 to 0.01) Significant at 5%**
**** (P value between 0.01 to 0.001) Significant at 1% as compared to control**
***** (P value less than 0.001) Significant at 0.1% as compared to control**

Field Trials



Field trial of chickpea plant and its three different stages of growth period [vegetative state (15 days), reproductive stage (45 days) and fruiting stage (60 days)].

Chickpea Field trial	Control	OG101	P-value	Significance
Root length (Cm)	4.37± 0.895	5.71± 1.352	0.048	*
Shoot length (Cm)	21.25± 1.89	25.5± 2.428	0.006	*
Total length (Cm)	25.68±1.823	31.2± 3.248	0.004	**
No. of leaves	40.66±10.55	112.8± 29.08	0.0001	***
No. of pods	0.6± 0.547	2± 1.032	0.008	*
Dry weight of Pod (gm)	0.078±0.033	0.12± 0.053	0.001	**

Field trials of chickpea showed 21% increase in production quality.

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