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Studies on efficacy of Biofertilizers for Nutrient Management in gladiolus

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ABSTRACT
An experiment was conducted at Precision Farming Development Centre, Department of Horticulture and Biocontrol laboratory, Department of Microbiology, CCS Haryana Agricultural University, Hisar to study the efficacy of biofertilizers for nutrient management in gladiolus cv Advance Red with nine treatments replicated thrice under Randomised Block Design for two consecutive years. The treatments include 100% RDF using inorganic fertilizers and reduced levels of inorganic fertilizers (75% and 50%) in combination with three rhizobacterial strains of biofertilizers viz. Azotobacter chroococcum (Mac27), Pseudomonas strain (WPS73) and Phosphate solubilizing bacteria PSB (P36) individually and in combinations. The results revealed that application of Pseudomonas strain (WPS73) with 75% RDF followed by Azotobacter chroococcum (Mac27) with 75% RDF recorded early sprouting, days to spike initiation and growth parameters like per cent sprouting, plant height, number of leaves was recorded maximum. Further same treatments were found to be superior in enhancing quality parameters like spike length, rachis length, number of florets and floret diameter. However, incompatibility was observed in co-inoculation of Azotobacter chroococcum (Mac27) with Pseudomonas strain (WPS73), which resulted in complete mortality of plants when applied in combination. It was found that use of compatible biofertilizers was beneficial for growth and quality of gladiolus.
Keywords: Azatobacter,Advance Red,Biofertilizers,Gladiolus,Pseudomonas, PSB.

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INTRODUCTION

Flowers are grown since ancient times and floriculture has emerged as a booming enterprise in India. In floriculture industry, gladiolus occupies prime position and occupies fourth place in the international trade ranking next to tulip among in European market. It is being cultivated in almost all the developing countries of the world including India. Gladiolus is cultivated as a commercial cut flower crop in India which is a costly enterprise involving land, water, planting material, fertilizers, pesticides etc. In modern agriculture, use of inorganic fertilizer is essential for sustainable yields and chemical fertilizers play a key role by contributing 50-60% increase in productivity. Due to the excessive and imbalance use of chemical fertilizers, problems of soil deterioration, ground water contamination and air pollution has been observed. This situation emphasized the need for developing alternate production systems that are eco-friendly and more judicious in managing soil health. Use of biofertilizers in crop cultivation may help in safeguarding the soil health [1]. It is now recognized that many rhizosphere bacteria including *Pseudomonas*, *Bacillus*, *Azotobacte*r and *Azospirillum* have ability to increase mobilization of insoluble nutrients, produce hormone like substances similar to auxins, suppression of phytopathogenic organism by production of antibiotics and hydrolytic enzymes [2]. The biofertilizers offer an economically attractive and ecologically sustainable means of reducing external inputs and improving the quality and quantity of natural land resources [1]. Keeping this in view the present investigation was carried out to study the efficacy of biofertilizers for nutrient management in gladiolus.

MATERIALS AND METHODS

The experiment was carried out at Precision Farming Development Centre, Department of Horticulture, CCS Haryana Agricultural University, Hisar. The experiment was conducted in randomized block design consisting of nine treatments and replicated thrice. Uniform sized corms of gladiolus cultivar Advance Red were used for trails. A plot size of 1 m x 1.2 m was made in which 20 corms at a spacing of 30 cm x 20 cm were sown per replication. The experiment was repeated twice in two consecutive years in the same season of sowing *i.e.,* October. The treatments include **T_1** -100% RDF (30:20:20 g NPK /m^2), **T_2** -75% RDF + *Azotobacter chroococcum* (Mac27), **T_3** -75% RDF + *Pseudomonas* strain (WPS73), **T_4** -75% RDF + *Azotobacter chroococcum* (Mac27). *+Pseudomonas* strain (WPS73), **T_5** -75% RDF + *Azotobacter chroococcum* (Mac27) + *Pseudomonas* strain (WPS73) + PSB (P36), **T_6** -50% RDF + *Azotobacter chroococcum* (Mac27), **T_7** -50% RDF + *Pseudomonas* strain (WPS73), **T_8** -50% RDF + *Azotobacter chroococcum* (Mac27) + *Pseudomonas* strain (WPS73) and **T_9** - 50% RDF + *Azotobacter chroococcum* (Mac27) + *Pseudomonas* strain (WPS73) + PSB (P36).

Biofertilizer preparation

Different broth media were prepared in single distilled water and autoclaved at 15 lbs pressure for 20 minutes. The following media were used for this experiment.

(i) Luria Bertani medium (Sambrook *et al.,* 1989) – for *Pseudomonas* and PSB.

Composition	**g/l**
Tryptone	10.0
Yeast extract	5.0
NaCl	2.0
Glucose	1.0
pH	7.0

(ii) Jenson's broth– for *Azotobacter* strain

Composition	**g/l**
Sucrose	20.0
K_2HPO_4	1.0
$MgSO_4$ 7 H_2O	0.5
NaCl	0.5
$FeSO_4.7H_2O$	0.1
NO_2MoO_4	5 mg
$CaCo_3$	2.0

The bacterial cultures used in the experiment were procured from the Department of Microbiology, Chaudhary Charan Singh Haryana Agricultural University, Hisar. The cultures of *Pseudomonas* and PSB bacterial strains were grown in LB broth and *Azotobacter* was grown in Jensen's broth for 3 days.

Biofertilizers application

The liquid biofertilizers were mixed according to the treatment combinations separately in plastic tubs with a little jaggery solution added to it to make the corms sticky and then corms were soaked in the liquid formulation for one hour. Later corms were removed from solution and planted immediately.

Observations were recorded for various vegetative, floral and corm characters by taking the average of five plants from each replication. The statistical method described by Pans and Sukhatme was followed for analysis and interpretation of the experimental results [4].

RESULTS AND DISCUSSION

In this experiment an attempt was made to determine the efficacy of biofertilizers in enhancing growth and quality of gladiolus. Three types of rhizobacterial strains were used *viz. Azotobacter chroococcum* (Mac27), *Pseudomonas* strain (WPS73) and Phosphate solubilizing bacteria PSB (P36) as biofertilizers in combination with reducing doses of inorganic fertilizers. The results are presented under different sub heads.

Vegatative characters

The data presented in Table 1 revealed that days to sprouting of corms was not influenced by different treatments but per cent corm sprouting was significantly influenced by various treatments. Application of 75% RDF+ *Azotobacter chroococcum* (Mac27) *+Pseudomonas* strain (WPS73) recorded minimum per cent sprouting (56.1%) whereas, maximum sprouting of corms (88.2%) was observed in 75% RDF+ *Pseudomonas* strain (WPS73) which was at par with all other treatments. Increased percentage sprouting can be attributed mainly due to availability of sufficient nutrients to the corms for its normal metabolic activities. Induced sprouting might be due to synthesis and secretion of thiamin, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, indole acetic acid (IAA) and gibberellins like substances [5]. It was also reported that combined application of chemical fertilizers, biofertilizers and biostimulants showed a significant influence on growth of gladiolus cv. Sancerre [6].

It was interesting to find that there was complete mortality of plants at an early stage in treatments containing a combination of *Azotobacter chroococcum* (Mac27) and *Pseudomonas* strain (WPS73) both at 75% and 50% RDF. This might be due to the incompatibility of the rhizobacterial strains used as biofertilizers or due to antagonistic effect and secretion of some toxic substances by the bacteria resulting in mortality of the plants. This area needs further research in detail (about the response of gladiolus with these two bacterial strains when used together) to know the actual cause behind the mortality of plants with the use of these two strains in combination as biofertilizers. During root colonization by introduced bacteria, introduced microorganisms have to compete with indigenous microflora for carbon source, mineral nutrients and infection sites on the roots. Sometimes, this competition is so severe that introduced microorganism fails to survive in the soil. Another factor that can contribute to inconsistent performance of PGPR is variable production or inactivation *in situ* of bacterial metabolites responsible for plant growth promotion [7].

Due to complete mortality of the plants these two treatments were not included in further observations for analysis and interpretation of results.

It is evident from the data presented in table 1 that the vegetative parameters like plant height and number of leaves were significantly influenced by the application of biofertilizers. Application of treatment 75% RDF + *Pseudomonas* strain (WPS73) resulted in significantly maximum plant height (60.8 cm) and (62.4 cm) and highest number of leaves (9.4) (9.5) under both the years of study respectively which was at par with 75 % RDF + *Azotobacter chroococcum* (Mac27). Application of *Pseudomonas* strain (WPS73) and *Azotobacter chroococcum* (Mac27) as single inoculation with 75% RDF resulted in increased plant height and number of leaves. Indole acetic acid is one of the naturally occurring auxins with broad physiological effects on plants. Many rhizosphere bacteria including *Pseudomonas*, *Azotobacter*, *Azospirillum* etc were found to have the ability to produce IAA or related auxins. Auxins have been implicated in initiation of lateral and adventitious roots, in stimulation of cell division and elongation of stems and roots [8]. Increased in number of leaves and plant height with the application of *Pseudomonas* and *Azotobacter* in gladiolus was also reported [9]. Increased plant height in gladiolus cv. Sancerre was reported with the application of biofertilizers due to better uptake of nutrient elements, solubilization and mobilization of insoluble forms of phosphorus in the soil by organic acids, better photosynthetic rate, and source-sink relationship, besides excellent physiological and biochemical activities due to the presence of biofertilizers[6].

Floral characters

The commercial value of a flower crop depends mainly on its specific quality attributes. In gladiolus floral characters like days to spike initiation and basal floret opening decides the availability of flowers to the market demand. Flower characters like spike length, rachis length, number of florets per spike and diameter of floret decides the value of flower spike. In the present investigation flower characters were significantly influenced by the use of biofertilizers.

The data pertaining to floral characters is presented in the Table 2. Earliness in spike initiation and basal floret opening was observed in plants applied with 75% RDF and *Pseudomonas* strain (WPS73) followed by *Azotobacter chroococcum* (Mac27). It is apparent from the data that 75 % RDF + *Pseudomonas* strain (WPS73) alone resulted in significantly minimum number of days (117.8) for spike initiation followed by *Azotobacter chroococcum* (Mac 27) with 75 % RDF (119.6) in first year of study But 75 % RDF either with *Azotobacter chroococcum* (Mac27) or *Pseudomonas* strain (WPS73) treatment resulted in early spike initiation (116.3 and 116.7), respectively in the later year which were at par with each other. 100% RDF (30:20:20 g NPK/m^2) application significantly recorded maximum number of days for spike initiation (125.3 and 121.8) during both the years under study. It is clear from the data (Table 2) that during the first year, 75 % RDF + *Pseudomonas* strain (WPS73) resulted in significantly earlier basal floret opening (131.4 days) among all other treatments however There were no significant differences between the treatments in the next year. Higher dose of nitrogen and phosphorus delayed spike emergence and basal floret opening due to prolonged vegetative phase and leaves regulate flower induction as they are site of auxin production and other hormones which are responsible for flower induction. Yogesh Singh reported that biofertilizers *Azotobacter* and *Pseudomonas* caused early spike initiation and basal floret opening in gladiolus [5]. Similar results were reported in gladiolus by the use of combination of chemical fertilizers, biofertilizers and biostimulants [6]. Whereas contradictory to the present investigation reported that 100% RDF recorded early spike initiation and basal floret opening in gladiolus cv. White prosperity [10]. However, delay in spike initiation with higher dose of NPK might be due to the fact that increased dose of N and P encouraged vigorous vegetative growth with more photosynthetic area and mobilization of photosynthates to sink, which ultimately delayed reproductive phase [11].

From the data presented in the Table 2 it is revealed that floral characters like length of spike, rachis length and number of florets per spike were recorded maximum by the application of 75% RDF and

Pseudomonas strain (WPS73) with the readings of (58.2 cm, 60.6 cm), (46.5 cm, 48.2 cm) and (17.1, 17.4) respectively which was followed by 75 % RDF+ *Azotobacter chroococcum* (Mac27) . Similar trend was observed in both the years. Whereas it is apparent from the data that all the treatments either containing *Azotobacter* strain (Mac27) or *Pseudomonas* strains (WPS73) were found to be superior and at par with each other with regards to floret diameter. Further during the second year no significant differences were observed among various treatments with respect to floret diameter. Application of *Pseudomonas* strain (WPS73) followed by *Azotobacter chroococcum* (Mac27) showed its superiority in enhancing the quality of flower spikes. This could be due to the fact that *Pseudomonas* strain and *Azotobacter* are known to produce growth promoting substances like IAA and GA_3 which helped in increasing flower stalk length. Further biofertilizers enhanced the availability of nutrients in the soil which increased the synthesis of amino acids and chlorophyll formation and in return carbohydrate formation which ultimately resulted in the better growth and length of spike and rachis. The present findings are in confirmation with the findings of [5] who reported that *Pseudomonas* strain (CPS63) and Bacillus strain showed increasing flower parameter in gladiolus. Similarly, increase in number of florets and size of floret in gladiolus was due to the fact that uptake and absorption of the nutrients was improved by the use of biofertilizers [12]. significant improvement in flower quality of gladiolus were recorded with the inoculation of *Azotobacter* + PSB, which might be due to increased availability of nitrogen and better mobilization, solubilization of phosphate and better uptake of micronutrients like Zn, which is a precursor of auxin ultimately improved plant growth and flower spike [11] in gladiolus cv. Sancerre it was reported that biofertilizers increased the plant height and number of leaves which ultimately resulted in increased flower attributes like spike length, rachis length and number of florets [6]. Similar results were reported in gladiolus cv. White Prosperity [13],[14].

Table 1: Effect of biofertilizers in combination with inorganic fertilizers on vegetative characters of gladiolus cv Advance Red.

Treatments	Days taken for sprouting		Per cent sprouting		Plant height (cm)		Number of leaves / plant	
	first year	second year	first year	second year	first year	second year	first year	second year
100% RDF (30:20:20 NPK g /m²)	14.7	22.4	96.6 (82.6)	93.6 (77.7)	50.5	53.7	8.0	7.8
75% RDF + *Azotobacter chroococcum* (Mac27).	14.3	16.3	100 (88.2)	96.8 (80.8)	59.7	60.5	8.9	8.9
75% RDF + *Pseudomonas* strain (WPS73)	14.9	17.2	100 (88.2)	96.6 (82.8)	60.8	62.4	9.4	9.5
75% RDF + *Azotobacter chroococcum* (Mac27). +*Pseudomonas* strain (WPS73)	14.7	16.0	68.3 (56.1)	62.4 (52.3)	-	-	-	-
75% RDF + *Azotobacter chroococcum* (Mac27) + *Pseudomonas* strain (WPS73) + PSB (P36)	13.5	17.0	100 (88.2)	91.6 (73.4)	48.6	50.9	8.3	8.4
50% RDF + *Azotobacter chroococcum* (Mac27).	12.8	16.8	100 (88.2)	98.0 (82.8)	55.3	56.3	8.6	8.7
50% RDF + *Pseudomonas* strain (WPS73)	12.4	17.9	100 (88.2)	98.3 (84.3)	57.5	58.2	8.7	8.7
50% RDF + *Azotobacter chroococcum* (Mac27) + *Pseudomonas* strain (WPS73)	14.0	20.4	76.6 (61.1)	83.6 (66.6)	-	-	-	-
50% RDF + *Azotobacter chroococcum* (Mac27). + *Pseudomonas* strain (WPS73) + PSB (P36)	13.1	18.9	95.0 (81.5)	95.0 (81.2)	47.8	48.4	8.2	8.2
SEm±	0.83	2.1	3.8	4.4	0.7	0.8	0.2	0.2
CD (P=0.05)	N.S.	N.S.	11.7	13.2	2.1	2.5	0.5	0.5

Table 2: Effect of biofertilizers in combination with inorganic fertilizers on floral characters of gladiolus cv Advance Red.

Treatments	Days taken for				Spike length (cm)		Rachis length (cm)		Number of florets per spike		Diameter of floret (cm)	
	Spike initiation		Basal floret opening									
	first year	second year	first year	second year	first year	second year	first year	second year	first year	second year	first year	second year
100% RDF (30:20:20 g /m²)	125.3	121.8	137.3	130.8	49.6	52.3	35.4	40.0	15.0	15.1	11.3	12.5

75% RDF + *Azotobacter chroococcum* (Mac27)	119.6	116.7	134.7	128.5	56.4	57.1	44.7	46.9	16.1	16.9	12.2	12.9
75% RDF + *Pseudomonas* strain (WPS73)	117.8	116.3	131.4	129.5	58.1	60.6	46.5	48.2	17.1	17.4	12.3	13.0
75% RDF + *Azotobacter chroococcum* (Mac27) + *Pseudomonas* strain (WPS73) + PSB (P36)	123.0	119.4	135.8	129.7	48.2	51.9	36.3	38.2	14.2	14.2	10.7	12.0
50% RDF + *Azotobacter chroococcum* (Mac27)	122.2	118.2	134.2	131.3	51.2	54.1	40.8	42.2	15.4	15.1	12.0	12.5
50% RDF + *Pseudomonas* strain (WPS73)	121.2	118.7	135.2	131.3	52.6	55.6	42.3	44.4	15.9	16.2	12.1	12.7
50% RDF + *Azotobacter chroococcum* (Mac27) + *Pseudomonas* strain (WPS73) + PSB (P36)	123.6	119.4	137.0	129.3	45.6	50.3	35.2	36.6	14.0	13.9	10.5	11.6
SEm±	0.5	1.8	0.5	0.7	0.6	0.4	0.3	0.5	0.2	0.1	0.2	0.5
CD (P =0.05)	1.7	2.3	1.5	N.S.	1.8	1.3	1.0	1.5	0.7	0.4	0.6	N.S.

CONCLUSION

From the present study we can conclude that the Application of *Pseudomonas* strain (WPS73) with 75% RDF followed by *Azotobacter chroococcum* (Mac27) was found to be beneficial for growth and quality of gladiolus thereby reduced 25% use of inorganic fertilizers.

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